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Biotherapies in neurological diseases

Biotherapies in stroke

Biothérapies dans l'accident vasculaire cérébral

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INFO ARTICLE

Article history:
Received 28 February 2014
Received in revised form
29 September 2014
Accepted 8 October 2014
Available online 6 November 2014

Keywords:
Plasticity
Neural repair
Cell therapy
Transplantation
Stem cells

Mots clés :
Plasticité
Régénération
Thérapie cellulaire
Transplantation
Cellule souche

ABSTRACT

Stroke is the second leading cause of death worldwide and the most common cause of severe disability. Neuroprotection and repair mechanisms supporting endogenous brain plasticity are often insufficient to allow complete recovery. While numerous neuroprotective drugs trials have failed to demonstrate benefits for patients, they have provided interesting translational research lessons related to neurorestorative therapy mechanisms in stroke. Stroke damage is not limited to neurons but involve all brain cell type including the extracellular matrix in a "glio-neurovascular niche". Targeting a range of host brain cells, biotherapies such as growth factors and therapeutic cells, currently hold great promise as a regenerative medical strategy for stroke. These techniques can promote both neuroprotection and delayed neural repair through neuro-synaptogenesis, angiogenesis, oligodendrogliogenesis, axonal sprouting and immunomodulatory effects. Their complex mechanisms of action are interdependent and vary according to the particular growth factor or grafted cell type. For example, while "peripheral" stem or stromal cells can provide paracrine trophic support, neural stem/progenitor cells (NSC) or mature neurons can act as more direct neural replacements. With a wide therapeutic time window after stroke, biotherapies could be used to treat many patients. However, guidelines for selecting the optimal time window, and the best delivery routes and doses are still debated and the answers may depend on the chosen product and its expected mechanism including early neuroprotection, delayed neural repair, trophic systemic transient effects or graft survival and integration. Currently, the great variety of growth factors, cell sources and cell therapy products form a therapeutic arsenal that is available for stroke treatment. Their effective clinical use will require prior careful considerations regarding safety (e.g. tumorgenicity, immunogenicity),

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http://dx.doi.org/10.1016/j.neurol.2014.10.005

potential efficacy, cell characterization, delivery route and in vivo biodistribution. Bone marrow-derived cell populations such as mesenchymal stromal/stem cells (MSC) or mononuclear cells (MNC), umbilical cord stem cells and NSC are most investigated notably in clinical trials. Finally, we discuss perspectives concerning potential novel biotherapies such as combinatorial approaches (growth factor combined with cell therapy, in vitro optimization of cell products, or co-transplantation) and the development of biomaterials, which could be used as injectable hydrogel scaffold matrices that could protect a cell graft or selectively deliver drugs and growth factors into the post-stroke cavity at chronic stages. Considering the remaining questions about the best procedure and the safety cautions, we can hope that future translational research about biotherapies will bring more efficient treatments that will decrease post-stroke disability for many patients.

RÉSUMÉ

Les accidents vasculaires cérébraux (AVC) représentent la seconde cause de mortalité et la première cause de handicap. En améliorant la neuroprotection et la plasticité endogènes, fréquemment insuffisantes pour assurer une récupération complète, les biothérapies semblent très prometteuses. Leurs mécanismes d'action dépendent du facteur de croissance ou des cellules choisis. On peut distinguer un effet neurotrophique paracrine pour les cellules souches « périphériques » et un effet de remplacement cellulaire plus direct pour les cellules souches neurales (NSC). Cependant, les délais, les doses et les voies d'administration restent débattus. Parmi les nombreuses cellules disponibles, les cellules de la moelle osseuse ou du cordon (par ex : cellules souches/stromales mésenchymateuses, ou cellules mononucléées) et les NSC sont les plus étudiées. En perspective, nous discutons ici les thérapies combinées (facteur de croissance et cellules, co-transplantations...) et l'utilisation de biomatériaux (hydrogels injectables) libérant l'agent thérapeutique ou protégeant le greffon. En tenant compte des questions en suspens sur la meilleure procédure et la sécurité (par ex : tumorigénicité, immunogénicité), nous pouvons espérer que les biothérapies deviennent une stratégie thérapeutique efficace, avec une large fenêtre thérapeutique, pour réduire le handicap post-AVC de nombreux patients.

1. Introduction: pathophysiology of stroke recovery

Stroke is the second leading cause of death worldwide with more than six million deaths per 17 million strokes each year. Stroke has an incidence from 1 to 4/1000/year with 31% occurring before the age of 64 [1]. Moreover, it is the most common source of severe disability in adults affecting 60–70% of stroke survivors. Ischemic strokes represent around 80% of all cases. Excepting early thrombolysis by alteplase (tPA), craniectomy for large strokes or admission to a stroke unit for intensive care and rehabilitation, no treatment currently exists to efficiently enhance recovery after stroke [2]. Therefore, the development of new therapies that can enhance brain remodeling processes is crucial and success in this endeavor requires a pathophysiological viewpoint [3–6]. It is well-known that stroke effects are not limited to neurons but involve both brain cells and the surrounding extracellular matrix in a ''glio-neurovascular niche'' that interacts with the peripheral immune system [7–11]. For these reasons, new therapies should target all these systems rather than narrowly targeting an individual damage process, perhaps then avoiding the failures of past clinical translational attempts to develop specific neuroprotective drugs.

Biotherapies for stroke, including growth factors and cell-based therapies, could achieve the goal of broad physiological action by simultaneously promoting both endogenous neuroprotection and neural repair, including neurogenesis, angiogenesis, oligodendrogliogenesis, axonal sprouting, and synaptogenesis [4,12–17]. These approaches also have the advantage of action over an extended therapeutic time-window after stroke and thereby might be effective in more patients than those helped by current acute strategies such as thrombolysis or neuroprotectant treatments.

1.1. *Endogenous neural protection after stroke*

Early after stroke, in addition to possible reperfusion via collateral arteries or fibrinolysis, several endogenous protective mechanisms are spontaneously engaged [18] (Fig. 1). During the acute phase, there is activation of ''anti-excitotoxic'' channels, receptors or regulators such as γ-aminobutyric acid (GABA) or K^+ receptors. These phenomena are followed by a transduction phase with amplification of kinases, transcription factors and growth factors (neurotrophin 3, granulocyte colony-stimulating factor [GCSF], vascular endothelial growth factor [VEGF], brain derived neurotrophic factor [BDNF]...). Finally, anti-apoptotic (heat shock protein [HSP 70], BCL2, inhibitors of apoptosis proteins [IAP],

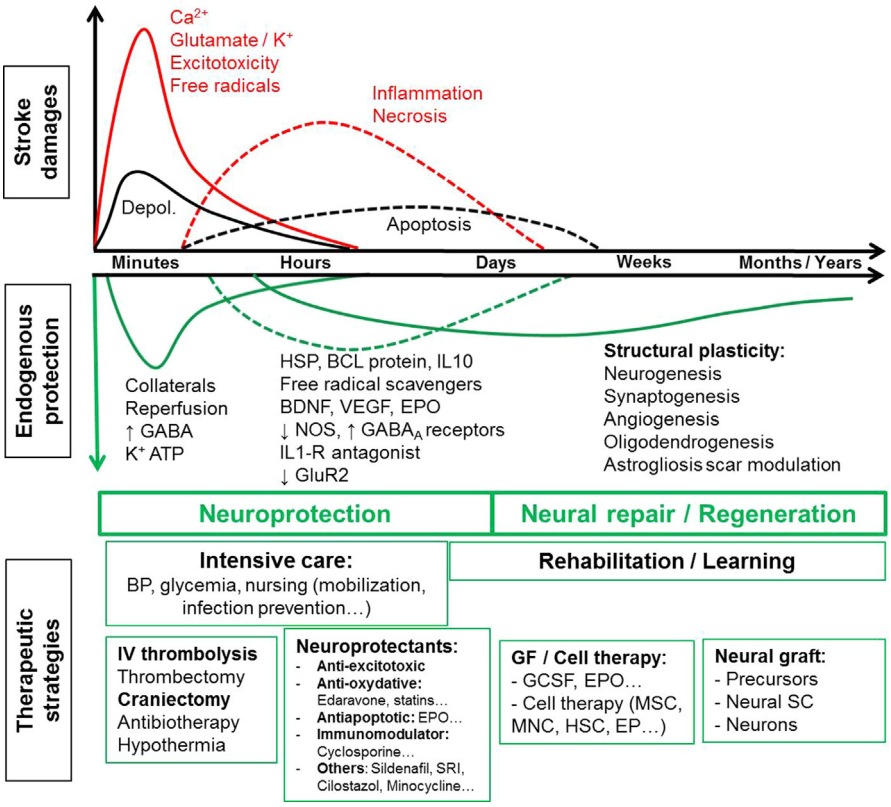

Fig. 1 – Time course of stroke and associated therapeutic time-windows. BCL: B-cell lymphoma protein; BDNF: brain derived neurotrophic factor; BP: blood pressure; Depol.: peri-infarct depolarisation; EP: endothelial progenitors (CD34+); EPO: erythropoietin; GCSF: granulocyte-colony stimulating factor; GF: growth factors; GluR2: glutamate receptor (subunit 2); HSC: hematopoietic stem cells (CD34+); HSP: heat-shock protein; IL: interleukin; IP3: inositol tri-phosphate; IV: intravenous; MNC: mononuclear cells; MSC: mesenchymal stromal/stem cells; NOS: NO synthase; SRI: serotonin reuptake inhibitors; VEGF: vascular endothelial growth factor.

hypoxia inducible factor [HIF1]...), anti-inflammatory (IL10, transforming growth factor β1 [TGFβ1]...) and anti-oxidative (α-tocopherol, vitamin C) molecules stimulate neural repair processes [19]. For example, in astrocytes, HIF1 notably induces erythropoietin production and then activates neuroprotective and neuro-angiogenic phosphoinositide-3-kinase/protein kinase B (PI3K/Akt) pathway [20].

1.2. *Post-stroke plasticity and neural repair*

The adult brain has a striking capacity for self-repair (Fig. 1) often resulting in re-emergence of childhood organizational patterns [21]. During stroke recovery, beyond phenomena related to adaptive functional compensation, there is "structural" brain plasticity [22,23] based on the surviving tissue participating in reorganization of damaged networks [24,25], and exhibiting neuro-synaptogenesis with axonal sprouting that can persist for several months [26–29]. This form of post-stroke plasticity notably occurs in elderly human brains [30–32]. Stroke also increases neurogenesis from neural stem cells (NSC) of the subventricular zone (SVZ) and hippocampal dentate gyrus, generating neuroblasts that migrate to the lesion and differentiate into mature neurons [29,33]. This post-stroke neurogenesis is closely linked to angio-vasculogenesis and glial function leading to the concept of a "glio-neurovascular niche" [11,34–36] as a favorable "stem cell niche" [37]. Endothelial cells, but not vascular smooth muscle cells, release factors that both stimulate the self-renewal of NSC and also enhance neuron production [38]. After stroke, microvessels size and density changes [39,40] stimulate the neuronal plasticity [41]. For several months following stroke, neuroblasts from the SVZ migrate close to vessels through an area exhibiting early vascular remodeling [42] stimulated by the release of neurotrophic factors such as angiopoietin 1 (Ang1) [43], stromal-derived factor (SDF1) [34], BDNF [44], VEGF [45,46] or metalloproteases [47]. Neuroblasts also enhance angiogenesis with the release of VEGF [48], a relationship underlining the bidirectional link between microvascular and neuronal remodeling.

Additionally, glial cells play a key role during post-stroke recovery, with astrocytes removing excitatory neurotransmitters (glutamate) and K^+ and thereby limiting excitotoxic damage. These cells also modulate synaptogenesis by enhancing the formation of functional synapses [49]. After injury, astrocytosis is linked to microvessels [50] and has a beneficial effect on NSCs via the sonic hedgehog pathway. Otherwise, oligodendrocyte progenitors that are resident in white matter or derived from SVZ NSCs differentiate after stroke into mature oligodendrocytes, thereby enabling axonal remyelination and strongly contributing to brain repair processes [36,51,52]. Microglia also contribute to this post-stroke remodeling

process, both acting as an inflammation modulator and also by releasing trophic factors such as BDNF that encourage synaptogenesis and neurite outgrowth [53].

Although the effects of these complex processes comprising post-stroke plasticity are reinforced by exercise and rehabilitation [54–58], post-stroke neurogenesis from endogenous NSCs is relatively weak and many new neurons die [27], resulting in incomplete and disappointing functional recovery. Thus, a deeper understanding of how post-stroke brain remodeling is affected by the integration neurons into their ''glio-vascular'' microenvironment is crucial to developing more effective neurorestorative therapies to amplify post-stroke plasticity and stimulate behavioral recovery.

2. Neuroprotective drugs for stroke: lessons for translational research

Most of the known acute damage processes have been targeted by one or more studies utilizing neuroprotectants [5,59] (Fig. 1). Despite thousands of positive results from animal experiments, it has been particularly disappointing that no clinical trial has demonstrated a clear benefit for any neuroprotective drug after stroke [60,61]. The drugs that have been investigated can be sorted in several types: anti-excitotoxics targeting glutamate toxicity [62], calcium-blockers [63], antioxidants [64,65], anti-apoptotics [66], angiotensin receptor blockers (sartans), prostaglandin receptor antagonists [67,68], anti-inflammatories or immunomodulators [69] such as cyclosporine A [70], immunotherapy to limit the tPA neurotoxicity [71,72], stimulants (amphetamine), or anti-edema agents (mannitol). Finally, some neuroprotective strategies have exhibited several or unclear effects. These include: induced hypothermia [73–75], hyperbaric or normobaric oxygen therapy [76], albumin [77], magnesium [78], the anti-aggregant cilostazol [79], statins [80], citicoline [81], sildenafil [82], or a treatment based on traditional Chinese medicine (MLC601, Neuroaid) [83].

