

## Review Article

# Pharmacological approaches promoting stem cell-based therapy following ischemic stroke insults

Shu-zhen ZHU<sup>1,2,3</sup>, Vivian SZETO<sup>1</sup>, Mei-hua BAO<sup>1,2</sup>, Hong-shuo SUN<sup>1,2,\*</sup>, Zhong-ping FENG<sup>1,\*</sup>

Departments of <sup>1</sup>Physiology and <sup>2</sup>Surgery, Faculty of Medicine, University of Toronto, <sup>1</sup>King's College Circle, Toronto, Ontario, Canada M5S 1A8; <sup>3</sup>Department of Neurology, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China

### Abstract

Stroke can lead to long-term neurological deficits. Adult neurogenesis, the continuous generation of newborn neurons in distinct regions of the brain throughout life, has been considered as one of the approaches to restore the neurological function following ischemic stroke. However, ischemia-induced spontaneous neurogenesis is not sufficient, thus cell-based therapy, including infusing exogenous stem cells or stimulating endogenous stem cells to help repair of injured brain, has been studied in numerous animal experiments and some pilot clinical trials. While the effects of cell-based therapy on neurological function during recovery remains unproven in randomized controlled trials, pharmacological agents have been administered to assist the cell-based therapy. In this review, we summarized the limitations of ischemia-induced neurogenesis and stem-cell transplantation, as well as the potential proneuroregenerative effects of drugs that may enhance efficacy of cell-based therapies. Specifically, we discussed drugs that enhance proliferation, migration, differentiation, survival and function connectivity of newborn neurons, which may restore neurobehavioral function and improve outcomes in stroke patients.

**Keywords:** stroke; neurobehavioral function; neurogenesis; cell-based therapy; stem-cell transplantation; granulocyte colony-stimulating factor (G-CSF); herbal medicine

Acta Pharmacologica Sinica advance (2018) 39: 695–712; doi: 10.1038/aps.2018.23

### Introduction

Cerebral infarction (CI) is a major health problem worldwide. It is the second leading cause of death and the third most common cause of disability<sup>[1]</sup>. Numerous efforts have been made to reduce ischemia-induced neuron injury and restore neurological function by various mechanisms, such as inhibition of neuroinflammation<sup>[2]</sup>, blocking *N*-methyl-*D*-aspartate (NMDA) receptor<sup>[3]</sup>, opening of  $K_{ATP}$  channel<sup>[4]</sup>, suppression of melastatin-like transient receptor potential cation channel, subfamily M, member 7 (TRPM7) channel<sup>[5]</sup>, and inhibition of postsynaptic density-95<sup>[6]</sup>. Despite of this, recombinant tissue plasminogen activator (rtPA) is still the only FDA-approved drug treatment for ischemic stroke and must be used within 4.5 h of onset<sup>[7]</sup>. Spontaneous neuroplasticity in perilesional tissue following ischemic insult may promote map reorganization abilities in human and animal models<sup>[8]</sup>. Neurogenesis was widely accepted as a fundamental mechanism of neural plasticity<sup>[9,10]</sup>. Recent studies suggest that after central ner-

vous system (CNS) injuries, regeneration and reparation may occur in the brain through adult neural stem/progenitor cells. Stem-cell-based therapies, including cell transplantation and stimulation of endogenous neurogenesis, are potential strategies to repair and regenerate the injured brain and may provide the second therapeutic time window for ischemic stroke treatment<sup>[11]</sup>. However, whether stem cell transplantation would be beneficial for neuronal function following stroke insults is still indefinite. So in this review we will discuss 1) the evidence of neurogenesis in adult brain; 2) the contributions and limitations of ischemia-induced neurogenesis for cerebral repair; 3) the potentials and limitations of stem cell transplantation therapies; and 4) the potential role of drugs to enhance efficacy of the cell-based therapy by enhancing the proliferation, migration, differentiation, survival, and functional connectivity of newborn neuron.

### The discovery of neurogenesis in normal adult brain

It was considered for a long time that neurogenesis ended in the period shortly after birth and adult neurogenesis was impossible. However, in 1992, adult neurogenesis in mouse brain was verified by Reynolds<sup>[12]</sup>. Only 6 years later, in 1998, adult neurogenesis in human brains was also found under

\*To whom correspondence should be addressed.

E-mail: hss.sun@utoronto.ca (Hong-shuo SUN);

zp.feng@utoronto.ca (Zhong-ping FENG)

Received 2017-12-21 Accepted 2018-03-13

physiologic conditions<sup>[13-17]</sup>. Then it was believed that proliferation of adult neural stem cells (NSCs) in the central nervous system (CNS) might have the ability to replace lost or damaged neural cells. Transplantation of exogenous or stimulation of endogenous stem cells could be potential treatments for human brain repair after an ischemic stroke or other neurodegeneration diseases<sup>[18-20]</sup>.

### **Ischemic stroke promotes neurogenesis**

#### **The evidence of ischemic stroke-induced neurogenesis**

Ischemic stroke is one of the most important causes of long-term disability and mortality worldwide. Vascular recanalization therapy is one of the few effective therapies; however, it can only be used within a narrow therapeutic time window (within 4.5–6 h post-stroke). The discovery of continuous adult neurogenesis in human brains provides a second therapeutic time window and gives hope to neural repair after ischemic stroke. Encouragingly, it was found that compared to quiescent state, the production of neuroblasts was significantly increased in the adult brain after ischemic stroke<sup>[21-26]</sup>. It was found that stroke gave rise to a 31-fold increase of the number of new-born neurons in the ipsilateral striatum<sup>[15]</sup>. The generated neuroblasts migrate toward the injured brain region, differentiate into mature striatal neurons, establish appropriate long-distance connections, integrate into the neuronal circuitry and may contribute to the recovery of ischemic stroke<sup>[27-29]</sup>.

#### **Cell responses associated with the ischemia-induced neurogenesis**

While a series of evidence showing the presence of ischemia-enhanced neurogenesis in rodents and human, their mechanisms remain to be elucidated. For a better understanding of the promise and limitations of ischemia-enhanced neurogenesis on brain repair, the mechanisms underlying ischemia-enhanced neurogenesis, especially the cell response, are discussed in this section (Figure 1).