Despite the past failure of their transfer to clinical environments, neuroprotective therapies are still actively investigated. In this context, some recent findings should be noted, such as the study utilizing the post-synaptic protein PSD95 inhibitor in monkeys [84]. Moreover, encouraging data about stroke recovery were obtained in clinical trials assessing fluoxetine, a serotonin reuptake inhibitor [85], edaravone, a free radical scavenger [65,86], or minocycline [87].

Lessons from past clinical trial failures should be carefully considered in designing future translational research stroke recovery studies, as the experience gained in these previous trials may be profitably inform the new trials and increase the chance of successful clinical biotherapy transfers [88]. Although rodent models allow convenient exploration of a range of putative pathophysiological mechanisms and potential therapies, the anatomic and pathophysiologic differences between rodent and human stroke arising from differences in cortical architecture, regional neuronal density, arterial network distribution, immunological mechanisms, pre-existing vascular risk factors, concomitant medical treatments, and recovery duration intervals could easily impede the effective translation of animal study results to the clinic. The effect of these inter-species' differences on clinical transfer potential might be anticipated by using computational modeling techniques. ''In silico'' mathematical stroke models can simulate neuroprotective drug [89] or cell therapy effects by implementing explicit models of neurogenesis [90]. The development of these models could facilitate the generation of mechanistic hypotheses that could enhance subsequent clinical transfer of promising treatments. In addition, effort could profitably be exerted to improve experimental methods and statistical data analysis of neuroscience preclinical experiments [91]. For this purpose, preclinical studies may be conducted following clinical trial guidelines, incorporating randomized treatment assignment, blinded assessments, multicenter design, and carefully defined inclusion and non-inclusion criteria [92,93]. Modeled on the format of the Stroke Therapy Academic Industry Roundtable (STAIR) [94], the stem cell therapeutics as an emerging paradigm in stroke (STEPS) consortium guidelines created in 2007 [95–98] summarize useful recommendations to enhance the translational development of biotherapies.

3. Growth factor treatment in stroke

Numerous growth factors have been investigated as potential regenerative biotherapies for stroke, particularly during the post-acute phase [15–17] (Fig. 1). Unfortunately, as neuroprotectants, none of them showed clear benefits in patients. Growth factors target enhancement of brain remodeling by either mobilizing endogenous stem/progenitor cells, such as cytokines like GCSF or chemokines like SDF1, or by direct trophic effects acting to support damaged neurons, as in the effects of BDNF or glial-derived neurotrophic factor (GDNF).

3.1. *Colony stimulating factors*

Colony stimulating factors, such as hematopoietic growth factors, can mobilize the release of bone marrow stem cells into the circulation and result in neuroprotective effects in experimental stroke [99]. Although erythropoietin [100] improves neuronal survival, angiogenesis, axonal sprouting, inflammation [101] and oligodendrogliogenesis [102], it appears to increase mortality in clinical trials [103]. Moreover, sequential administration of erythropoietin and β-human chorionic gonadotropin failed to show a benefit despite good safety results during their pilot clinical trial [104]. Concerning GCSF, its experimental effects on neural repair remains controversial [105,106] as it could increase macrophage infiltration into ischemic brain regions [107]. Combining GCSF and stem cell factor (SCF) treatment could enhance post-stroke angiogenesis, neuronal survival and functional outcome [108]. However, GCSF injection alone did not provide any significant clinical benefit during the acute (<9 h) [109] or subacute (<48 h) phase after stroke [110].

3.2. *''Neurotrophic'' growth factors*

In the same manner, administration of neural angio-trophic supports such as SDF1 [111,112], BDNF [44,113,114], GDNF [115], angiopoietin [116–118], VEGF [119–121], fibroblast growth

factor (FGF) [122,123], or hepatocyte growth factor [124] have not yet been evaluated in clinical trial despite encouraging results from preclinical studies. Thus, additional studies are warranted to further evaluate the use of growth factors as useful stroke treatments. In the future, an interesting strategy would be the combination of growth factors with cell therapy or biomaterials, as discussed below in ''Perspectives concerning the use of biotherapies for stroke''.

4. Cell therapy after stroke

Cell-based therapies are particularly relevant as neurorestorative treatment for stroke [4,12–17,125–130]. With a wide therapeutic time-window, they could be used to treat many stroke patients, potentially generating significant increments in societal value [131]. Transplanted cells, an example of ''plastic'' biological products, can adapt to different local conditions in damaged brain tissue while not being limited to a unique target. They can act on a wide range of endogenous neuroprotective and neural repair processes including immunomodulation, neuronal, vascular and glial remodeling (Fig. 1). Traditionally, we distinguish two main treatment strategies, either paracrine trophic support using ''peripheral'' stem or stromal cells, or direct neural replacement using neural stem/progenitor cells or mature cells such as neurons. The route, dose, and timing for cell transplantation after stroke are still debated, depending on the chosen cell product and the expected therapeutic effect.

4.1. *Cell sources and therapeutic cells*

Today, the great variety of available cell types and sources form a rich therapeutic arsenal for stroke which requires, prior to clinical use, careful consideration regarding their respective preclinical safety and efficacy profiles, cell characterization, mechanisms of action, delivery routes and in vivo biodistribution properties [125,132]. Currently, among the many available cell types, bone marrow-derived cell populations such as mesenchymal stromal/stem cells (MSC), umbilical cord stem cells and neural stem cells (NSC) are the most commonly investigated in clinical trials.

4.1.1. *Cell sources*

Cell sources can be sorted by their adult, fetal (extra-embryonic), or in vitro cell-culture origin [133]. While adult sources, such as bone marrow [134–136], are widely used in clinical trials (Table 1), adipose tissue containing MSC [137–140], peripheral blood [141–144], olfactory mucosa [145], menstrual blood [146], brain tissue [147], breast milk [148] or dental tissue [149] are interesting alternatives for neurorestorative therapy. ''Fetal'' sources, such as umbilical cord, are relatively easy to collect for banking and can provide cell products for stroke therapy from either the cord itself (Wharton's jelly) [150] or from cord blood samples [151]. Placenta [152,153], amniotic fluid [154], or fetal brain samples, including striatum or 1st trimester cerebral cortex [155,156] are already used as cell sources in current clinical trials. Finally, in vitro sources such as NSCs [157] or neural cell cultures [155,158] have already been used in clinics. From in vitro cultures, pluripotent cells such as embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC) from adult tissue cannot be directly used due to their associated high tumorgenicity risk [159]. However, as sources, ESC [160–162] and iPSC [163,164] can be expanded over many passages, thereby providing a virtually unlimited supply of multipotent or mature derived cells usable for cell therapy.

4.1.2. *Cell therapy products*

Cell therapy products harvested from these different sources can be used for xenogenic, allogenic or autologous treatments. Three main therapeutic cell categories can be distinguished:

- mesoderm-derived stromal or stem cells;
- ectoderm-derived neural stem/progenitor cells;
- hematopoietic/endothelial stem cells.

Stromal/stem cells isolated from bone marrow, umbilical cord, blood or adipose tissue are widely used in cell therapy trials for stroke. MSC and multipotent adult progenitor cells (MAPC) can be used under autologous or allogenic conditions without concomitant immunosuppressive drugs owing to their immunomodulatory properties [135,136,165,166]. For MSC, precautions must be taken when using ex vivo cultures. Indeed, excessive MSC expansion with several passages could affect their therapeutic features [167] and one study suggest that cell culture media, either fetal bovine serum or autoserum, would modify the final MSC phenotype [168]. Mobilized into blood from bone marrow after stroke, very small embryonic-like stem cells require further study to assess their therapeutic potential for stroke [142].

The second category includes neural stem/progenitor cells (NSC) harvested from brain tissue, immortalized neural cell lines or cells derived from ESC or iPSC cultures [155,169,170]. NSC grafts provide functional benefit but usually require immunosuppressant treatments. An immortalized neural cell line (ReNeuron) is currently being studied in a clinical trial for stroke in the United Kingdom [171]. We can also include in this heterogeneous ''NSC group'' the olfactory ensheathing cells [145], oligodendrocyte progenitors [172], and immature neurons [158,173].

The third category of therapeutic cells employed in stroke studies includes the hematopoietic stem cells (HSC CD34+) [174,175] and endothelial progenitors (EP CD34+) [176]. They are easily harvested from cord blood, bone marrow or peripheral blood after mobilization and represent a type of mononuclear cells (MNC) usable, without amplification, to enhance microvascular repair [177,178]. Despite unclear cell characterization, MNC have been used in several clinical trials with encouraging results particularly as a treatment applied a few days after stroke onset (Table 1). Additionally, mature endothelial cells could be an interesting and well-characterized product improving post-stroke vasculogenesis and neurogenesis [147].

4.2. *Mechanisms of action*

The functional benefits of cell therapy on stroke recovery are well established in animal models using the above-mentioned cell types coupled with different delivery routes [179–181].

Table 1 – Restorative cell therapy after stroke: completed clinical trials.

Reference	Location	Patients (age)	Source	Cell type	Delay stroke to treatment	Dose	Route	Results
Kondziolka et al, 2000 [173]	Pittsburgh USA	12 IS (61 years, 44–74 years)	Allo/Human embryonic teratocarcinoma cell line (NT2/D1)	LBS-neurons (Layton BioScience)	2.5 years (7 months–4.5 years)	2 (60 μL; $n = 8$) or 6 million (180 μL; $n = 4$) + Cyclo. A (9 weeks)	IC	Feasible Safe (single seizure at 6 months, $n = 1$; remote stroke at 5 months, $n = 1$)
Kondziolka et al., 2005 [158]	Pittsburgh, Stanford USA	6 IS/8 ICH (58 years) 4 controls (3 IS/1 ICH; 46 years)	Allo/NT2/D1	LBS-neurons (Layton BioScience)	3.5 years (1–5 years)	5 ($n = 7$) or 10 million ($n = 7$) (250 μL) + Cyclo. A (6 months)	IC	Feasible Safe (single post-operative seizure, $n = 1$; asymptomatic SDH, $n = 1$)
Savitz et al., 2005 [256]	Boston USA	5 IS (25–52 years)	Xeno/Primordial porcine striatum + antiMHC1	LGE cells (Genvec)	5 years (1.5–10 years)	1 million/cm^3 of infarct 50 ($n = 4$) or 80 million ($n = 1$)	IC	Feasible Adverse events (cortical vein occlusion, $n = 1$; hyperglycemic seizures, $n = 1$; both with MRI transient abnormalities) FDA termination
Rabinovitch et al., 2005 [257]	Novosibirsk Russia	7 IS/3 ICH (46 years; 35–56 years) 11 controls (6 IS/5 ICH; 55 years)	Allo/"immature" nervous and hemopoietic tissues	Immature cells (NSC?)	4–24 months	1 injection ($n = 5$) 2 injections ($n = 5$)	IThec (LP)	Feasible Safe (transient meningeal syndrome)
Bang et al., 2005 [258]	Suwon South Korea	5 IS (63 years; 54–72 years) 25 controls (59 years)	Auto BM	MSC Expansion in FCS	4-5 and 7-9 weeks	50 million × 2	IV	Feasible Safe
Lee et al., 2010 [260]	Suwon South Korea	16 IS (65 ± 14 years) 36 controls (64 ± 12 years) including the previous trial	Auto BM	MSC Expansion in FCS	2.5–5 and 5–9 weeks	50 million × 2	IV	Feasible (fever after first injection, $n = 1$: no second injection) Safe (3–5 year follow-up) Beneficial: recovery, increase of serum SDF1α
Mendonça et al., 2006 [264]	Rio de Janeiro Brazil	1 IS (54 years)	Auto BM	MNC	4 days	300 million (3 mL/10 min)	IA	Feasible Safe
Suarez-Monteagudo et al., 2009 [263]	Habana Cuba	5 IS (?)	Auto BM	MNC	1–10 days	?	IC	Feasible Safe
Battistella et al., 2011 [265] Barbosa et al., 2010 [272]	Rio de Janeiro Brazil	6 IS (24–65 years)	Auto BM	MNC	2–3 months	125–500 million including 20 million ^{99m}Tc-labeled	IA	Feasible Safe Biodistribution: brain, liver, lungs, spleen, kidneys
Savitz et al., 2011 [268]	Houston USA	10 IS (55 ± 15 years) 79 historical controls (63 ± 12 years)	Auto BM	MNC	1–3 days	7 ($n = 1$) or 8.5 ($n = 1$) or 10 million/kg	IV	Feasible Safe (death/pulmonary embolism at 40 days, $n = 1$)
Honmou et al., 2011 [259]	Sapporo Japan	12 IS (59 ± 8 years, 41–73 years)	Auto BM	MSC Expansion in auto serum	10 weeks (5–19 weeks)	110 million (60–160 million)	IV	Feasible Safe (transient fever, $n = 1$)

Table 1 (*Continued*)