#### **Ischemia-induced astrocyte-to-neuron conversion**

After cerebral ischemia, astrocytes are activated, which indeed can give rise to neurons *in vivo* in the adult mouse striatum through Notch signaling pathway<sup>[30]</sup>. By local transduction of striatal astrocytes with adenoviruses expressing Cre under regulatory elements of the GFAP promoter in Connexin-30-CreER transgenic mice, researchers were able to visualize doublecortin (DCX)-positive neuroblasts striatal astrocyte origin<sup>[31]</sup>. Another study showed that striatal astrocytes could transdifferentiate into immature neurons at 1 week and mature neurons at 2 weeks after middle cerebral artery occlusion (MCAO). In addition, these astrocyte origin neurons could form synapses with other neurons at 13 weeks after MCAO. It has been shown that these astrocyte origin newborn neurons could produce connections with other neurons in the injured brain<sup>[32]</sup>. VEGF helps striatal astrocytes transdifferentiate into new mature neurons<sup>[33]</sup>. These results indicate that astrocytes were one of the sources of new-born neurons after ischemic stroke.

#### **Astrocyte-derived neurotrophic factors involved in ischemia-induced neurogenesis**

Recently astrocytes are considered to be involved in adult neurogenesis through the releasing of neurotrophic factors<sup>[34, 35]</sup>. In stroke model, activated astrocytes enhanced the expression of BDNF<sup>[36]</sup>, which enhanced the differentiation of CNS stem cell-derived neuronal precursors<sup>[37]</sup>, resulted in higher initial NSCs engraftment and survival<sup>[38]</sup>. Glial cell line-derived neurotrophic factor (GDNF), another neurotrophic factor secreted by astrocytes, induces neural differentiation in neural progenitor cells<sup>[39]</sup>, promotes striatal neurogenesis after stroke in adult rats<sup>[40]</sup>. Nerve growth factor (NGF) expressed in astrocytes and enhanced after ischemic stroke in peri-infarct area<sup>[41]</sup>, has been shown to improve survival of newly generated cells in the ipsilateral striatum and subventricular zone (SVZ)<sup>[42]</sup>.

#### **Vasculature is associated with neurogenesis**

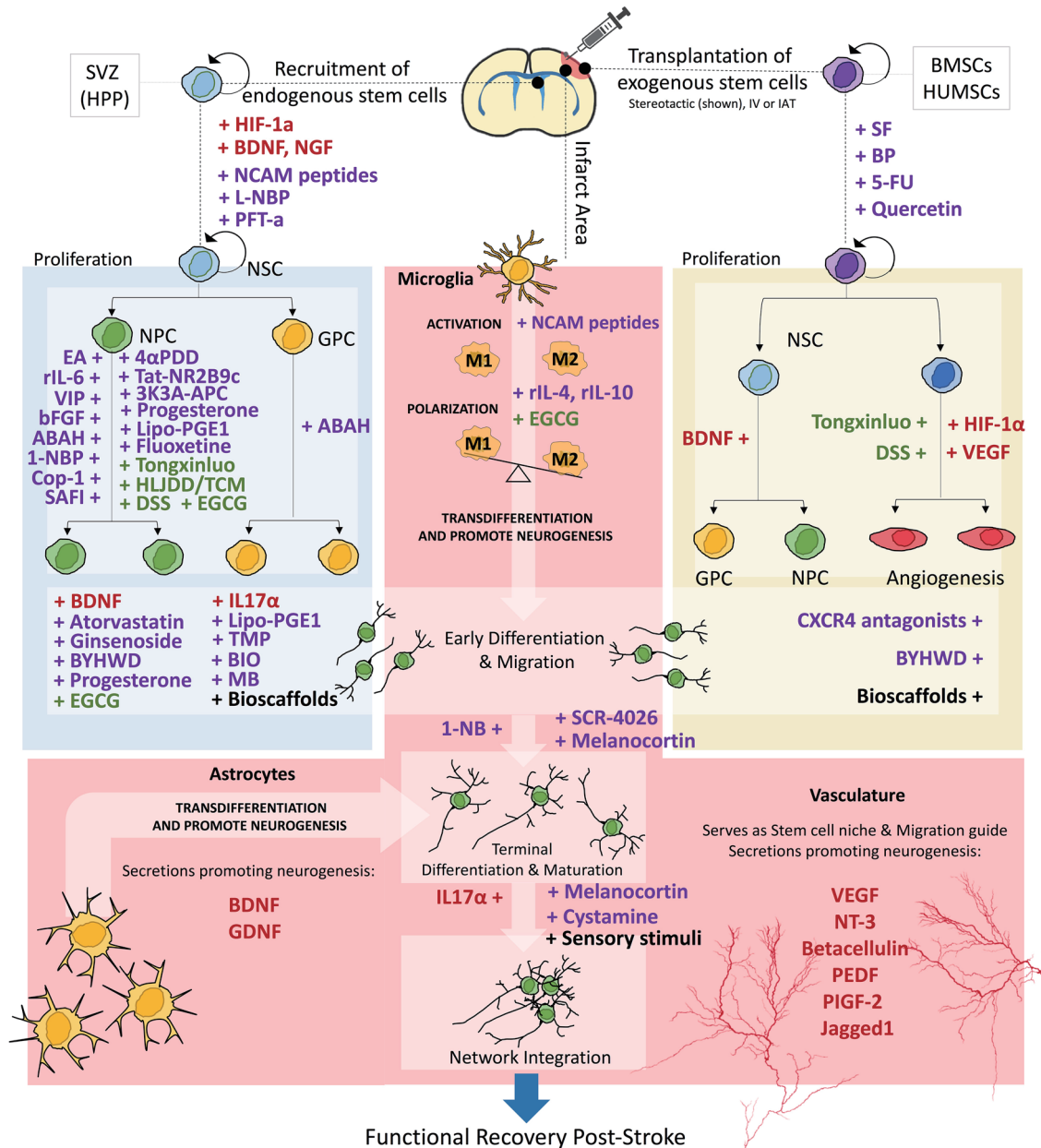
The vasculature is an important component of the adult neural stem cell niche. After cerebral ischemia, neurotrophic factors secreted by endothelial and pericyte affect the neurogenesis in a variety of aspects, such as promoting the proliferation, neuronal differentiation of NSCs<sup>[43]</sup>. Vascular endothelial growth factor (VEGF), which is secreted by endothelial cells and pericytes, is one of the most important neurotrophic factors stimulating cell proliferation in the SVZ<sup>[44, 45]</sup>, facilitating the migration of immature neurons towards the ischemic tissue<sup>[46]</sup>. Besides VEGF, several other cytokines or growth factors have been implicated in poststroke neurogenesis. Betacellulin (BTC), placenta growth factor (PlGF-2) and Jagged1 were also found to induce NSCs proliferation during postnatal and adult neurogenesis<sup>[43, 47, 48]</sup>. Neurotrophin-3 (NT-3), a mediator of quiescence in the SVZ adult neural stem cell niche, promotes newly differentiated neurons in hippocampal dentate gyrus (DG)<sup>[49, 50]</sup> and cholinergic neuronal differentiation of bone marrow-derived neural stem cells<sup>[51]</sup>. Another endothelial-derived neurotrophic factor, pigment epithelium-derived factor (PEDF), was shown to promote the self-renewing cell division and multipotency maintenance of neural stem cells<sup>[52, 53]</sup>.