Reference	Location	Patients (age)	Source	Cell type	Delay stroke to treatment	Dose	Route	Results
Bhasin et al., 2011 [261]	New Delhi India	4 IS/2 ICH (42 years, 20–59 years) 6 controls (5 IS/1 ICH; 46 years)	Auto BM	MSC Expansion in animal serum-free media	9 months (7–12 months)	50–60 million	IV	Feasible Safe
Bhasin et al., 2013 [262]	New Delhi India	18 IS/2 ICH (45 ± 12 years) 20 controls (19 IS/1 ICH; 45 ± 10 years) including the previous trial	Auto BM	MSC: expansion in animal serum-free media MNC	10 months (3 months–2 years)	MSC: 50–60 million ($n = 6$) MNC: 50–60 million ($n = 14$)	IV	Feasible Safe Beneficial: recovery (for MNC), activation fMRI
Prasad et al., 2012 [269]	New Delhi India	11 IS (30–70 years)	Auto BM	MNC	7–30 days	80 million	IV	Feasibility = 11/11 (target-dose = 9/11) Safe
Moniche et al., 2012 [266]	Seville Spain	10 IS (67 ± 14 years) 10 controls (67 ± 13 years)	Auto BM	MNC	6 days (5–9 days)	159 million	IA	Feasible Safe (seizure at 3 months, $n = 2$) Increase of serum βNGF
Friedrich et al., 2012 [267]	Porto Alegre Brazil	20 IS (?)	Auto BM	MNC	3–7 days	220 million	IA	Feasible Safe
Li et al., 2013 [270]	Shandong China	60 ICH (56 years, 39–74 years) 40 controls (56 years, 35–72 years)	Auto BM	MNC	6 days (5–7 days)	2.4–23 million (3.5 mL)	IC	Feasible Safe (transient fever, = 5; lung cancer, $n = 1$) Beneficial
NCT01028794 (Taguchi 2008–13)	Osaka, Kobe Japan	12 IS (20–75 years)	Auto BM	MNC	7–10 days	Full from 25 mL of BM Full from 50 mL of BM	IV	Feasible Safe
NCT00473057 (Andre et al.[a], 2005–11)	Rio de Janeiro Brazil	12 IS (18–75 years)	Auto BM	MNC	< 90 days	500 million 500 million	IA IV	Unknown
NCT00950521 (Lin 2009–10)	Taichung Taiwan	30 IS (35–70 years) including controls	Auto blood	CD34+ SC	6–60 months	2–8 million	IC	Unknown
NCT01501773 (INVEST, Prasad 2008–11)	India[a]	120 IS (18–70 years) including controls	Auto BM	MNC	7–30 days	30–500 million	IV	Unknown
NCT01310114 (Celgene, 2011–13)	Chattanooga USA	44 IS (18–80 years) including controls	Allo placenta	Placenta derived SC (Celgene PDA001)	Subacute	200 million 200 million × 2 800 million × 2 (interval = 7 days)	IV	Unknown

Sorted by date (black: intracerebral [IC], red: intra-arterial [IA], or blue: intravenous [IV] cell-delivery route studies). Keywords on PubMed-NCBI and clinicaltrials.gov: ''cell therapy'' or ''stem cell'' or ''transplantation'' or ''bone marrow'' AND ''stroke'' or ''cerebral ischemia''.

Allo: allogeneic; Auto: autologous; BM: bone marrow; Cyclo: cyclosporine; FCS: fetal calf serum; ICH: intracerebral hemorrhage; IThec (LP): intrathecal (lumbar puncture); IS: ischemic stroke; LGE: lateral ganglionic eminence; MHC: major histocompatibility complex; MNC: mononuclear cells; MSC: mesenchymal stromal/stem cells; βNGF: nerve growth factor; NSC: neural stem cells; SC: stem cells; SDF1α: stromal cell derived factor 1α; SDH: subdural hematoma; Xeno: xenogeneic

[a] de Freitas, Mendez-Otero, Barbosa (investigators).

However, their respective mechanisms of action are complex and vary according to the transplanted cell type [13,14,132]. A common characteristic of these cell products is that they simultaneously target many different host brain cell types, including NSCs, neurons, endothelial and glial cells, leading to improvements via several endogenous repair processes, such as neuro-glio-angiogenesis, axonal sprouting, synaptogenesis. Direct replacement of injured neurons (''homotopic'' repair) has been suggested after NSC IC [182,183] or IA injection [184] encouraging possible long-term survival of implanted neurons in human [185], after iPSC derived neuron [186], bone marrow cell [187] or ESC-derived MSC injection [160]. However, only a few grafted cells can be expected to express neuronal markers, so long-term graft survival is relatively poor [188–192]. Moreover, despite possible integration of grafted NSCs [193–195] into the host circuitry, functional recovery occurs too early to be caused by newly formed neurons and synapses. Additionally, as emphasized for IV-injected cord blood cells, cell entry or integration into the host brain would not be required to obtain neural repair enhancement [196,197]. Concerning HSC, the possibility of their efficient differentiation into neurons is still debated [198–200]. Direct replacement of all damaged brain cells, including cells from ectoderm such as neurons or glia, and mesoderm such as microglia or endothelium), would require transplantation of ''native'' pluripotent cells (ESC or iPSC) which might result in tumor formation [161].

Thus, it seems more realistic to expect that cell therapy, notably employing ''non-neural'' cells, such as MSC or MNC, works through paracrine trophic support (''by stander'') effects on the injured brain by secreting various growth factors [201,202]. The improvement in host brain plasticity and associated recruitment of endogenous progenitors has been identified after injection of MSC [203] via the sonic hedgehog pathway [204], NSC [157] notably by enhancing dendritic plasticity [205], or olfactory ensheathing cells [145]. Moreover, the effects of cell therapies on post-stroke vasculogenesis and angiogenesis seem to be crucial in explaining early post-graft benefits. IC injection of endothelial cells can improve vasculogenesis linked to neurogenesis via VEGF release mechanisms [147]. Proangiogenic effects were also observed early after injection of MSC [206–209] that can contribute to VEGF-induced angiogenesis by supplying metalloprotease MMP-9 [210], after injection of NSC [211,212], EP [213], or cord-blood MNC CD34+ [175]. These MNCs contain EP and smooth muscle progenitors which may collaborate to form a mature vascular network supporting and enhancing neuroblast survival and migration after stroke [214]. Moreover, EP, MSC or NSC could also facilitate protection or restoration of the blood-brain barrier after stroke [126,211,215].

Another important effect of cell therapy is enhanced glial remodeling and limitations in anterograde degeneration [216–218]. For example, IV injection of MSC has beneficial effects on both post-stroke glial remodeling and axonal remyelination [219]. It also increases GDNF levels, creating a hospitable environment for neural repair and neuroblast migration from the SVZ [220].

Finally, cell therapies can also limit host cell death through anti-apoptotic [153,221,222] and immunomodulatory mechanisms. Although MSCs are known to attenuate microglia and leukocyte inflammatory responses after stroke [223–225], some immunomodulation properties were also observed for cord blood cells [226] or NSC [227,228], which can both influence splenic inflammatory responses after stroke [229].

4.3. *What is the optimal route for cell delivery?*

The best combination of delivery route and dose for cell therapy after stroke still remains debated, depending strongly on the chosen cell products and expected therapeutic effects. Theoretically, we can expect that intracerebral (IC) delivery would be preferred for cell replacement while intravenous (IV) or intra-arterial (IA) injection would be better for systemic and trophic support.

Systemic intravascular injections are less invasive and easier to implement than surgical implantation, properties that are relevant in stroke therapy [230]. This administration route allows cell distribution into vascularized and viable areas of the lesion, not only into localized graft sites. Because cytokine, chemokine and inflammatory signals are released by compromised brain tissue [231,232] such as SDF1/CXCR4 [233], IV-injected MSCs can preferentially migrate to the damaged brain regions despite initial and transient lung trapping [234]. In a model of hemorrhagic stroke, compared to IC injection, only IV-injected NSCs were found to have an efficient anti-inflammatory effect, measured by the degree of attenuation of splenic activation of tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL6), and nuclear factor-kappa B (NF-κB) [229]. In the same manner, cord blood cells were more effective after IV compared to IC administration [235], a result contrary to other studies showing poor efficacy associated with use of the IV route [236,237]. As only 1 to 4/10,000 IV-injected cells reach the arterial system and the brain tissue targets (1 to 4/10,000 IV-injected cells) [234], the acute pulmonary passage after IV injection is a major obstacle for IV delivery of NSCs, MAPCs and particularly for larger cells such as MSCs [238]. This pulmonary passage can be increased by inhibition of MSC CD49d, infusion via two boluses [238], or sodium nitroprusside preadministration [239].

Other graft routes, such as IA [190,240,241] or IC [236,237,242,243], could avoid the lung entrapment problem and thereby increase the number of grafted cells in the target tissue. ^{111}In-labeled NSCs can be detected in the ischemic hemisphere after IA but not IV injection [244]. This is consistent with an in vivo MRI study showing brain penetration of MSCs only after IA injection [245]. However, this study reported a risk of vascular occlusion after IA cell delivery, which could be linked to the large size of MSCs [246]. For NSC delivery, although the IA route seems to be efficient [247], it can increase mortality [248]. To continue the debate, it was recently reported that IV and IA injection of MNCs lead to the same benefit [249]. Thus, whereas IA or IC grafts are feasible administration routes for stroke therapy, IV cell injection has been selected for the majority of clinical trials (Table 1). Further discussion can be seen in the ''Clinical trials of cell therapy for stroke''.

4.4. *What is the optimal therapeutic time-window?*

As in route and dose, the optimal timing for cell transplantation after stroke remains debated, depending on the cell type,

allowing a potential delay for in vitro amplification, and their specific mechanism of action, involving acute neuroprotection vs. delayed neural repair, or trophic systemic transient effects vs. graft survival or integration [230] (Fig. 1). For example, early (6–24 h post-stroke) IA injection of NSCs leads to greater differentiation into astrocytes, whereas injection at later time points (7–14 days post-stroke) leads to greater differentiation into neurons [184]. MNCs, obtained from bone marrow or cord blood without amplification delay, could be good candidates for acute delivery in the first 72 hours after stroke [177,197,250], possibly serving as an adjunct to thrombolysis or mechanical thrombectomy [251].

A great clinical advantage of cell therapies would be to delay stroke treatment until the post-acute and rehabilitation phases in a majority of patients. Whereas some studies suggested that only early (3–24 h) IV injection of MSCs could be effective [252], others have shown that treatment delays up to one month after stroke can also result in improvements in recovery [192,253]. Give up to one month after experimental stroke, IC transplantation of immortalized neurons [254] or IV injection of umbilical cord derived cells [222] both enhance recovery. While a 6-week-transplantation delay in NSC administration does not influence cell migration, proliferation or neuronal differentiation, it could result in poorer graft survival compared to early transplantation (48 h) [183].

Thus, the issue of optimal route, dose, and time-window for cell therapy persists. Different cell products can be used at different time points. Further investigations that take into account the difference between animal models and human stroke in terms of recovery timing and duration are clearly warranted.

5. Clinical trials of cell therapy for stroke

Seventeen clinical trials have been published and 5 others are complete but not yet published (Table 1). The cell source was most commonly autologous bone marrow ($n = 16$ trials), followed by NSC/neuron cultures ($n = 4$), and autologous peripheral blood ($n = 1$) or placenta ($n = 1$). Several cell products and routes were investigated: IC or intrathecal transplantation of NSC or immortalized neurons [158,173,255–257], IV injection of autologous MSC [258–262], IC [263], IA [264–267] or IV [268,269] injections of autologous MNC, IC transplantation of HSC/EP CD34+ from autologous blood (Lin, NCT00950521), and IV injection of placenta derived stem cells (Celgene, NCT01310114). Only one unpublished trial directly compared IA vs. IV injection of autologous MNC (Andre et al., NCT00473057). Recently, one trial focused on intracerebral hemorrhage using IC injection of MNC from autologous bone marrow [270].

Direct comparisons are difficult due to the important differences in cell products, routes and post-stroke delays among the trials. However, these first studies of cell therapy for stroke have reported encouraging results regarding safety and feasibility [271,272]. From the available studies, we can conclude that:

- NSC transplantation requires the use of immunosuppressants or pretreatment of pig xenograft cells;
- xenografts can induce deleterious inflammatory effects;
- MSC or MNC (including HSC/EP) injections are easier to employ with possible monitoring of IA-injected MNCs [273].

Currently, around 30 on-going trials utilize MNC or MSC as cell products and are exploring the potential utility of allogenic administration and of using umbilical cord blood as the cell source. New biomarkers of stroke recovery, such as multimodal MRI [274,275] and biological blood markers, such as SDF1, βNGF or circulating EP, will provide additional important data about cell therapy functional effects and its mechanism of action in human stroke.

6. Perspectives concerning the use of biotherapies for stroke

6.1. *Careful translation*

For the development of neurorestorative therapies for stroke, additional translational studies should be conducted regarding the influences related to the stroke type and localization, the usual neurovascular risk factors, such as hypertension, diabetes, and cerebral small artery disease, and concomitant treatments, such as tPA thrombolysis or statins. Lesion location and size will be important factors to determine which patients are suitable for cell therapy. For example, IV MSC could be less efficient in treating stroke patients with a lesion including the SVZ [260]. Concerning stroke type, little data are available concerning hemorrhagic stroke. Despite benefits seen in experimental studies of MSC IA or IV injections [139,241], it remains unclear if neurorestorative therapy clinical trials should include both hemorrhagic and ischemic strokes regarding differences in pathophysiology and recovery. Concerning risk factors, recent studies have suggested that IV injection of MSC given 24 h post-stroke induces adverse effects in diabetic rats increasing blood-brain barrier leakage and vascular damage via increased expression of angiogenin [276]. Negative results were also reported in hypertensive rats after acute IV injection of bone marrow MNCs [277] or cord blood cells [278,279]. Otherwise, the combination of simvastatin and cord blood cell IV injection 24 h post-stroke increased BDNF/TrkB expression, enhanced cell migration towards the ischemic brain tissue, and amplified neuro-synaptogenesis, improving recovery [280].