#### **Ischemia-induced pericytes-to-neuron conversion**

Besides glial cells, pericytes were also found to be involved in neurogenesis. Studies found that 3 days after transient ischemia/reperfusion platelet-derived growth factor receptor beta-positive (PDGFR beta<sup>+</sup>) pericytes within injured areas began to express the NSCs marker Nestin, and at day 7, some of them expressed the immature neuronal marker DCX. These findings suggest that brain pericytes may contribute to new neurons in response to ischemia condition<sup>[54, 55]</sup>.

#### **The polarization of microglia adjusts neurogenesis**

Microglia, one of the resident immune cells in CNS, plays a crucial role in neurogenesis, which includes 1) Resting microglia in the neurogenic niche releasing neurotrophic factors such as insulin-like growth factor 1 (IGF-1) which are essential for new neurons proliferation and survival<sup>[56]</sup>; 2) activated microglia converting to neuron<sup>[57]</sup>, and 3) bidirectionally adjusting



**Figure 1.** Schematic diagram of two major routes of stem cells and neurogenesis in stroke. Endogenous stem cells (Left) and transplanted exogenous stem cells (Right). Important processes towards improved neurological outcomes as shown are proliferation, differentiation, migration, and functional connection. Drug and peptides (Purple) and herbal medicines (Green) tested in animal and cell culture models are shown along side their suspected targeted processes. Endogenous compounds are denoted in Red text.

neurogenesis through polarization. In this section, we mainly discuss the third role of microglia, which is closely related to the regulation of neurogenesis and the recovery of neurological function.

Under physiological circumstances, microglia retain a relative quiescent surveillance phenotype for constant monitoring of the brain parenchyma<sup>[58]</sup>. Shortly after ischemic stroke, due to the change of cellular environments, such as the deletion of ATP, microglia were activated to clear the cell debris<sup>[59]</sup>. The activated microglia present two polarization phenotypes, M1

and M2, which exhibit distinct roles in influencing neurogenesis. Acute M1 microglial activation along with secreted pro-inflammatory cytokines [interleukin 6(IL-6), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interferon gamma (IFN- $\gamma$ ), interleukin 23(IL-23), interleukin 12 (IL-12) and interleukin 1 $\beta$  (IL-1 $\beta$ ), etc.]<sup>[60-62]</sup> and reactive oxygen species (ROS)<sup>[63, 64]</sup>. It is widely considered that M1 microglia causes neuronal death, neurogenesis inhibition and exacerbates neuronal injury<sup>[65]</sup>. However, some studies do not fully support this notion. For instance, the neuro-inflammatory environment is not entirely harmful and

may have dual roles in regulating neurogenesis after stroke<sup>[66]</sup>. Advantages or disadvantages of neuroinflammation on neurogenesis depends on its severity and location. During mild, acute inflammation, activated, ramified or intermediate microglia in ipsilateral SVZ has been shown to accompany neuroblast migration after stroke, indicating a beneficial role in neurogenesis, while amoeboid microglia in the peri-infarct, accompanied with the uncontrolled inflammation, induced the death of newborn neuron, and inhibited neural progenitors from differentiating into neurons which were detrimental to neurogenesis<sup>[67-69]</sup>.

In contrast, an increase in activated M2 microglia promotes neurogenesis<sup>[70]</sup>. In addition to cytokines and chemokines, microglial cells also synthesized and secreted neurotrophic factors like basic fibroblast growth factor (bFGF), brain derived neurotrophic factor (BDNF) and interleukin 4 (IL-4)<sup>[71]</sup> which are known to stimulate the proliferation, migration, differentiation, the survival of neuron<sup>[72]</sup> and regulate synaptic maturation<sup>[73]</sup>. According to the above studies, promoting M1 to M2 phenotype transition may be a promising strategy to minimize detrimental effects and/or maximizing protective effects.

In summary, ischemia induces astrocyte, pericytes and microglia to neuron conversion (Figure 1). Activated microglia bi-directionally adjusted the process of neurogenesis partly through the polarization. During the course of neurogenesis, molecules, such as ATP, glucose, signaling pathways, such as the notch, Ras/MAPK and PI3K/TOR/PTEN, and transcription-related factors, such as Hes1, miRNA 210, help regulate the process of neurogenesis<sup>[74]</sup>. Strategies to promote neuron conversion, microglia M2 polarization by targeting molecular pathways and transcription factors may promote neurogenesis.

#### **The role of ischemia-induced neurogenesis in brain repair and recovery: favorable or harmful**

Ischemia stroke enhances cell proliferation. Stroke-generated new neurons migrate into the severely damaged area, partly replenish the damaged or lost neuron, and promote the repair of injured-brain<sup>[15, 75]</sup>. Most studies suggest that ischemia-induced neurogenesis is a means of self-repairing which partly contributes to the neurological recovery and may be related to the spontaneous recovery after ischemic stroke insults. However, whether this injury-induced neurogenesis contributes to recovery after brain injury remains controversial.

#### **Ischemia-induced neurogenesis promotes brain recovery**

Most of the studies provided evidence that ischemia-induced neurogenesis is helpful to brain repair and recovery. After ischemia, spontaneous neurogenesis is enhanced and accompanied by the course of spontaneous recovery of neurological function, suggesting a possible relationship between neurogenic potential and recovery after injury<sup>[76, 77]</sup>. Once ischemia-induced neurogenesis and associated neuromigration was abolished in transgenic mice expressing herpes simplex virus thymidine kinase under control of DCX promoter by the antiviral drug ganciclovir (GCV), infarct size was enlarged, and

post-ischemic sensorimotor behavioral deficits were measured by rotarod, limb placing, and elevated body swing tests were exacerbated<sup>[78]</sup>. In another study, when neuroprogenitor cells were conditionally ablated using a transgenic mouse model containing modified Herpes Simplex Virus Thymidine Kinase Gene (HSV-TK gene) driven by Nestin promoter, learning and memory outcomes were worsened and synaptic connectivity in the performant pathway reduced<sup>[79]</sup>. The above two studies present evidence that spontaneous ischemia-induced neurogenesis contributes to the recovery of neurological function and might therefore be a target for stroke therapy.

#### **Spontaneous Ischemia-induced neurogenesis has its own limitation**

Studies suggested the major limitation of spontaneous neurogenesis in the brain is the lack of surviving high-quality newborn neuron. Three major possibilities support this notion.

First, there are few surviving neurons. Despite the large number of new-born neurons that are generated following stroke, more than 80% of them die during the first 2 weeks, most of them do not differentiate to mature neurons after 4 weeks post-stroke<sup>[80]</sup>, and no surviving differentiated mature neural cells were observed by 90 days<sup>[26]</sup>. These data indicate that although many neuroblasts are produced and migrate to the site of injury, the ability of new-born neurons to replace lost neurons is limited. The limited number of surviving NCSs is partly due to unfavorable microenvironment post stroke attack (high levels of detrimental inflammatory factors and lack of trophic factors). In addition, normal aging may lead to further decreases in the number and maturation of newly generated neurons in the ischemic penumbra. To this day, the mechanisms underlying the low survival rate of new-born neurons remain unclear.

Second, the morphological features of the new neurons remain abnormal. Despite the fact that stroke enhanced SVZ neurogenesis and attracted new-born neurons to the injury area in rodents<sup>[81, 82]</sup> and patients<sup>[23]</sup>, approximately 5% to 10% of newborn granule cells display significant morphological abnormalities. The main features are additional basal dendrites, ectopic cell position, and an increased portion of mushroom spines in aberrant neurons, which suggests stable synaptic integration<sup>[83]</sup>.

Finally, there is a lack of diversity in the new neurons. Ischemia-induced neurogenesis generates predominantly GABAergic interneurons in SVZ, which cannot replace the broad spectrum of neuronal subtypes damaged by stroke. Therefore, SVZ neurogenesis may not be sufficient to replenish the loss of neuron after ischemic stroke<sup>[16]</sup>

In summary, ischemia-induced neurogenesis promotes brain recovery to some extent; however, it has been proven weak. Endogenous neurogenesis by itself is insufficient for effective brain repair after stroke. More ideal strategies are needed to enhance the number of surviving neurons, alleviate morphological abnormalities, enrich the cell subtype and construct the new neural network.



### Potential of cell-based therapy for clinical transformation

Numerous animal experiments provide evidence that promoting neurogenesis is a potential way to protect and repair damaged brain tissues post-stroke<sup>[82, 84-87]</sup>. However, the role of cell-based therapy in ischemic stroke still needs to be established, because of the demonstrated challenges of cell-based therapies for ischemic stroke. It has been proposed that transplantation of neurons could improve neurological function by a variety of mechanisms including neuron replacement, alleviation of the neuroinflammation<sup>[88]</sup>, inhibition of MMP-9 activation<sup>[89]</sup>, secretion of neurotrophic factors, thereby survival of newborn neurons in injured-brain. In recent years, many pre-clinical studies and clinical trials on stem cells transplantation have been performed (Figure 1). Stem cell-based therapies were found to reduce infarct size and improve neural functional recovery in pre-clinical studies; however, its efficacy in humans still needs to be determined.

### Stem cells

Bone marrow stem cells (BMSCs) are an array of different types of multipotent and pluripotent cells homed in the spongy tissue of almost all bones. Three basic lineages prevail: mesenchymal stem cells (MSCs), bone marrow mononuclear cells (BM-MNCs) and immortalized human neural stem-cell line. BMSCs were widely used to treat cerebral ischemic stroke in animal experiments and clinical trials for their advantages, such as easy collection, lack of ethical issues, pluripotency, and safely transplanted. MSCs transplantation was found to inhibit microglia activation, secrete growth factors, enhance angiogenic factor expression and vascular density, reduce scar size, limit apoptosis, and exert beneficial function on neurological recovery after ischemic brain injury in rats<sup>[11]</sup>. In the clinic, a long-term follow-up study for 5 years study of 16 patients showed that MSCs intravenous injection decreased modified Rankin score (mRS)<sup>[90]</sup>. Another study of 40 stroke patients by Bhasin group showed statistically significant improvement in modified Barthel Index (mBI) in stem cell group 6 months post-stroke<sup>[91]</sup>. MSCs also improved mBI at 39 and 52 months after transplantation<sup>[92]</sup>. Despite the efficacy of MSCs proved in some clinical trials, the results from different clinical trials are partly contradictory (Table 1). For instance, a small clinical trial of 5 patients with acute middle cerebral artery (MCA) infarction by Band group reported a better Barthel index (BI) at 3 or 6 months but not at 12 months post-stroke when the patients received intravenous injection with autologous MSCs. In addition, MSCs transplantation did not improve mRS at 3, 6, 12 months post-stroke<sup>[93]</sup>. A randomized blinded phase II clinical trial showed at 6 months, there is no difference in BI, mRS, NIHSS and infarct volume between treatment group and control group<sup>[94]</sup>. In this study, 120 sub-acute stroke patients were enrolled, among them, 58 received  $2.8 \times 10^8$  MSCs intravenously injected at a medium of 18.5 days post-stroke.

BM-MNCs are another type of stem cells widely used in clinical trials. In 2012, a prospective clinical trial was performed by Friedrich group. In this study, 20 patients with

acute MCA infarct, spontaneous recanalization but persistent deficits were enrolled. BM-MNCs were intra-arterially injected between 3 and 7 days after stroke onset. At 3 months, clinical improvement occurred in 30% patients<sup>[95]</sup>. In the same year, a single-blinded (outcomes assessor) controlled Phase I/II trial was performed and a total of 20 MCA infarction patients were enrolled. Ten of them received MNCs injection. Results showed that BM-MNCs enhanced  $\beta$ -Nerve growth factor ( $\beta$ -NGF), however, did not improve neurological function at 6 months<sup>[96]</sup>. In 2015, a Phase I/IIa clinical Trial enrolled 12 severe embolic stroke patients<sup>[97]</sup>. At 6 months, intravenous MNCs enhanced cerebral blood flow (CBF), metabolic rate of oxygen consumption and neurologic outcomes. Another randomized, controlled, dose-finding, multicenter trial, IBIS, has sought to further test the efficacy of MNCs. This trial has just started the recruitment phase<sup>[98]</sup>. The findings are summarized in Table 1.

Besides mesenchymal stem cells, recently, immortalized human neural stem-cell lines were also used in clinical trials. Kalladka D enrolled 13 patients, among them, 11 received cell transplantation, and results showed that stem cell therapy with single intracerebral doses of up to 20 million cells significantly improved neurological function and was not associated with adverse events<sup>[99]</sup>.