6.2. *Safety issues related to tumorgenicity and immunogenicity*

In considering translation study characteristics, beyond determining the presence of good cell characterization, safety concerns must be emphasized. While tumorgenicity was clearly identified following injection of "native" ESCs or iPSCs, it could also be a safety issue for MSCs according to their specific source, whether adult bone marrow or umbilical cord [281]. Even so, across numerous studies, there has never been a report of tumor emergence, even after long-term follow-up [190,260]. However, the first case of a human brain tumor occurred four years after a NSC therapy in a child [282,283] indicates that tumorgenicity must be carefully taken into

consideration for all cell therapy products using long-term surveys.

Concerning immunogenicity, we previously discussed the immunomodulation properties of MSCs from autologous or allogenic sources [219]. MSCs or MNCs harvested from human sources are widely used in animal models without immunosuppression. In contrast, for allogenic NSCs, the use of concomitant immunosuppression is needed [158,171] to avoid severe inflammatory reaction notably after local delivery [284]. Even so, some experimental studies have reported potential ''anti-inflammatory'' effects associated with NSCs [227–229]. A small trial has indicated that anti-MHC1 pretreatment of porcine NSC cultures is insufficient to fully avoid human host immunoreactivity [256]. Moreover, autologous transplantation of iPSC-derived neurons elicited only a minimal immune brain response, in contrast to allografts, which caused an acquired immune response with a microglia activation and leukocyte infiltration [285]. Thus, the development of iPSC-derived cell therapy will probably require an autologous source, such as skin, or HLA-haplotyped iPSC banking.

In the future, the development of neurorestorative therapies for stroke should carefully take into consideration all these translational and safety concerns to allow an efficient transfer from bench to bedside, particularly for the emerging cell products such as genetically-modified cells.

6.3. *Combinatorial approaches: co-treatment, cell modification, co-transplantation*

Enhancing the graft survival and cell therapy benefits is the common aim of several on-going experimental strategies, including combinations of neuroprotectants or growth factors with cell therapy, modification of therapeutic cells to increase a specific feature, or co-transplantations of ''synergic'' cells.

Concerning combinations, we have already noted that statins can increase the benefits seen with IV injection of cord blood cells [280]. Erythropoietin [286] or NO donor [287] could also act in synergistic ways with MSC IV injection, as seen when VEGF is combined with IV injection of NSC [288] resulting in a pro-angiogenic effect, or when GCSF is combined with MNC [289]. Currently, biotechnology allows genetic cell modifications or improvements in cell culture conditions [134] allowing delivery of cell products overexpressing interesting factors. Thus, genetically modified-MSC overexpressing Ang1 [208], BDNF [290,291], GDNF [292,293], VEGF or VEGF-Ang1 [294] can all increase the effects of MSC IV injection on post-stroke neural repair and recovery. Moreover, without gene modification, MSC cultures exposed to hypoxia [295] or co-cultured with depolarized astrocytes [296] could be interesting to respectively enhance their HIF1 and growth factor expression or their neuronal differentiation, thus improving their therapeutic potential after stroke.

Co-transplantation of synergic cells would be another strategy to increase the benefit of cell therapy. For example, IV co-injection of MSC and EP 24 h post-stroke enhance expressions of βFGF, VEGF and BNDF [297]. IV injection of cord blood EP and smooth muscle progenitors 24 h post-stroke, which collaborate to enhance angiogenesis, results in the maintenance of neurogenesis and neuroblast migration to the peri-ischemic cortex [214].

6.4. *Biomaterials enhancing neurorestorative therapy in stroke*

The combination of drugs, growth factors and cells with biopolymer scaffolds (i.e. matrix) of extracellular matrix molecules, including collagen and hyaluronic acid, may protect the graft and provide localized and controlled delivery of the therapeutic agent [298,299]. The use of such ''carrier'' scaffolds are particularly relevant for injections into the stroke cavity at a chronic stage, as they might help to avoid a deleterious injection into the adjacent brain tissue where important recovery processes may be underway. Thus, close to the plastic brain regions, grafted scaffolds could provide a favorable environment for drug delivery and graft cell survival.

After stroke, different hydrogel scaffolds have already been used for IC delivery of cyclosporine [300], VEGF [301,302], erythropoietin [303], epidermal growth factor [304], BDNF for enhancing endogenous NSC survival [305], or anti-receptor Nogo to improve neuro-synaptogenesis [306].

For cell therapy, we noted that most grafted cells die after IC graft into the damaged area. To improve graft cell survival, proliferation, migration and differentiation, different hydrogels such as hyaluronic acid or collagen gels, or matrigel have been assessed as cell-seeded scaffolds injected at chronic stages (1–3 weeks post-stroke). The approaches include ESC-derived NSC incorporated into Matrigel [307], embryonic cortex NSC into hyaluronan-heparin-collagen hydrogel [308], MSC into thermoreversible hydrogel [309], cord MSC into gelatin-laminin scaffold [310] and MSC-derived endothelial cells into hyaluronic acid hydrogel [311]. The cell scaffolds protect the grafted cells from the host immune system, form a functional vascular network integrated with the host vascular system [311], prevent glial scar formation and reduce neuroinflammation [310]. Interestingly, after the biodegradation of the scaffold, the remaining grafted umbilical cord MSCs could still survive [310]. However, further optimization of hydrogel compositions is warranted to avoid possible inflammatory responses as observed in immunocompetent mouse brain two weeks after IC injection of a hyaluronic acid hydrogel pre-seeded with human NSCs or glial precursors [312].

Currently, biomaterials researchers are seeking to optimize injectable hydrogels by combining cell seeding with the incorporation of growth factors or tracers. Specific features such as gelation rate, cell adhesion, delivery properties, biocompatibility and degradation must be investigated in experimental studies prior to transferring this promising procedure to clinical trials.

7. Conclusion

Neurorestorative therapies include both growth factors and cell-based techniques that can be combined or used with an injectable biomaterial. They hold great promises for stroke treatment, as they both enhance several processes involved in post-stroke recovery and also can be used across a wide therapeutic time-window. However, preclinical studies must still be conducted in parallel with the clinical trials. Indeed, related to the rapidly expanding therapeutic arsenal of biological treatments, many interdependent

questions remain to be answered about specific mechanisms of action and the details of the procedures themselves. Which growth factor or cell type is most appropriate for patients that may vary in their lesion extent, location, age, or neurovascular risk factors? Which are the optimal dose and delivery routes? What is the therapeutic time-window for which target? Will the expansion of ''biological'' therapy use be associated with increased long-term tumorgenicity and immunogenicity? Assuming that these remaining questions and safety concerns can be adequately addressed, is hoped that the emerging biotherapies will prove to be efficient and effective means to decrease post-stroke disability for many patients.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

The authors acknowledge Thomas Zeffiro (Neural Systems Group, Massachusetts General Hospital, Charlestown, USA) for proofreading. This work is supported by the French National Research Agency as part of the ''Investments for the future'' (ANR-11-INBS-0005) for ECell-France national network for regenerative medicine (ecellfrance.com).

REFERENCES

[1] Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, et al. Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. Lancet 2014;383(9913):245–54.
[2] Sarraj A, Grotta JC. Stroke: new horizons in treatment. Lancet Neurol 2014;13(1):2–3.
[3] Chollet F, Cramer SC, Stinear C, Kappelle LJ, Baron JC, Weiller C, et al. Pharmacological therapies in post stroke recovery: recommendations for future clinical trials. J Neurol 2013 [epub (PMID 24221642)].
[4] Deb P, Sharma S, Hassan KM. Pathophysiologic mechanisms of acute ischemic stroke: an overview with emphasis on therapeutic significance beyond thrombolysis. Pathophysiology 2010;17(3):197–218.
[5] Vosler PS, Chen J. Potential molecular targets for translational stroke research. Stroke 2009;40(3 Suppl.): S119–20.
[6] Carmichael ST. Targets for neural repair therapies after stroke. Stroke 2010;41(10 Suppl.):S124–6.
[7] Dirnagl U. Pathobiology of injury after stroke: the neurovascular unit and beyond. Ann N Y Acad Sci 2012;1268:21–5.
[8] Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. Neuron 2010;67(2):181–98.
[9] Xing C, Arai K, Lo EH, Hommel M. Pathophysiologic cascades in ischemic stroke. Int J Stroke 2012;7(5):378–85.
[10] Mergenthaler P, Dirnagl U, Meisel A. Pathophysiology of stroke: lessons from animal models. Metab Brain Dis 2004;19(3–4):151–67.
[11] Zhang JH, Badaut J, Tang J, Obenaus A, Hartman R, Pearce WJ. The vascular neural network – a new paradigm in stroke pathophysiology. Nat Rev Neurol 2012;8(12):711–6.
[12] Sahota P, Savitz SI. Investigational therapies for ischemic stroke: neuroprotection and neurorecovery. Neurotherapeutics 2011;8(3):434–51.
[13] Hermann DM, Chopp M. Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol 2012;11(4):369–80.
[14] Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol 2009;8(5):491–500.
[15] Onteniente B, Polentes J. Regenerative medicine for stroke – are we there yet? Cerebrovasc Dis 2011;31(6): 544–51.
[16] Savitz SI, Mattle HP. Advances in stroke: emerging therapies. Stroke 2013;44(2):314–5.
[17] Gutierrez M, Merino JJ, De Lecinana MA, Diez-Tejedor E. Cerebral protection, brain repair, plasticity and cell therapy in ischemic stroke. Cerebrovasc Dis 2009;27(Suppl. 1):177–86.
[18] Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. Trends Neurosci 2003;26(5):248–54.
[19] Lo EH. A new penumbra: transitioning from injury into repair after stroke. Nat Med 2008;14(5):497–500.
[20] Prass K, Scharff A, Ruscher K, Lowl D, Muselmann C, Victorov I, et al. Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. Stroke 2003;34(8):1981–6.
[21] Sanai N, Nguyen T, Ihrie RA, Mirzadeh Z, Tsai HH, Wong M, et al. Corridors of migrating neurons in the human brain and their decline during infancy. Nature 2011;478(7369):382–6.
[22] Murphy TH, Corbett D. Plasticity during stroke recovery: from synapse to behaviour. Nat Rev Neurosci 2009;10(12):861–72.
[23] Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. Ann Neurol 2006;59(5):735–42.
[24] Jaillard A, Martin CD, Garambois K, Lebas JF, Hommel M. Vicarious function within the human primary motor cortex? A longitudinal fMRI stroke study. Brain 2005;128(Pt 5):1122–38.
[25] Rehme AK, Eickhoff SB, Rottschy C, Fink GR, Grefkes C. Activation likelihood estimation meta-analysis of motor-related neural activity after stroke. Neuroimage 2012;59(3):2771–82.
[26] Zhang RL, Zhang ZG, Zhang L, Chopp M. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 2001;105(1):33–41.
[27] 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(9):963–70.
[28] Thored P, Arvidsson A, Cacci E, Ahlenius H, Kallur T, Darsalia V, et al. Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem Cells 2006;24(3):739–47.
[29] Darsalia V, Heldmann U, Lindvall O, Kokaia Z. Stroke-induced neurogenesis in aged brain. Stroke 2005;36(8):1790–5.
[30] 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(35):13198–202.
[31] Macas J, Nern C, Plate KH, Momma S. Increased generation of neuronal progenitors after ischemic injury in the aged adult human forebrain. J Neurosci 2006;26(50):13114–9.