### Neural progenitor cells (NPCs) transplant

Neural progenitor cells (NPCs) derived from adult brain and embryonic/fetal tissues can differentiate into neurons, astrocytes, or oligodendrocytes<sup>[100-102]</sup>. The transplantation of NPCs into brains with cerebral infarction increased dendritic length and the number of branch points and improved sensorimotor function<sup>[103]</sup>. These beneficial effects are thought to be associated with the secretion of trophic factors such as BDNF<sup>[104]</sup>, vascular endothelial growth factor (VEGF)<sup>[105, 106]</sup>, glial cell derived neurotrophic factor (GDNF)<sup>[107]</sup>, basic fibroblast growth factor (FGF-2)<sup>[108, 109]</sup> and others by transplanted NPCs. Despite numerous animal experiments, the efficacy of progenitor cells on brain repair after stroke still need to be determined in more clinical trials. Recently a phase 2, randomized, double-blind, placebo-controlled, dose-escalation trial of intravenous NPCs was performed in 33 centers in the UK and the USA. Patients aged 18–83 years with moderately severe acute ischemic stroke were enrolled to treatment with intravenous NPCs (400 million or 1200 million cells) between 24 h and 48 h after symptom onset. Results showed that NPCs showed good efficacy in improvements in clinical functional scores (mRS and NIHSS score) and reductions in lesion volume<sup>[110]</sup>.

### Neuronal precursors transplant

Collective evidence showed that neuronal precursor cells improved animal survival following ischemic brain injury. Grafted neuronal precursor cells in ischemic stroke rats survived 3 months after transplantation and differentiated into neurons of diverse neurotransmitter-subtypes and the surviving neurons exhibit electrophysiological properties and ability to fire action potentials<sup>[111]</sup>. An independent group further

Table 1. Cell transplantation for ischemic stroke therapy (clinical trials in the recent five years).

Author	Name of Trial	Design	Patient	Cells	Time	Dosage	Deliver	Follow-up	Efficacy	Adverse Effects
Hess, 2017 [110] USA	Safety and efficacy of multipotent adult progenitor cells in acute ischemic stroke (MASTERS) (NCT 01436487)	RCT	Acute n=129 (n=67) Age: 18–83 Y	MAPC	24–48 h	4×10 <sup>6</sup> 1.2×10 <sup>8</sup>	IV	3 m	No	Safe
Bhasin [92] 2017, Indian	Safety and Feasibility of Autologous Mesenchymal Stem Cell Transplantation in Chronic Stroke in Indian patients. A four-year follow up	open-label	Chronic n=12 (n=6) Mean age: 42.8 Y	MSCs	3 m–2 y	N/A	IV	52 m	Clinical outcomes ↑	Safe
Steinberg [147] 2016 USA	Clinical Outcomes of Transplanted Modified Bone Marrow-Derived Mesenchymal Stem Cells in Stroke: A Phase 1/2a Study (NCT 01287936)	Open-label, single-arm	Stable, chronic stroke n=18 Mean age: 61 Y	BMSCs	6–60 m	2.5×10 <sup>6</sup> , 5.0×10 <sup>6</sup> , 10×10 <sup>6</sup>	Peri-infarct	24 m	ESS, NIHSS, F-M total score ↑	Emergent adverse event ↑
Kalladka, [99] 2016 UK	Human neural stem cells in patients with chronic ischemic stroke (PISCES): a phase 1, first-in-man study (NCT 01151124)	Open-label	Stroke n=13 (n=11) Age: ≥60	CTX0E03	6–60 m	2×10 <sup>6</sup> , 5×10 <sup>6</sup> , 10×10 <sup>6</sup> , 20×10 <sup>6</sup>	Putamen	≥24 m	NIHSS ↓	Hyperintensity in brain ↑
Taguchi, [148] 2015 Japan	Intravenous Autologous Bone Marrow Mononuclear Cell Transplantation for Stroke: Phase 1/2a Clinical Trial in a Homogeneous Group of Stroke Patients	open-label study	Severe embolic stroke n=12, Age: 20–75	BM-MNCs	7–10 d	2.5×10 <sup>8</sup> , 3.4×10 <sup>8</sup>	IV	6 m	Clinical outcomes ↑	Safe
Moniche [98] 2015 Spain	IBIS trial (NCT 02178657)	RCT	MCA infarction n=76 (n=38) Age: 18–80	BM-MNCs	1–7 d	2×10 <sup>6</sup> 5×10 <sup>6</sup>	IAT	6–24 m	Going on	N/A
Qiao [149], 2014 China	A two-year follow-up study of cotransplantation with neural stem/progenitor cells, mesenchymal stromal cells in ischemic stroke patients	Case report	MCA or ACA infarction n=6 Age: 3–85	NSPCs or MSCs	1 w to 2 y	1) MSCs 0.5×10 <sup>6</sup> *4 2) NSPCs 6×10 <sup>6</sup> *3	CMCI	24 m	Clinical outcomes ↑	Low fever, dizziness
Prasad [150] 2014 India	Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial (NCT 0150177)	RCT	Subacute n=120 (n=58) Age: N/A	BMSCs	18.5 d (medium)	2.8×10 <sup>8</sup> (Mean)	IV	6 m	No	Safe
Moniche [151] 2014 Spain	Intra-arterial bone marrow mononuclear cell transplantation correlates with GM-CSF, PDGF-BB, and MMP-2 serum levels in stroke patients: results from a clinical trial	RCT	Subacute MCA stroke n=17 (n=8)	BM-MNCs	5–9 d	1.59×10 <sup>8</sup>	IAT	6 m	No	N/A

(To be continued)

Author	Name of Trial	Design	Patient	Cells	Time	Dosage	Deliver	Follow-up	Efficacy	Adverse Effects
Diez-Tejedor [152] 2014 Spain	Reparative therapy for acute ischemic stroke with allogeneic mesenchymal stem cells from adipose tissue: a safety assessment: a phase II randomized, double-blind, placebo-controlled, single-center, pilot clinical trial	RCT	Acute stroke n=20 (n=10)	MSCs	≤2 w	N/A	IV	24 m	Going on	N/A
Chen[153], 2014, China Taiwan.	Intracerebral implantation of autologous peripheral blood stem cells in stroke patients: a randomized phase II study	Randomized Controlled Trial	MCA infarction n=30 (n=15)	PBSCs	6 m-5 y	(3-8)×10 <sup>6</sup>	N/A	12 m	Clinical outcomes ↑	Safe
Banerjee [154] 2014 UK	Intra-Arterial Immunoselected CD34 <sup>+</sup> Stem Cells for Acute Ischemic Stroke	Prospective, open-label,	Severe anterior circulation ischemic stroke n= 5 NIHSS ≥8	Autologous CD34 <sup>+</sup> selected stem/progenitor cell	≤7 d	1×10 <sup>8</sup>	IAT	6 m	Clinical outcome ↑	Safe
Kim [155] 2013 South Korea	Intravenous transplantation of mesenchymal stem cells preconditioned with early phase stroke serum: current evidence and study protocol for a randomized trial STARTING-2 (NCT 01716481)	PROBE	Acute and chronic stroke n=60 (n=40) Age: 30-75	MSCs	≤90 d	N/A	IV	3 m	Going on	N/A
Jiang [156] 2013 China	Feasibility of delivering mesenchymal stem cells via catheter to the proximal end of the lesion artery in patients with stroke in the territory of the middle cerebral artery	Case report	Ischemic stroke n=3	UCMSCs	90-180 d	2×10 <sup>7</sup>	IAT	6 m	Clinical outcomes ↑	Safe
Chen [157] 2013 China	Multiple cell transplantation based on an intraparenchymal approach for patients with chronic phase stroke	Case report	Ischemic stroke n=6 Age: 42-87	OECs NPCs UC-MSCs SCs	6 m- 20 y	OECs (1.0-2.0)×10 <sup>6</sup> ; NPCs: (2.0-5.0)×10 <sup>6</sup> ; SCs: 2.0×10 <sup>6</sup> ; UCMSCs (1-2.3)×10 <sup>7</sup>	intracranial	24 m	Clinical outcomes ↑	N/A
Bhasin [91] 2013 India	Stem cell therapy: a clinical trial of stroke	Prospective	Stroke, n=40	MSCs	3 m-2 y	(5-6)×10 <sup>7</sup>	intravenously	6 m	mBI ↑	Safe
Prasad [158] 2012 India	Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischemic stroke: a pilot study	Non-randomized phase I clinical study	Subacute ischemic stroke n=11	MNCs	7-30 d	8×10 <sup>7</sup>	intravenously	13 m	Clinical outcome ↑ Neurological function ↑	Safe Seizure

(To be continued)

Author	Name of Trial	Design	Patient	Cells	Time	Dosage	Deliver	Follow-up	Efficacy	Adverse Effects
Moniche [96] 2012	Intra-arterial bone marrow mononuclear cells in ischemic stroke: a pilot clinical trial (NCT 00761982)	A single-blind Phase I/II trial	MCA stroke n=20 (n=10)	BM-MNCs	5–9 d	1.59×10 <sup>8</sup>	Intra-arterially	6 m	Clinical outcomes†	Safe
Spain Friedrich [95] 2012	Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke	Prospective	Acute MCA infarcts n=20	BM-MNCs	3–7 d		IAT	6 m		

Abbreviation: BDNF, brain-derived neurotrophic factor;  $\beta$ -NGF,  $\beta$ -nerve growth factor; BMSCs, Modified Bone Marrow-Derived Mesenchymal Stem Cells; BI, Barthel index; CBF, cerebral blood flow; CMCI, cerebellomedullary cistern injection; DTI, diffusion tensor image; ESS, European Stroke Scale; FLAIR, Fluid-attenuated inversion recovery; F-M: Fugl-Meyer total score; FNA, fiber numbers asymmetry; GM-CSF, granulocyte-macrophage colony-stimulating factor; IAT, Intra-arterial therapy; IV, Intravenous; MAPC, multipotent adult progenitor cell; MCA, middle cerebral artery; MEP, motor-evoked potential; MMP-2, matrix metalloproteinase 2; MMP-9, matrix metalloproteinase 9; mRS, modified Rankin Scale; MRI, magnetic resonance imaging; MSC, Mesenchymal stem cells; N/A, not available; NIHSS, National Institutes of Health Stroke Scale; NPCs, Neural progenitor cells; OECs, Olfactory ensheathing cells; PDGF-BB, platelet-derived growth factor-BB; PROBE, Prospective, randomized, open-label, blinded-endpoint; SCs, Schwann cells; TMS, transcranial magnetic stimulation; UCMSCs, Umbilical cord mesenchymal stromal cells; VEGF, vascular endothelial growth factor.

showed that cell transplantation increased neurogenesis in the ipsilateral SVZ in both young adult (3 months old) and aged (24 months old) rats with focal cerebral ischemia<sup>[112]</sup>, and improve sensory recovery after ischemic stroke<sup>[113]</sup>. Unfortunately, there is no clear clinical evidence to demonstrate the efficacy of neuronal precursors on cerebral ischemia recovery<sup>[92]</sup>.

Despite significant improvements found in some case reports, prospective studies and single arm studies, no clear difference has been found in randomized, controlled, blinded clinical trials (RCT). These differences could be due, at least in part, to the diverse designs of experiments, such as the dose, route of administration, initial time of stem cells therapy, severity of disease, choice of neurological evaluation scales and design types of clinical trials. Yet it is possible that the limited capacity for neurogenesis in humans is the primary reason for the failure of stem cells on cerebral ischemia recovery in RCT clinical trials. Therefore, there is a pressing need to provide novel measures to improve the cell-based therapies for ischemic stroke. Because the consecutive process of cell-based therapy includes neuronal differentiation, migration, survival and functional connection, more effective measures need to be developed to successfully go through the full process and help us get out of the plight.

### Drugs assisting stem cell-based therapy

Neurogenesis following ischemic stroke has been considered as a potential mechanism for neuronal restoration, however, endogenous neurogenesis by itself is insufficient for effective brain repair as most newborn neurons do not survive. Replenishment of stem cells does not perfectly solve the problem of neurogenesis and neurofunction restoration following ischemic stroke insults. Mobilization, promotion of migration, improvement of differentiation of neural stem/progenitor cells, and promotion of connection of newly-developed mature neurons may be a potential way for brain repair. In view of the clinical transformation of exogenous cells is not easy, using pharmacological drugs to improve stem cell-based therapies has recently become a new focus. In this section, we will discuss the drugs that promote neurogenesis via different ways in recent studies (Figure 1). Some drugs enhancing neurogenesis and improving neurological outcomes in animal experiments are shown in Table 2.

### Chemical drugs

#### Proliferation of new stem cells

The proliferation of stem cells was the first step of neurogenesis. Neuroinflammation, neurotrophic factors and apoptosis-related signal pathway are involved in the process of proliferation (Figure 1).