[32] Minger SL, Ekonomou A, Carta EM, Chinoy A, Perry RH, Ballard CG. Endogenous neurogenesis in the human brain following cerebral infarction. Regen Med 2007;2(1):69–74.
[33] Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol 2002;52(6):802–13.
[34] Ohab JJ, Fleming S, Blesch A, Carmichael ST. A neurovascular niche for neurogenesis after stroke. J Neurosci 2006;26(50):13007–16.
[35] Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, Goderie SK, et al. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 2008;3(3):289–300.
[36] Arai K, Lo EH. An oligovascular niche: cerebral endothelial cells promote the survival and proliferation of oligodendrocyte precursor cells. J Neurosci 2009;29(14):4351–5.
[37] Walker MR, Patel KK, Stappenbeck TS. The stem cell niche. J Pathol 2009;217(2):169–80.
[38] Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, et al. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 2004;304(5675):1338–40.
[39] Boehm-Sturm P, Farr TD, Adamczak J, Jikeli JF, Mengler L, Wiedermann D, et al. Vascular changes after stroke in the rat: a longitudinal study using optimized magnetic resonance imaging. Contrast Media Mol Imaging 2013;8(5):383–92.
[40] Lin C-Y, Chang C, Cheung W-M, Lin M-H, Chen J-J, Hsu CY, et al. Dynamic changes in vascular permeability, cerebral blood volume, vascular density, and size after transient focal cerebral ischemia in rats: evaluation with contrast-enhanced magnetic resonance imaging. J Cereb Blood Flow Metab 2008;28(8):1491–501.
[41] Ergul A, Alhusban A, Fagan SC. Angiogenesis: a harmonized target for recovery after stroke. Stroke 2012;43(8):2270–4.
[42] Thored P, Wood J, Arvidsson A, Cammenga J, Kokaia Z, Lindvall O. Long-term neuroblast migration along blood vessels in an area with transient angiogenesis and increased vascularization after stroke. Stroke 2007;38(11):3032–9.
[43] Valable S, Bellail A, Lesné S, Liot G, Mackenzie ET, Vivien D, et al. Angiopoietin-1-induced PI3-kinase activation prevents neuronal apoptosis. FASEB J 2003;17(3):443–5.
[44] Grade S, Weng YC, Snapyan M, Kriz J, Malva JO, Saghatelyan A. Brain-derived neurotrophic factor promotes vasculature-associated migration of neuronal precursors toward the ischemic striatum. PLoS One 2013;8(1):e55039.
[45] Hermann DM, Zechariah A. Implications of vascular endothelial growth factor for postischemic neurovascular remodeling. J Cereb Blood Flow Metab 2009;29(10):1620–43.
[46] Slevin M, Krupinski J, Slowik A, Kumar P, Szczudlik A, Gaffney J. Serial measurement of vascular endothelial growth factor and transforming growth factor-beta1 in serum of patients with acute ischemic stroke. Stroke 2000;31(8):1863–70.
[47] Chopp M, Zhang ZG, Jiang Q. Neurogenesis, angiogenesis, and MRI indices of functional recovery from stroke. Stroke 2007;38(2 Suppl.):827–31.
[48] Teng H, Zhang ZG, Wang L, Zhang RL, Zhang L, Morris D, et al. Coupling of angiogenesis and neurogenesis in cultured endothelial cells and neural progenitor cells after stroke. J Cereb Blood Flow Metab 2008;28(4):764–71.
[49] Pannasch U, Vargova L, Reingruber J, Ezan P, Holcman D, Giaume C, et al. Astroglial networks scale synaptic activity and plasticity. Proc Natl Acad Sci U S A 2011;108(20):8467–72.
[50] Bardehle S, Kruger M, Buggenthin F, Schwausch J, Ninkovic J, Clevers H, et al. Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nat Neurosci 2013;16(5):580–6.
[51] Zhang R, Chopp M, Zhang ZG. Oligodendrogenesis after cerebral ischemia. Front Cell Neurosci 2013;7:201.
[52] Pham LD, Hayakawa K, Seo JH, Nguyen MN, Som AT, Lee BJ, et al. Crosstalk between oligodendrocytes and cerebral endothelium contributes to vascular remodeling after white matter injury. Glia 2012;60(6):875–81.
[53] Madinier A, Bertrand N, Mossiat C, Prigent-Tessier A, Beley A, Marie C, et al. Microglial involvement in neuroplastic changes following focal brain ischemia in rats. PLoS One 2009;4(12):e8101.
[54] Zhang QW, Deng XX, Sun X, Xu JX, Sun FY. Exercise promotes axon regeneration of newborn striatonigral and corticonigral projection neurons in rats after ischemic stroke. PLoS One 2013;8(11):e80139.
[55] Schmidt A, Wellmann J, Schilling M, Strecker JK, Sommer C, Schabitz WR, et al. Meta-analysis of the efficacy of different training strategies in animal models of ischemic stroke. Stroke 2014;45(1):239–47.
[56] Liepert J, Bauder H, Wolfgang HR, Miltner WH, Taub E, Weiller C. Treatment-induced cortical reorganization after stroke in humans. Stroke 2000;31(6):1210–6.
[57] Pekna M, Pekny M, Nilsson M. Modulation of neural plasticity as a basis for stroke rehabilitation. Stroke 2012;43(10):2819–28.
[58] Langhorne P, Bernhardt J, Kwakkel G. Stroke rehabilitation. Lancet 2011;377(9778):1693–702.
[59] Hossmann KA. Pathophysiological basis of translational stroke research. Folia Neuropathol 2009;47(3):213–27.
[60] De Keyser J, Sulter G, Luiten PG. Clinical trials with neuroprotective drugs in acute ischaemic stroke: are we doing the right thing? Trends Neurosci 1999;22(12):535–40.
[61] O'collins VE, Macleod MR, Donnan GA, Horky LL, Van Der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. Ann Neurol 2006;59(3):467–77.
[62] Villmann C, Becker CM. On the hypes and falls in neuroprotection: targeting the NMDA receptor. Neuroscientist 2007;13(6):594–615.
[63] Xiong ZG, Zhu XM, Chu XP, Minami M, Hey J, Wei WL, et al. Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. Cell 2004;118(6):687–98.
[64] Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007;357(6):562–71.
[65] Nakase T, Yoshioka S, Suzuki A. Free radical scavenger, edaravone, reduces the lesion size of lacunar infarction in human brain ischemic stroke. BMC Neurol 2011;11:39.
[66] Yuan J. Neuroprotective strategies targeting apoptotic and necrotic cell death for stroke. Apoptosis 2009;14(4):469–77.
[67] Abe T, Kunz A, Shimamura M, Zhou P, Anrather J, Iadecola C. The neuroprotective effect of prostaglandin E2 EP1 receptor inhibition has a wide therapeutic window, is sustained in time and is not sexually dimorphic. J Cereb Blood Flow Metab 2009;29(1):66–72.
[68] Kawano T, Anrather J, Zhou P, Park L, Wang G, Frys KA, et al. Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. Nat Med 2006;12(2):225–9.
[69] Macrez R, Ali C, Toutirais O, Le Mauff B, Defer G, Dirnagl U, et al. Stroke and the immune system: from pathophysiology to new therapeutic strategies. Lancet Neurol 2011;10(5):471–80.
[70] Yu G, Hess DC, Borlongan CV. Combined cyclosporine-A and methylprednisolone treatment exerts partial and transient neuroprotection against ischemic stroke. Brain Res 2004;1018(1):32–7.

[71] Macrez R, Obiang P, Gauberti M, Roussel B, Baron A, Parcq J, et al. Antibodies preventing the interaction of tissue-type plasminogen activator with N-methyl-D-aspartate receptors reduce stroke damages and extend the therapeutic window of thrombolysis. Stroke 2011;42(8):2315–22.
[72] Yu CY, Ng G, Liao P. Therapeutic antibodies in stroke. Transl Stroke Res 2013;4:477–83.
[73] Lyden PD, Hemmen TM, Grotta J, Rapp K, Raman R. Endovascular therapeutic hypothermia for acute ischemic stroke: ICTuS 2/3 protocol. Int J Stroke 2013 [epub (PMID 24206528)].
[74] Yenari MA, Han HS. Neuroprotective mechanisms of hypothermia in brain ischaemia. Nat Rev Neurosci 2012;13(4):267–78.
[75] Piironen K, Tiainen M, Mustanoja S, Kaukonen KM, Meretoja A, Tatlisumak T, et al. mild hypothermia after intravenous thrombolysis in patients with acute stroke: a randomized controlled trial. Stroke 2014 [epub (PMID 24436240)].
[76] Poli S, Veltkamp R. Oxygen therapy in acute ischemic stroke – experimental efficacy and molecular mechanisms. Curr Mol Med 2009;9(2):227–41.
[77] Ginsberg MD, Palesch YY, Hill MD, Martin RH, Moy CS, Barsan WG, et al. High-dose albumin treatment for acute ischaemic stroke (ALIAS) part 2: a randomised, double-blind, phase 3, placebo-controlled trial. Lancet Neurol 2013;12(11):1049–58.
[78] Saver JL, Kidwell C, Eckstein M, Starkman S. Prehospital neuroprotective therapy for acute stroke: results of the Field Administration of Stroke Therapy-Magnesium (FAST-MAG) pilot trial. Stroke 2004;35(5):e106–8.
[79] Miyamoto N, Tanaka R, Shimura H, Watanabe T, Mori H, Onodera M, et al. Phosphodiesterase III inhibition promotes differentiation and survival of oligodendrocyte progenitors and enhances regeneration of ischemic white matter lesions in the adult mammalian brain. J Cereb Blood Flow Metab 2010;30(2):299–310.
[80] Chen J, Zhang ZG, Li Y, Wang Y, Wang L, Jiang H, et al. Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Ann Neurol 2003;53(6):743–51.
[81] Davalos A, Alvarez-Sabin J, Castillo J, Diez-Tejedor E, Ferro J, Martinez-Vila E, et al. Citicoline in the treatment of acute ischaemic stroke: an international, randomised, multicentre, placebo-controlled study (ICTUS trial). Lancet 2012;380(9839):349–57.
[82] Zhang RL, Chopp M, Roberts C, Wei M, Wang X, Liu X, et al. Sildenafil enhances neurogenesis and oligodendrogenesis in ischemic brain of middle-aged mouse. PLoS One 2012;7(10):e48141.
[83] Chen CL, Young SH, Gan HH, Singh R, Lao AY, Baroque AC, et al. Chinese medicine neuroaid efficacy on stroke recovery: a double-blind, placebo-controlled, randomized study. Stroke 2013;44(8):2093–100.
[84] Cook DJ, Teves L, Tymianski M. Treatment of stroke with a PSD-95 inhibitor in the gyrencephalic primate brain. Nature 2012;483(7388):213–7.
[85] Chollet F, Tardy J, Albucher JF, Thalamas C, Berard E, Lamy C, et al. Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. Lancet Neurol 2011;10(2):123–30.
[86] Kimura K, Aoki J, Sakamoto Y, Kobayashi K, Sakai K, Inoue T, et al. Administration of edaravone, a free radical scavenger, during t-PA infusion can enhance early recanalization in acute stroke patients – a preliminary study. J Neurol Sci 2012;313(1–2):132–6.
[87] Fagan SC, Waller JL, Nichols FT, Edwards DJ, Pettigrew LC, Clark WM, et al. Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. Stroke 2010;41(10):2283–7.
[88] Savitz SI. Cell therapies: careful translation from animals to patients. Stroke 2013;44(6 Suppl. 1):S107–9.
[89] Dronne MA, Grenier E, Chapuisat G, Hommel M, Boissel JP. A modelling approach to explore some hypotheses of the failure of neuroprotective trials in ischemic stroke patients. Prog Biophys Mol Biol 2008;97(1):60–78.
[90] Aimone JB, Weick JP. Perspectives for computational modeling of cell replacement for neurological disorders. Front Comput Neurosci 2013;7:150.
[91] Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 2013;14(5):365–76.
[92] Dirnagl U. Bench to bedside: the quest for quality in experimental stroke research. J Cereb Blood Flow Metab 2006;26(12):1465–78.
[93] Minnerup J, Wersching H, Diederich K, Schilling M, Ringelstein EB, Wellmann J, et al. Methodological quality of preclinical stroke studies is not required for publication in high-impact journals. J Cereb Blood Flow Metab 2010;30(9):1619–24.
[94] Stair. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 1999;30(12):2752–8.
[95] Steps. Stem cell therapies as an emerging paradigm in stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. Stroke 2009;40(2):510–5.
[96] Borlongan CV. Cell therapy for stroke: remaining issues to address before embarking on clinical trials. Stroke 2009;40(3 Suppl.):S146–8.
[97] Savitz SI, Chopp M, Deans R, Carmichael ST, Phinney D, Wechsler L. Stem cell therapy as an emerging paradigm for stroke (STEPS) II. Stroke 2011;42(3):825–9.
[98] Savitz SI, Cramer SC, Wechsler L. Stem cells as an emerging paradigm in stroke 3: enhancing the development of clinical trials. Stroke 2013 [epub (PMID 24368562)].
[99] Ghezzi P, Bernaudin M, Bianchi R, Blomgren K, Brines M, Campana W, et al. Erythropoietin: not just about erythropoiesis. Lancet 2010;375(9732):2142.
[100] Minnerup J, Heidrich J, Rogalewski A, Schabitz WR, Wellmann J. The efficacy of erythropoietin and its analogues in animal stroke models: a meta-analysis. Stroke 2009;40(9):3113–20.
[101] Reitmeir R, Kilic E, Kilic U, Bacigaluppi M, Elali A, Salani G, et al. Post-acute delivery of erythropoietin induces stroke recovery by promoting perilesional tissue remodelling and contralesional pyramidal tract plasticity. Brain 2011;134(Pt 1):84–99.
[102] Zhang L, Chopp M, Zhang RL, Wang L, Zhang J, Wang Y, et al. Erythropoietin amplifies stroke-induced oligodendrogenesis in the rat. PLoS One 2010;5(6):e11016.
[103] Bath PM, Sprigg N, England T. Colony stimulating factors (including erythropoietin, granulocyte colony stimulating factor and analogues) for stroke. Cochrane Database Syst Rev 2013;6(CD005207).
[104] Cramer SC, Fitzpatrick C, Warren M, Hill MD, Brown D, Whitaker L, et al. The beta-hCG + erythropoietin in acute stroke (BETAS) study: a 3-center, single-dose, open-label, noncontrolled, phase IIa safety trial. Stroke 2010;41(5):927–31.
[105] Shyu WC, Lin SZ, Yang HI, Tzeng YS, Pang CY, Yen PS, et al. Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. Circulation 2004;110(13):1847–54.

[106] Taguchi A, Wen Z, Myojin K, Yoshihara T, Nakagomi T, Nakayama D, et al. Granulocyte colony-stimulating factor has a negative effect on stroke outcome in a murine model. Eur J Neurosci 2007;26(1):126–33.
[107] Wagner DC, Posel C, Schulz I, Schicht G, Boltze J, Lange F, et al. Allometric dose retranslation unveiled substantial immunological side effects of granulocyte colony-stimulating factor after stroke. Stroke 2014 [epub (PMID 24407949)].
[108] Toth ZE, Leker RR, Shahar T, Pastorino S, Szalayova I, Asemenew B, et al. 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 2008;111(12):5544–52.
[109] Ringelstein EB, Thijs V, Norrving B, Chamorro A, Aichner F, Grond M, et al. Granulocyte colony-stimulating factor in patients with acute ischemic stroke: results of the AX200 for ischemic stroke trial. Stroke 2013;44(10):2681–7.
[110] Alasheev AM, Belkin AA, Leiderman IN, Ivanov RA, Isakova TM. Granulocyte-colony-stimulating factor for acute ischemic stroke: a randomized controlled trial (STEMTHER). Transl Stroke Res 2011;2(3):358–65.
[111] Kokovay E, Goderie S, Wang Y, Lotz S, Lin G, Sun Y, et al. Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. Cell Stem Cell 2010;7(2):163–73.
[112] Shyu W-C, Lin S-Z, Yen P-S, Su C-Y, Chen D-C, Wang H-J, et al. Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. J Pharmacol Exp Ther 2008;324(2):834–49.
[113] Bejot Y, Mossiat C, Giroud M, Prigent-Tessier A, Marie C. Circulating and brain BDNF levels in stroke rats. Relevance to clinical studies. PLoS One 2011;6(12):e29405.
[114] Schabitz WR, Sommer C, Zoder W, Kiessling M, Schwaninger M, Schwab S. Intravenous brain-derived neurotrophic factor reduces infarct size and counterregulates Bax and Bcl-2 expression after temporary focal cerebral ischemia. Stroke 2000;31(9):2212–7.
[115] Kobayashi T, Ahlenius H, Thored P, Kobayashi R, Kokaia Z, Lindvall O. Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats. Stroke 2006;37(9):2361–7.
[116] Zhao Y, Li Z, Wang R, Wei J, Li G, Zhao H. Angiopoietin 1 counteracts vascular endothelial growth factor-induced blood-brain barrier permeability and alleviates ischemic injury in the early stages of transient focal cerebral ischemia in rats. Neurol Res 2010;32(7):748–55.
[117] Bouleti C, Mathivet T, Coqueran B, Serfaty JM, Lesage M, Berland E, et al. Protective effects of angiopoietin-like 4 on cerebrovascular and functional damages in ischaemic stroke. Eur Heart J 2013;34(47):3657–68.
[118] Zhang ZG, Zhang L, Croll SD, Chopp M. Angiopoietin-1 reduces cerebral blood vessel leakage and ischemic lesion volume after focal cerebral embolic ischemia in mice. Neuroscience 2002;113(3):683–7.
[119] Herz J, Reitmeir R, Hagen SI, Reinboth BS, Guo Z, Zechariah A, et al. Intracerebroventricularly delivered VEGF promotes contralesional corticorubral plasticity after focal cerebral ischemia via mechanisms involving anti-inflammatory actions. Neurobiol Dis 2012;45(3):1077–85.
[120] Reitmeir R, Kilic E, Reinboth BS, Guo Z, Elali A, Zechariah A, et al. Vascular endothelial growth factor induces contralesional corticobulbar plasticity and functional neurological recovery in the ischemic brain. Acta Neuropathol 2012;123(2):273–84.
[121] Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, et al. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest 2000;106(7):829–38.
[122] Ellsworth JL, Garcia R, Yu J, Kindy MS. Fibroblast growth factor-18 reduced infarct volumes and behavioral deficits after transient occlusion of the middle cerebral artery in rats. Stroke 2003;34(6):1507–12.
[123] Li Q, Stephenson D. Postischemic administration of basic fibroblast growth factor improves sensorimotor function and reduces infarct size following permanent focal cerebral ischemia in the rat. Exp Neurol 2002;177(2):531–7.
[124] Doeppner TR, Kaltwasser B, Elali A, Zechariah A, Hermann DM, Bahr M. Acute hepatocyte growth factor treatment induces long-term neuroprotection and stroke recovery via mechanisms involving neural precursor cell proliferation and differentiation. J Cereb Blood Flow Metab 2011;31(5):1251–62.
[125] Shinozuka K, Dailey T, Tajiri N, Ishikawa H, Kaneko Y, Borlongan CV. Stem cell transplantation for neuroprotection in stroke. Brain Sci 2013;3(1):239–61.
[126] Shinozuka K, Dailey T, Tajiri N, Ishikawa H, Kim DW, Pabon M, et al. Stem cells for neurovascular repair in stroke. J Stem Cell Res Ther 2013;4(4):12912.
[127] Sinden JD, Muir KW. Stem cells in stroke treatment: the promise and the challenges. Int J Stroke 2012;7(5):426–34.
[128] Bliss TM, Andres RH, Steinberg GK. Optimizing the success of cell transplantation therapy for stroke. Neurobiol Dis 2010;37(2):275–83.
[129] Burns TC, Steinberg GK. Stem cells and stroke: opportunities, challenges and strategies. Expert Opin Biol Ther 2011;11(4):447–61.
[130] Haas S, Weidner N, Winkler J. Adult stem cell therapy in stroke. Curr Opin Neurol 2005;18(1):59–64.
[131] Svensson J, Ghatnekar O, Lindgren A, Lindvall O, Norrving B, Persson U, et al. Societal value of stem cell therapy in stroke – a modeling study. Cerebrovasc Dis 2012;33(6):532–9.
[132] Onteniente B. The multiple aspects of stroke and stem cell therapy. Curr Mol Med 2013;13(5):821–31.
[133] Eve DJ, Fillmore RW, Borlongan CV, Sanberg PR. Stem cell research in cell transplantation: sources, geopolitical influence, and transplantation. Cell Transplant 2010;19(11):1493–509.
[134] Wagner J, Kean T, Young R, Dennis JE, Caplan AI. Optimizing mesenchymal stem cell-based therapeutics. Curr Opin Biotechnol 2009;20(5):531–6.
[135] Honmou O, Onodera R, Sasaki M, Waxman SG, Kocsis JD. Mesenchymal stem cells: therapeutic outlook for stroke. Trends Mol Med 2012;18(5):292–7.
[136] Eckert MA, Vu Q, Xie K, Yu J, Liao W, Cramer SC, et al. Evidence for high translational potential of mesenchymal stromal cell therapy to improve recovery from ischemic stroke. J Cereb Blood Flow Metab 2013;33(9):1322–34.
[137] Ikegame Y, Yamashita K, Hayashi S, Mizuno H, Tawada M, You F, et al. Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 2011;13(6):675–85.
[138] Gutierrez-Fernandez M, Rodriguez-Frutos B, Ramos-Cejudo J, Teresa Vallejo-Cremades M, Fuentes B, Cerdan S, et al. Effects of intravenous administration of allogenic bone marrow- and adipose tissue-derived mesenchymal stem cells on functional recovery and brain repair markers in experimental ischemic stroke. Stem Cell Res Ther 2013;4(1):11.
[139] Kim J-M, Lee S-T, Chu K, Jung K-H, Song E-C, Kim S-J, et al. Systemic transplantation of human adipose stem cells attenuated cerebral inflammation and degeneration in a hemorrhagic stroke model. Brain Res 2007;1183:43–50.

[140] Gao S, Zhao P, Lin C, Sun Y, Wang Y, Zhou Z, et al. Differentiation of human-adipose derived stem cells into neuron-like cells which are compatible with photocurable three-dimensional scaffolds. Tissue Eng Part A 2013 [epub (PMID 24251600)].
[141] Kim S, Honmou O, Kato K, Nonaka T, Houkin K, Hamada H, et al. Neural differentiation potential of peripheral blood- and bone-marrow-derived precursor cells. Brain Res 2006;1123(1):27–33.
[142] Paczkowska E, Kucia M, Koziarska D, Halasa M, Safranow K, Masiuk M, et al. Clinical evidence that very small embryonic-like stem cells are mobilized into peripheral blood in patients after stroke. Stroke 2009;40(4):1237–44.
[143] Ukai R, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Mesenchymal stem cells derived from peripheral blood protects against ischemia. J Neurotrauma 2007;24(3): 508–20.
[144] Willing AE, Vendrame M, Mallery J, Cassady CJ, Davis CD, Sanchez-Ramos J, et al. Mobilized peripheral blood cells administered intravenously produce functional recovery in stroke. Cell Transplant 2003;12(4):449–54.
[145] Shyu W-C, Liu DD, Lin S-Z, Li W-W, Su C-Y, Chang Y-C, et al. Implantation of olfactory ensheathing cells promotes neuroplasticity in murine models of stroke. J Clin Invest 2008;118(7):2482–95.
[146] Borlongan CV, Kaneko Y, Maki M, Yu SJ, Ali M, Allickson JG, et al. Menstrual blood cells display stem cell-like phenotypic markers and exert neuroprotection following transplantation in experimental stroke. Stem Cells Dev 2010;19(4):439–52.
[147] Ishikawa H, Tajiri N, Shinozuka K, Vasconcellos J, Kaneko Y, Lee HJ, et al. Vasculogenesis in experimental stroke after human cerebral endothelial cell transplantation. Stroke 2013;44(12):3473–81.
[148] Hassiotou F, Beltran A, Chetwynd E, Stuebe AM, Twigger AJ, Metzger P, et al. Breastmilk is a novel source of stem cells with multilineage differentiation potential. Stem Cells 2012;30(10):2164–74.
[149] Leong WK, Henshall TL, Arthur A, Kremer KL, Lewis MD, Helps SC, et al. Human adult dental pulp stem cells enhance poststroke functional recovery through non-neural replacement mechanisms. Stem Cells Transl Med 2012;1(3):177–87.
[150] Kim DW, Staples M, Shinozuka K, Pantcheva P, Kang SD, Borlongan CV. Wharton's jelly-derived mesenchymal stem cells: phenotypic characterization and optimizing their therapeutic potential for clinical applications. Int J Mol Sci 2013;14(6):11692–712.
[151] Park DH, Borlongan CV, Willing AE, Eve DJ, Cruz LE, Sanberg CD, et al. Human umbilical cord blood cell grafts for brain ischemia. Cell Transplant 2009;18(9):985–98.
[152] Barlow S, Brooke G, Chatterjee K, Price G, Pelekanos R, Rossetti T, et al. Comparison of human placenta- and bone marrow-derived multipotent mesenchymal stem cells. Stem Cells Dev 2008;17(6):1095–107.
[153] Chen J, Shehadah A, Pal A, Zacharek A, Cui X, Cui Y, et al. Neuroprotective effect of human placenta-derived cell treatment of stroke in rats. Cell Transplant 2013;22(5): 871–9.
[154] Tajiri N, Acosta S, Glover LE, Bickford PC, Jacotte Simancas A, Yasuhara T, et al. Intravenous grafts of amniotic fluid-derived stem cells induce endogenous cell proliferation and attenuate behavioral deficits in ischemic stroke rats. PLoS One 2012;7(8):e43779.
[155] Stroemer P, Patel S, Hope A, Oliveira C, Pollock K, Sinden J. The neural stem cell line CTX0E03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. Neurorehabil Neural Repair 2009;23(9):895–909.
[156] Darsalia V, Kallur T, Kokaia Z. Survival, migration and neuronal differentiation of human fetal striatal and cortical neural stem cells grafted in stroke-damaged rat striatum. Eur J Neurosci 2007;26(3):605–14.
[157] Park DH, Eve DJ, Sanberg PR, Musso 3rd J, Bachstetter AD, Wolfson A, et al. Increased neuronal proliferation in the dentate gyrus of aged rats following neural stem cell implantation. Stem Cells Dev 2010;19(2):175–80.
[158] Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, et al. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. J Neurosurg 2005;103(1):38–45.
[159] Koch P, Kokaia Z, Lindvall O, Brustle O. Emerging concepts in neural stem cell research: autologous repair and cell-based disease modelling. Lancet Neurol 2009;8(9):819–29.
[160] Liu YP, Seckin H, Izci Y, Du ZW, Yan YP, Baskaya MK. Neuroprotective effects of mesenchymal stem cells derived from human embryonic stem cells in transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 2009;29(4):780–91.
[161] Seminatore C, Polentes J, Ellman D, Kozubenko N, Itier V, Tine S, et al. The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. Stroke 2010;41(1):153–9.
[162] Daadi MM, Maag AL, Steinberg GK. Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLoS One 2008;3(2):e1644.
[163] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131(5):861–72.
[164] Polentes J, Jendelova P, Cailleret M, Braun H, Romanyuk N, Tropel P, et al. Human induced pluripotent stem cells improve stroke outcome and reduce secondary degeneration in the recipient brain. Cell Transplant 2012;21(12):2587–602.
[165] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2007;447(7146):880–1.
[166] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007;25(11):2739–49.
[167] Li WY, Choi YJ, Lee PH, Huh K, Kang YM, Kim HS, et al. Mesenchymal stem cells for ischemic stroke: changes in effects after ex vivo culturing. Cell Transplant 2008;17(9):1045–59.
[168] Shahdadfar A, Fronsdal K, Haug T, Reinholt FP, Brinchmann JE. In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cells 2005;23(9):1357–66.
[169] Newman MB, Misiuta I, Willing AE, Zigova T, Karl RC, Borlongan CV, et al. Tumorigenicity issues of embryonic carcinoma-derived stem cells: relevance to surgical trials using NT2 and hNT neural cells. Stem Cells Dev 2005;14(1):29–43.
[170] Andres RH, Horie N, Slikker W, Keren-Gill H, Zhan K, Sun G, et al. Human neural stem cells enhance structural plasticity and axonal transport in the ischaemic brain. Brain 2011;134(Pt 6):1777–89.
[171] Mack GS. ReNeuron and stem cells get green light for neural stem cell trials. Nat Biotechnol 2011;29(2):95–7.
[172] Bulte JW, Zhang S, Van Gelderen P, Herynek V, Jordan EK, Duncan ID, et al. Neurotransplantation of magnetically