#### Modulation of neuroinflammation

C-X-C chemokine receptor type 4 (CXCR4) is a receptor for a pleiotropic chemokine CXCL12. CXCR4 antagonist AMD3100 and CX549 mobilized bone marrow hematopoietic stem cells (HSCs) for transplantation by reducing neuroinflammation in stroke brain<sup>[114]</sup>. The neural cell adhesion molecule-derived



Table 2. Promotion neurogenesis with drugs after ischemic stroke.

Intervention	Related mechanism	Therapeutic effects	Neurological outcome
EGCG (predominant constituent of green tea) Zhang, Xu et al 2017 [84]	M2 phenotype of microglia	Proliferation of SVZ NPCs↑ Migration of SVZ neuroblasts↑	Functional recovery↑
MC-2J (the anti-CCR2 antibody), Laterza, et al, 2017 [159]	Depletion of circulating monocytes; Reduced astrocyte activation in SVZ and adjacent striatum	Enhances striatal neurogenesis at one week post-insult, most likely by increasing short-term survival of the newly formed neuroblasts in the SVZ and adjacent striatum.	
Bumetanide (a selective Na <sup>+</sup> -K <sup>+</sup> -Cl <sup>-</sup> -co-transporter inhibitor), Xu, Mu et al, 2017 [160]	Effects on inflammation----	Migration of neuroblasts in the SVZ towards the infarct area↑ Long-term survival of newborn neurons↑	Sensorimotor recovery↑
6-Bromoindirubin-3'-oxime (BIO)(GSK3β specific inhibitor), Wang, Li et al, 2017 [161]	N/A	Generation of neuroblasts in the SVZ↑ Neuroblasts migrated to the peri-infarct region↑ Newly formed neurons ↑	Sensorimotor recovery↑
Guanosine (GUO), Deng, Qiu et al, 2017 [162]	BDNF, VEGF↑	Neurogenesis and angiogenesis ↑	Functional recovery↑
4αlpha-PDD (TRPV4 agonist), Chen, Hsu et al, 2017 [163]	eNOS expression and phosphorylation (serine 1177) ↑	NPC proliferation ↑ NPC migration in the ischemic hemisphere ↑	Functional outcomes on day 5↑
Fluoxetine ①, Sun, Zhou et al, 2016 [164]	N/A	Proliferation of newborn neurons in the SVZ↑ SGZ ---- Perilesional apoptosis ↓ Survival or differentiation of newly generated cells in the SVZ----	Behavioral outcome ----
Fluoxetine ②, Sun, Sun et al, 2015 [165]	N/A	Neuroblasts in both the SVZ and DG↑ Dendritic complexity of newborn dentate granule cells↑ Survival or differentiation of newly generated cells----	Sensorimotor recovery ----
Lipo-PGE4, Ling, Zhang et al, 2016 [166]	N/A	Proliferation↑ Migration of endogenous neural stem cells in the ipsilateral SVZ ↑	Neurological recovery ↑
100K/bFGF①, Li, Tsai et al, 2016 [167]	N/A	NSPCs proliferation↑ MAP-2 cells ↑ GFAP cells at the SVZ area and in the infarcted regions ----	Motor coordination ↑
bFGF②, Wang, et al 2008[168]	N/A	Infarct size ---- Proliferation of progenitor cells in the subventricular zone and the subgranular zone of the dentate gyrus (DG) ↑	Neurobehavioral recovery ↑
ABAH (MPO inhibitor), Kim, Wei et al, 2016 [169]	BDNF, Phosphorylation of cAMP response element-binding protein (Ser 133)↑, Acetylated H3 ↑, Chemokine CXCR2 receptor 4↑	Neural stem cells ↑ Astrocytes↑ Neuroprogenitor cells↑ Neuroblasts ↑ in the ischemic SVZ, anterior SVZ striatum, and cortex	N/A
Progesterone Intervention, Jiang, Zuo et al, 2016 [170]	VEGF↑, BDNF↑	Newly generated neurons in the SVZ↑ Neuroblast cells in the peri-infarct region ↑	Neurologic function on days 7 and 14 post-occlusion ↑
MB, Ahmed, Tucker et al, 2016 [171]	Reactive gliosis↓, pro-inflammatory ↓, cytokines cytochrome c oxidase activity↑, ATP production in peri-infarct regions↑	Cell proliferation and neurogenesis in the peri-infarct zone ↓	Neurological deficits ↓

(To be continued)

Intervention	Related mechanism	Therapeutic Effects	Neurological outcome
I-NBP, Yang, Li <i>et al</i> 2015 [119]	PKA↑, Akt↑, CREB↑, STAT3↓, cleaved Caspase-3↓, Bax↓	Neurogenesis (DG) ↑ Newborn cells and newly Mature neurons ↑	Behavioral recovery ↑
VIP, Yang, Shi <i>et al</i> 2015 [172]	VEGF in the SVZ ↑	Stem cells and neuroblast in the SVZ at 7, 14 and 28 days after ischemia ↑	Neurological severity score ↓ infarct volume ↓ Learning, memory ↑
Melanocortin, Giuliani, Zaffe <i>et al</i> 2011 [173]	Wnt-3A signaling pathways ↑	Stem cells co-localized with NeuN (used as indicator of mature neurons) and Zif268 (used as indicator of functionally integrated neurons) Day 50 post-stroke in the DG ↑	
Ginsenoside Rd 5, Liu, Zhou <i>et al</i> 2015 [174]	p-Akt ↑, VEGF↑, BDNF↑, P-ERK↑, PC12 cell apoptosis ↓	BrdU/DCX and Nestin/GFAP double-positive cells in ischemic area ↑	N/A
Copolymer-1 (COP-1) (Glatiramer acetate), Cruz, Lorea <i>et al</i> 2015 [175]	NT-3 ↑	Neurogenesis (at 7 and 60 days) in the SVZ, SGZ, and cerebral cortex ↑	Neurological outcome ↑
Huang-Lian-Jie-Du-Decoction (HLJDD) (TCM), Zou, Long <i>et al</i> 2016 [139]	VEGF, Ang-1, Ang-2 ↑ phosphorylation of AKT, and GSK-3beta ↓	Alkaloids and Iridoids: neuronal differentiation in the cortex ↑ Alkaloids: neurogenesis ↑	N/A
Huatuo Zaizao pill (TCM), Duan, Wang <i>et al</i> 2017 [140]	BDNF, phosphorylated PKA, CREB ↑	Neurogenesis ↑	Functional recovery ↑
Tongxinluo (TCM), Chen, Wang <i>et al</i> 2016 [176]	N/A	Neurogenesis ↑ angiogenesis in the peri-infarct area and SVZ ↑	Neurological function deficit ↑
Danggui-Shaoyao-San (TCM), Ren, Wang <i>et al</i> 2015 [142]	vascular endothelial growth factor ↑ eNOS phosphorylation ↑	Microvessel density in the peri focal region ↑ Stem cells and neuroblast in the SVZ ↑	Neurobehavioral outcomes ↑