labeled oligodendrocyte progenitors: magnetic resonance tracking of cell migration and myelination. Proc Natl Acad Sci U S A 1999;96(26):15256–61.
[173] 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(4):565–9.
[174] Schwarting S, Litwak S, Hao W, Bähr M, Weise J, Neumann H. Hematopoietic stem cells reduce postischemic inflammation and ameliorate ischemic brain injury. Stroke 2008;39(10):2867–75.
[175] Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest 2004;114(3):330–8.
[176] Chen Z-Z, Jiang X-D, Zhang L-L, Shang J-H, Du M-X, Xu G, et al. Beneficial effect of autologous transplantation of bone marrow stromal cells and endothelial progenitor cells on cerebral ischemia in rabbits. Neurosci Lett 2008;445(1):36–41.
[177] Yang B, Strong R, Sharma S, Brenneman M, Mallikarjunarao K, Xi X, et al. Therapeutic time window and dose response of autologous bone marrow mononuclear cells for ischemic stroke. J Neurosci Res 2011;89(6):833–9.
[178] Brenneman M, Sharma S, Harting M, Strong R, Cox Jr CS, Aronowski J, et al. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow Metab 2010;30(1):140–9.
[179] Lees JS, Sena ES, Egan KJ, Antonic A, Koblar SA, Howells DW, et al. Stem cell-based therapy for experimental stroke: a systematic review and meta-analysis. Int J Stroke 2012;7(7):582–8.
[180] Hicks A, Schallert T, Jolkkonen J. Cell-based therapies and functional outcome in experimental stroke. Cell Stem Cell 2009;5(2):139–40.
[181] Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. Stroke 2007;38(2):817–26.
[182] Smith EJ, Stroemer RP, Gorenkova N, Nakajima M, Crum WR, Tang E, et al. Implantation site and lesion topology determine efficacy of a human neural stem cell line in a rat model of chronic stroke. Stem Cells 2012;30(4):785–96.
[183] Darsalia V, Allison SJ, Cusulin C, Monni E, Kuzdas D, Kallur T, et al. Cell number and timing of transplantation determine survival of human neural stem cell grafts in stroke-damaged rat brain. J Cereb Blood Flow Metab 2011;31(1):235–42.
[184] Rosenblum S, Wang N, Smith TN, Pendharkar AV, Chua JY, Birk H, et al. Timing of intra-arterial neural stem cell transplantation after hypoxia-ischemia influences cell engraftment, survival, and differentiation. Stroke 2012;43(6):1624–31.
[185] 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(4):1201–6.
[186] Tornero D, Wattananit S, Gronning Madsen M, Koch P, Wood J, Tatarishvili J, et al. Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. Brain 2013;136(Pt 12):3561–77.
[187] Crain BJ, Tran SD, Mezey E. Transplanted human bone marrow cells generate new brain cells. J Neurol Sci 2005;233(1–2):121–3.
[188] Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 2001;32(4):1005–11.
[189] Lu D, Sanberg PR, Mahmood A, Li Y, Wang L, Sanchez-Ramos J, et al. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. Cell Transplant 2002;11(3):275–81.
[190] Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, et al. One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. Stroke 2007;38(7):2150–6.
[191] Ramos-Cabrer P, Justicia C, Wiedermann D, Hoehn M. Stem cell mediation of functional recovery after stroke in the rat. PLoS One 2010;5(9):e12779.
[192] Shen LH, Li Y, Chen J, Zacharek A, Gao Q, Kapke A, et al. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab 2007;27(1):6–13.
[193] 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(2):215–23.
[194] Englund U, Bjorklund A, Wictorin K, Lindvall O, Kokaia M. Grafted neural stem cells develop into functional pyramidal neurons and integrate into host cortical circuitry. Proc Natl Acad Sci U S A 2002;99(26):17089–94.
[195] Daadi MM, Lee SH, Arac A, Grueter BA, Bhatnagar R, Maag A-L, et al. Functional engraftment of the medial ganglionic eminence cells in experimental stroke model. Cell Transplant 2009;18(7):815–26.
[196] Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke 2004;35(10):2385–9.
[197] Boltze J, Schmidt UR, Reich DM, Kranz A, Reymann KG, Strassburger M, et al. Determination of the therapeutic time window for human umbilical cord blood mononuclear cell transplantation following experimental stroke in rats. Cell Transplant 2012;21(6):1199–211.
[198] Roybon L, Ma Z, Asztely F, Fosum A, Jacobsen SE, Brundin P, et al. Failure of transdifferentiation of adult hematopoietic stem cells into neurons. Stem Cells 2006;24(6):1594–604.
[199] Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 2002;297(5590):2256–9.
[200] Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H, Shine HD. Failure of bone marrow cells to transdifferentiate into neural cells in vivo. Science 2002;297(5585):1299.
[201] Teixeira FG, Carvalho MM, Sousa N, Salgado AJ. Mesenchymal stem cells secretome: a new paradigm for central nervous system regeneration? Cell Mol Life Sci 2013;70(20):3871–82.
[202] Hsieh JY, Wang HW, Chang SJ, Liao KH, Lee IH, Lin WS, et al. Mesenchymal stem cells from human umbilical cord express preferentially secreted factors related to neuroprotection, neurogenesis, and angiogenesis. PLoS One 2013;8(8):e72604.
[203] Song M, Mohamad O, Gu X, Wei L, Yu SP. Restoration of intracortical and thalamocortical circuits after transplantation of bone marrow mesenchymal stem cells into the ischemic brain of mice. Cell Transplant 2013;22(11):2001–15.
[204] Ding X, Li Y, Liu Z, Zhang J, Cui Y, Chen X, et al. The sonic hedgehog pathway mediates brain plasticity and subsequent functional recovery after bone marrow stromal cell treatment of stroke in mice. J Cereb Blood Flow Metab 2013;33(7):1015–24.

[205] Minnerup J, Kim JB, Schmidt A, Diederich K, Bauer H, Schilling M, et al. Effects of neural progenitor cells on sensorimotor recovery and endogenous repair mechanisms after photothrombotic stroke. Stroke 2011;42(6):1757–63.
[206] Moisan A, Pannetier N, Grillon E, Richard MJ, De Fraipont F, Remy C, et al. Intracerebral injection of human mesenchymal stem cells impacts cerebral microvasculature after experimental stroke: MRI study. NMR Biomed 2012;25(12):1340–8.
[207] Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, et al. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res 2003;92(6): 692–9.
[208] Onda T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. J Cereb Blood Flow Metab 2008;28(2):329–40.
[209] Omori Y, Honmou O, Harada K, Suzuki J, Houkin K, Kocsis JD. Optimization of a therapeutic protocol for intravenous injection of human mesenchymal stem cells after cerebral ischemia in adult rats. Brain Res 2008;1236:30–8.
[210] Hao Q, Su H, Palmer D, Sun B, Gao P, Yang GY, et al. Bone marrow-derived cells contribute to vascular endothelial growth factor-induced angiogenesis in the adult mouse brain by supplying matrix metalloproteinase-9. Stroke 2011;42(2):453–8.
[211] Horie N, Pereira MP, Niizuma K, Sun G, Keren-Gill H, Encarnacion A, et al. Transplanted stem cell-secreted vascular endothelial growth factor effects poststroke recovery, inflammation, and vascular repair. Stem Cells 2011;29(2):274–85.
[212] Roitbak T, Li L, Cunningham LA. Neural stem/progenitor cells promote endothelial cell morphogenesis and protect endothelial cells against ischemia via HIF-1alpha-regulated VEGF signaling. J Cereb Blood Flow Metab 2008;28(9):1530–42.
[213] Rosell A, Morancho A, Navarro-Sobrino M, Martinez-Saez E, Hernandez-Guillamon M, Lope-Piedrafita S, et al. Factors secreted by endothelial progenitor cells enhance neurorepair responses after cerebral ischemia in mice. PLoS One 2013;8(9):e73244.
[214] Nih LR, Deroide N, Lere-Dean C, Lerouet D, Soustrat M, Levy BI, et al. Neuroblast survival depends on mature vascular network formation after mouse stroke: role of endothelial and smooth muscle progenitor cell co-administration. Eur J Neurosci 2012;35(8):1208–17.
[215] Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C, et al. Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. Brain Res 2004;1010(1–2):108–16.
[216] Li Y, Liu Z, Xin H, Chopp M. The role of astrocytes in mediating exogenous cell-based restorative therapy for stroke. Glia 2014;62(1):1–16.
[217] Chopp M, Li Y, Zhang ZG. Mechanisms underlying improved recovery of neurological function after stroke in the rodent after treatment with neurorestorative cell-based therapies. Stroke 2009;40(3 Suppl.):S143–5.
[218] Gao Q, Li Y, Chopp M. Bone marrow stromal cells increase astrocyte survival via upregulation of phosphoinositide 3-kinase/threonine protein kinase and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathways and stimulate astrocyte trophic factor gene expression after anaerobic insult. Neuroscience 2005;136(1):123–34.
[219] Li Y, Mcintosh K, Chen J, Zhang C, Gao Q, Borneman J, et al. Allogeneic bone marrow stromal cells promote glial-axonal remodeling without immunologic sensitization after stroke in rats. Exp Neurol 2006;198(2):313–25.
[220] Shen LH, Li Y, Chopp M. Astrocytic endogenous glial cell derived neurotrophic factor production is enhanced by bone marrow stromal cell transplantation in the ischemic boundary zone after stroke in adult rats. Glia 2010;58(9):1074–81.
[221] Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, et al. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res 2003;73(6):778–86.
[222] Zhang L, Li Y, Zhang C, Chopp M, Gosiewska A, Hong K. Delayed administration of human umbilical tissue-derived cells improved neurological functional recovery in a rodent model of focal ischemia. Stroke 2011;42(5): 1437–44.
[223] Sheikh AM, Nagai A, Wakabayashi K, Narantuya D, Kobayashi S, Yamaguchi S, et al. Mesenchymal stem cell transplantation modulates neuroinflammation in focal cerebral ischemia: contribution of fractalkine and IL-5. Neurobiol Dis 2010;41(3):717–24.
[224] Li Y, Chen J, Zhang CL, Wang L, Lu D, Katakowski M, et al. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. Glia 2005;49(3):407–17.
[225] Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, et al. Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci U S A 2008;105(38):14638–43.
[226] Vendrame M, Gemma C, De Mesquita D, Collier L, Bickford PC, Sanberg CD, et al. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev 2005;14(5):595–604.
[227] Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci 2006;7(5):395–406.
[228] Ben-Hur T. Immunomodulation by neural stem cells. J Neurol Sci 2008;265(1–2):102–4.
[229] Lee S-T, Chu K, Jung K-H, Kim S-J, Kim D-H, Kang K-M, et al. Anti-inflammatory mechanism of intravascular neural stem cell transplantation in haemorrhagic stroke. Brain 2008;131(Pt 3):616–29.
[230] Guzman R, Choi R, Gera A, De Los Angeles A, Andres RH, Steinberg GK. Intravascular cell replacement therapy for stroke. Neurosurg Focus 2008;24(3–4):E15.
[231] Newman MB, Willing AE, Manresa JJ, Davis-Sanberg C, Sanberg PR. Stroke-induced migration of human umbilical cord blood cells: time course and cytokines. Stem Cells Dev 2005;14(5):576–86.
[232] Park DH, Eve DJ, Musso Iii J, Klasko SK, Cruz LE, Borlongan CV, et al. Inflammation and stem cell migration to the injured brain in higher organisms. Stem Cells Dev 2009;18(5):693–702.
[233] Chang Y-C, Shyu W-C, Lin S-Z, Li H. Regenerative therapy for stroke. Cell Transplant 2007;16(2):171–81.
[234] Detante O, Moisan A, Dimastromatteo J, Richard MJ, Riou L, Grillon E, et al. Intravenous administration of 99mTc-HMPAO-labeled human mesenchymal stem cells after stroke: in vivo imaging and biodistribution. Cell Transplant 2009;18(12):1369–79.
[235] Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S, et al. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. J Neurosci Res 2003;73(3):296–307.
[236] Borlongan CV, Evans A, Yu G, Hess DC. Limitations of intravenous human bone marrow CD133+ cell grafts in stroke rats. Brain Res 2005;1048(1–2):116–22.
[237] Kawabori M, Kuroda S, Sugiyama T, Ito M, Shichinohe H, Houkin K, et al. Intracerebral, but not intravenous, transplantation of bone marrow stromal cells enhances

functional recovery in rat cerebral infarct: an optical imaging study. Neuropathology 2012;32(3):217–26.
[238] Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev 2009;18(5):683–92.
[239] Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. Transplant Proc 2007;39(2):573–6.
[240] Shen LH, Li Y, Chen J, Zhang J, Vanguri P, Borneman J, et al. Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. Neuroscience 2006;137(2):393–9.
[241] Seyfried DM, Han Y, Yang D, Ding J, Savant-Bhonsale S, Shukairy MS, et al. Mannitol enhances delivery of marrow stromal cells to the brain after experimental intracerebral hemorrhage. Brain Res 2008;1224:12–9.
[242] 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(32):11839–44.
[243] Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J Neurol Sci 2001;189(1–2):49–57.
[244] Lappalainen RS, Narkilahti S, Huhtala T, Liimatainen T, Suuronen T, Närvänen A, et al. The SPECT imaging shows the accumulation of neural progenitor cells into internal organs after systemic administration in middle cerebral artery occlusion rats. Neurosci Lett 2008;440(3):246–50.
[245] Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG, et al. Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. Stroke 2008;39(5):1569–74.
[246] Ge J, Guo L, Wang S, Zhang Y, Cai T, Zhao RC, et al. The size of mesenchymal stem cells is a significant cause of vascular obstructions and stroke. Stem Cell Rev 2014 [epub (PMID 24390934)].
[247] Guzman R, De Los Angeles A, Cheshier S, Choi R, Hoang S, Liauw J, et al. Intracarotid injection of fluorescence activated cell-sorted CD49d-positive neural stem cells improves targeted cell delivery and behavior after stroke in a mouse stroke model. Stroke 2008;39(4):1300–6.
[248] Li L, Jiang Q, Ding G, Zhang L, Zhang ZG, Li Q, et al. Effects of administration route on migration and distribution of neural progenitor cells transplanted into rats with focal cerebral ischemia, an MRI study. J Cereb Blood Flow Metab 2010;30(3):653–62.
[249] Yang B, Migliati E, Parsha K, Schaar K, Xi X, Aronowski J, et al. Intra-arterial delivery is not superior to intravenous delivery of autologous bone marrow mononuclear cells in acute ischemic stroke. Stroke 2013;44(12):3463–72.
[250] Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis JD. A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. Brain Res 2004;1007(1–2):1–9.
[251] Misra V, Ritchie MM, Stone LL, Low WC, Janardhan V. Stem cell therapy in ischemic stroke: role of IV and intra-arterial therapy. Neurology 2012;79(13 Suppl. 1): S207–12.
[252] Wang LQ, Lin ZZ, Zhang HX, Shao B, Xiao L, Jiang HG, et al. Timing and dose regimens of marrow mesenchymal stem cell transplantation affect the outcomes and neuroinflammatory response after ischemic stroke. CNS Neurosci Ther 2014 [epub (PMID 24393245)].
[253] Komatsu K, Honmou O, Suzuki J, Houkin K, Hamada H, Kocsis JD. Therapeutic time window of mesenchymal stem cells derived from bone marrow after cerebral ischemia. Brain Res 2010;1334:84–92.
[254] Borlongan CV, Tajima Y, Trojanowski JQ, Lee VM, Sanberg PR. Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2 N cells) promotes functional recovery in ischemic rats. Exp Neurol 1998;149(2):310–21.
[255] Bonn D. First cell transplant aimed to reverse stroke damage. Lancet 1998;352(9122):119.
[256] 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(2):101–7.
[257] Rabinovich SS, Seledtsov VI, Banul NV, Poveshchenko OV, Senyukov VV, Astrakov SV, et al. Cell therapy of brain stroke. Bull Exp Biol Med 2005;139(1):126–8.
[258] Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol 2005;57(6):874–82.
[259] Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, et al. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain 2011;134(Pt 6):1790–807.
[260] Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells 2010;28(6):1099–106.
[261] Bhasin A, Srivastava MV, Kumaran SS, Mohanty S, Bhatia R, Bose S, et al. Autologous mesenchymal stem cells in chronic stroke. Cerebrovasc Dis Extra 2011;1(1):93–104.
[262] Bhasin A, Srivastava MV, Mohanty S, Bhatia R, Kumaran SS, Bose S. Stem cell therapy: a clinical trial of stroke. Clin Neurol Neurosurg 2013;115(7):1003–8.
[263] Suárez-Monteagudo C, Hernández-Ramírez P, Alvarez-González L, García-Maeso I, De La Cuétara-Bernal K, Castillo-Díaz L, et al. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study. Restor Neurol Neurosci 2009;27(3):151–61.
[264] Mendonca MLFD, Freitas GRD, Silva SaD, Manfrim A, Falcao CHE, Gonzales C, et al. Safety of intra-arterial autologous bone marrow mononuclear cell transplantation for acute ischemic stroke. Arq Bras Cardiol 2006;86(1):52–5.
[265] Battistella V, De Freitas GR, Da Fonseca LM, Mercante D, Gutfilen B, Goldenberg RC, et al. Safety of autologous bone marrow mononuclear cell transplantation in patients with nonacute ischemic stroke. Regen Med 2011;6(1): 45–52.
[266] Moniche F, Gonzalez A, Gonzalez-Marcos JR, Carmona M, Pinero P, Espigado I, et al. Intra-arterial bone marrow mononuclear cells in ischemic stroke: a pilot clinical trial. Stroke 2012;43(8):2242–4.
[267] Friedrich MA, Martins MP, Araujo MD, Klamt C, Vedolin L, Garicochea B, et al. Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke. Cell Transplant 2012;21(Suppl. 1):S13–21.
[268] Savitz SI, Misra V, Kasam M, Juneja H, Cox Jr CS, Alderman S, et al. Intravenous autologous bone marrow mononuclear cells for ischemic stroke. Ann Neurol 2011;70(1):59–69.
[269] Prasad K, Mohanty S, Bhatia R, Srivastava MV, Garg A, Srivastava A, et al. Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischaemic stroke: a pilot study. Indian J Med Res 2012;136(2):221–8.
[270] Li ZM, Zhang ZT, Guo CJ, Geng FY, Qiang F, Wang LX. Autologous bone marrow mononuclear cell implantation for intracerebral hemorrhage-a prospective clinical observation. Clin Neurol Neurosurg 2013;115(1):72–6.

[271] Lemmens R, Steinberg GK. Stem cell therapy for acute cerebral injury: what do we know and what will the future bring? Curr Opin Neurol 2013 [epub (PMID 24136128)].
[272] Wechsler LR. Clinical trials of stroke therapy: which cells, which patients? Stroke 2009;40(3 Suppl.):S149–51.
[273] Barbosa Da Fonseca LM, Gutfilen B, Rosado De Castro PH, Battistella V, Goldenberg RC, Kasai-Brunswick T, et al. Migration and homing of bone-marrow mononuclear cells in chronic ischemic stroke after intra-arterial injection. Exp Neurol 2010;221(1):122–8.
[274] Grefkes C, Fink GR. Connectivity-based approaches in stroke and recovery of function. Lancet Neurol 2014;13(2):206–16.
[275] Carey LM, Seitz RJ. Functional neuroimaging in stroke recovery and neurorehabilitation: conceptual issues and perspectives. Int J Stroke 2007;2(4):245–64.
[276] Chen J, Ye X, Yan T, Zhang C, Yang XP, Cui X, et al. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. Stroke 2011;42(12):3551–8.
[277] Minnerup J, Wagner DC, Strecker JK, Posel C, Sevimli-Abdis S, Schmidt A, et al. Bone marrow-derived mononuclear cells do not exert acute neuroprotection after stroke in spontaneously hypertensive rats. Front Cell Neurosci 2014;7:288.
[278] Riegelsberger UM, Deten A, Posel C, Zille M, Kranz A, Boltze J, et al. Intravenous human umbilical cord blood transplantation for stroke: impact on infarct volume and caspase-3-dependent cell death in spontaneously hypertensive rats. Exp Neurol 2011;227(1):218–23.
[279] Weise G, Lorenz M, Posel C, Maria Riegelsberger U, Storbeck V, Kamprad M, et al. Transplantation of cryopreserved human umbilical cord blood mononuclear cells does not induce sustained recovery after experimental stroke in spontaneously hypertensive rats. J Cereb Blood Flow Metab 2014;34(1) [epub (PMID 24169850)].
[280] Cui X, Chopp M, Shehadaha A, Zachareka A, Nicholsc NK, Sanbergc CD, et al. Therapeutic benefit of treatment of stroke with simvastatin and human umbilical cord blood cells: neurogenesis, synaptic plasticity and axon growth. Cell Transplant 2012;21(5):845–56.
[281] Subramanian A, Shu-Uin G, Kae-Siang N, Gauthaman K, Biswas A, Choolani M, et al. Human umbilical cord Wharton's jelly mesenchymal stem cells do not transform to tumor-associated fibroblasts in the presence of breast and ovarian cancer cells unlike bone marrow mesenchymal stem cells. J Cell Biochem 2012;113(6): 1886–95.
[282] Amariglio N, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, Trakhtenbrot L, et al. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med 2009;6(2):e1000029.
[283] Amariglio N, Rechavi G. On the origin of glioneural neoplasms after neural cell transplantation. Nat Med 2010;16(2):157 [author reply -8].
[284] Hurst RW, Peter Bosch E, Morris JM, Dyck PJ, Reeves RK. Inflammatory hypertrophic cauda equina following intrathecal neural stem cell injection. Muscle Nerve 2013;48(5):831–5.
[285] Morizane A, Doi D, Kikuchi T, Okita K, Hotta A, Kawasaki T, et al. Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a nonhuman primate. Stem Cell Reports 2013;1(4):283–92.
[286] Esneault E, Pacary E, Eddi D, Freret T, Tixier E, Toutain J, et al. Combined therapeutic strategy using erythropoietin and mesenchymal stem cells potentiates neurogenesis after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 2008;28(9):1552–63.
[287] Chen J, Li Y, Zhang R, Katakowski M, Gautam SC, Xu Y, et al. Combination therapy of stroke in rats with a nitric oxide donor and human bone marrow stromal cells enhances angiogenesis and neurogenesis. Brain Res 2004;1005(1–2):21–8.
[288] Chu K, Park K-I, Lee S-T, Jung K-H, Ko S-Y, Kang L, et al. Combined treatment of vascular endothelial growth factor and human neural stem cells in experimental focal cerebral ischemia. Neurosci Res 2005;53(4):384–90.
[289] Zhang XM, Du F, Yang D, Wang R, Yu CJ, Huang XN, et al. Granulocyte colony-stimulating factor increases the therapeutic efficacy of bone marrow mononuclear cell transplantation in cerebral ischemia in mice. BMC Neurosci 2011;12:61.
[290] Nomura T, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. I.V. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Neuroscience 2005;136(1):161–9.
[291] Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Kobune M, Hirai S, et al. BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. Mol Ther 2004;9(2):189–97.
[292] Kurozumi K, Nakamura K, Tamiya T, Kawano Y, Ishii K, Kobune M, et al. Mesenchymal stem cells that produce neurotrophic factors reduce ischemic damage in the rat middle cerebral artery occlusion model. Mol Ther 2005;11(1):96–104.
[293] Horita Y, Honmou O, Harada K, Houkin K, Hamada H, Kocsis JD. Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. J Neurosci Res 2006;84(7):1495–504.
[294] Toyama K, Honmou O, Harada K, Suzuki J, Houkin K, Hamada H, et al. Therapeutic benefits of angiogenetic gene-modified human mesenchymal stem cells after cerebral ischemia. Exp Neurol 2009;216(1):47–55.
[295] Wei L, Fraser JL, Lu ZY, Hu X, Yu SP. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. Neurobiol Dis 2012;46(3):635–45.
[296] Tu J, Yang F, Wan J, Liu Y, Zhang J, Wu B, et al. Light-controlled astrocytes promote human mesenchymal stem cells toward neuronal differentiation and improve the neurological deficit in stroke rats. Glia 2014;62(1):106–21.
[297] He XY, Chen ZZ, Cai YQ, Xu G, Shang JH, Kou SB, et al. Expression of cytokines in rat brain with focal cerebral ischemia after grafting with bone marrow stromal cells and endothelial progenitor cells. Cytotherapy 2010;13(1):46–53.
[298] Delcroix GJ, Schiller PC, Benoit JP, Montero-Menei CN. Adult cell therapy for brain neuronal damages and the role of tissue engineering. Biomaterials 2010;31(8):2105–20.
[299] Moshayedi P, Carmichael ST. Hyaluronan, neural stem cells and tissue reconstruction after acute ischemic stroke. Biomatter 2013;3(1) [epub (PMID 23507922)].
[300] Caicco MJ, Cooke MJ, Wang Y, Tuladhar A, Morshead CM, Shoichet MS. A hydrogel composite system for sustained epi-cortical delivery of cyclosporin A to the brain for treatment of stroke. J Control Release 2013;166(3):197–202.
[301] Yamashita T, Deguchi K, Nagotani S, Abe K. Vascular protection and restorative therapy in ischemic stroke. Cell Transplant 2011;20(1):95–7.
[302] Emerich DF, Silva E, Ali O, Mooney D, Bell W, Yu SJ, et al. Injectable VEGF hydrogels produce near complete neurological and anatomical protection following cerebral ischemia in rats. Cell Transplant 2010;19(9):1063–71.
[303] Wang Y, Cooke MJ, Morshead CM, Shoichet MS. Hydrogel delivery of erythropoietin to the brain for endogenous

stem cell stimulation after stroke injury. Biomaterials 2012;33(9):2681–92.
[304] Cooke MJ, Wang Y, Morshead CM, Shoichet MS. Controlled epi-cortical delivery of epidermal growth factor for the stimulation of endogenous neural stem cell proliferation in stroke-injured brain. Biomaterials 2011;32(24):5688–97.
[305] Nakaji-Hirabayashi T, Kato K, Iwata H. Hyaluronic acid hydrogel loaded with genetically-engineered brain-derived neurotrophic factor as a neural cell carrier. Biomaterials 2009;30(27):4581–9.
[306] Ma J, Tian WM, Hou SP, Xu QY, Spector M, Cui FZ. An experimental test of stroke recovery by implanting a hyaluronic acid hydrogel carrying a Nogo receptor antibody in a rat model. Biomed Mater 2007;2(4):233–40.
[307] Jin K, Mao X, Xie L, Galvan V, Lai B, Wang Y, et al. Transplantation of human neural precursor cells in Matrigel scaffolding improves outcome from focal cerebral ischemia after delayed postischemic treatment in rats. J Cereb Blood Flow Metab 2010;30(3):534–44.
[308] Zhong J, Chan A, Morad L, Kornblum HI, Fan G, Carmichael ST. Hydrogel matrix to support stem cell survival after brain transplantation in stroke. Neurorehabil Neural Repair 2010;24(7): 636–44.
[309] Osanai T, Kuroda S, Yasuda H, Chiba Y, Maruichi K, Hokari M, et al. Noninvasive transplantation of bone marrow stromal cells for ischemic stroke: preliminary study with a thermoreversible gelation polymer hydrogel. Neurosurgery 2010;66(6):1140–7 [discussion 7].
[310] Sarnowska A, Jablonska A, Jurga M, Dainiak M, Strojek L, Drela K, et al. Encapsulation of mesenchymal stemcells by bio-scaffolds protects cell survival and attenuates neuroinflammatory reaction in injured brain tissue after transplantation. Cell Transplant 2013 [epub (PMID 24070175)].
[311] Portalska KJ, Teixeira LM, Leijten JC, Jin R, Van Blitterswijk C, De Boer J, et al. Boosting angiogenesis and functional vascularization in injectable dextran-hyaluronic acid hydrogels by endothelial-like mesenchymal stromal cells. Tissue Eng Part A 2013 [epub (PMID 24070233)].
[312] Liang Y, Walczak P, Bulte JW. The survival of engrafted neural stem cells within hyaluronic acid hydrogels. Biomaterials 2013;34(22):5521–9.