Abbreviation: ABAH, 4-aminobenzoic acid hydrazide; Akt, protein kinase B; Ang-1, Angiopoietin-1; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BrdU, 5-bromo-2'-deoxyuridine; CREB, cAMP response element-binding protein; DCX, doublecortin; DG, dentate gyrus; EGCG, Epigallocatechin-3-gallate; GDNF, Glial cell-derived neurotrophic factor; HBM-MSC, Human bone marrow stem cell; H<sub>2</sub>S, hydrogen sulfide; L-NBP, L-3-n-butylphthalide; MB, methylene blue; N/A, non-available; NT-3, neurotrophin 3; NPC, neural progenitor cell; PKA, protein kinase A; PGE<sub>1</sub>, Prostaglandin E<sub>1</sub>; SGZ, subgranular zone; STAT3, signal transducer and activation of transcription 3; SVZ, subventricular zone; TCM, Traditional Chinese Medicine; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide. ↑: enhanced or improved; ---: no change; ↓: decreased.

peptide FG loop significantly increased endogenous NSC mobilization in the neurogenic niches, which is associated with the modulation of the activation of microglia and modulation of neuroinflammation<sup>[115]</sup>. rIL-6 significantly increased the proliferation of NPCs in the ipsilateral SVZ<sup>[116]</sup>.

#### Modulation of neurotrophic factors

Salvianolic acids for injection (SAFI) promoted the proliferation of NPCs, enhanced the number of surviving newborn neurons in the SVZ and led to the improvement of neurological outcome. In addition, SAFI activated sonic hedgehog-Patched-Gli (Shh-Ptch-Gli) signal pathway and induced the production of BDNF and NGF. The beneficial effect of SAFI was abolished by Cyclopamine (CYC) significantly through decreasing BDNF and NGF level. These data indicated that SAFI significantly improved long-term functional recovery by enhancing BDNF and NGF production and promoting neurogenesis<sup>[117]</sup>. Besides SAFI, in another study, cystamine was also found to significantly enhance neuronal progenitor cell proliferation and plasticity through BDNF/TrkB pathway after stroke<sup>[118]</sup>.

#### Modulation of apoptosis-related signal pathway

L-3-*n*-butylphthalide (L-NBP) was found to markedly increase 5-bromo-2'-deoxyuridine (BrdU)-positive cells in the hippocampal dentate gyrus (DG) on day 28 after ischemia by activating CREB and Akt and inhibiting STAT3 signaling<sup>[119]</sup>. Sodium ferulate (SF) and *n*-butylidenephthalide (BP) combined with BMSC can significantly improve neurogenesis following stroke through the enhancement of VEGF and BDNF expressions and activation of AKT/mTOR signal pathway<sup>[120]</sup>.

3K3A-APC (3K3A-activated protein C) has been demonstrated to stimulate transplanted NSCs to neurons and promote neurological recovery via a protease-activated receptor-1 (PAR1)-protease-activated receptors(PAR3)-sphingosine-1-phosphate-receptor 1 (S1PRs)-Protein Kinase B (Akt) pathway *in vitro*<sup>[121]</sup>.

Polyphenol ellagic acid (EA) was found to enhance the proliferation of NSCs and the content of nestin protein in the brain semidarkness zone through the Wnt/beta-catenin signaling pathway<sup>[122]</sup>.

Tat-NR2B9c, a peptide disrupting the *N*-methyl-*D*-aspartate receptor-postsynaptic density protein-95 interaction, substantially increased neurogenesis in the dentate gyrus by reversing the ischemia-induced formation of *S*-nitrosylation-cyclin-dependent kinase 5 and increasing cyclin-dependent kinase 5 (CDK5) activity in the ipsilateral hippocampus<sup>[123]</sup>.

#### **Enhancement of migration of NPCs**

##### Neurotrophic factors

BDNF enhanced the recruitment of NPCs into the lesioned site after ischemic stroke. Atorvastatin, a chemical that activates the expression of BDNF, enhanced migration of SVZ cells<sup>[124]</sup>. Similarly, overexpression of BDNF through gene therapy via Adeno-associated virus (AAV) infection facilitated endogenous NPC migration from the SVZ<sup>[125]</sup>.

##### PI3K/AKT signal pathway

Tetramethylpyrazine (TMP) was found to promote NPC migration, this effect was reversed by inhibiting the molecular, such as phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK). These data show that the PI3K/AKT signaling pathway is involved in the process of migration of NPCs<sup>[126]</sup>.

##### **Promoting cell differentiation**

nNOS-PSD-95 was found to be involved in the course of differentiation during ischemic stroke. A small-molecular inhibitor of nNOS-PSD-95 interaction, SCR-4026, was found to promote neural stem cells to differentiate into neuron-like cells<sup>[127]</sup>. Complement-derived peptide C3a regulates neural progenitor cell migration and differentiation *in vitro*<sup>[128]</sup>.

##### **Enhancing the survival of stem cells**

Transplantation of cells is a promising strategy for neuroregeneration, however cell survival is one of the key barriers to the success of cell implantation treatment. Studies have explored the protective effects of pharmacological agent preconditioning to enhance the viability of stem cells.

##### Modulation of neuroinflammation

Cerebral ischemia stimulated inflammatory processes and affected NSCs in multiple ways. Drugs modulating neuroinflammation are likely to provide neuroprotection during neurogenesis.

5-Fluorouracil (5-FU) pre-treatment enhanced the viability of transplanted bone marrow mononuclear cells (BMMNCs) in the hippocampus, which was found to be associated with the increased microvessel density (MVD), reduced levels of pro-inflammatory cytokines and increased levels of growth factors in the penumbra. These results indicate that 5-FU improve the local microenvironment and increase number of viable cells<sup>[129]</sup>.

Quercetin is another drug found to be effective in improving the survival rate of human umbilical cord mesenchymal stromal cells (HUMSCs) in the injury site after local cerebral ischemia. The protective mechanisms include reducing proinflammatory cytokines (IL-1 $\beta$  and IL-6), increasing anti-inflammatory cytokines (IL-4, IL-10, and transforming growth factor- $\beta$ 1) and inhibiting cell apoptosis (caspase-3 expression)<sup>[130]</sup>.

##### Apoptosis-related signal pathway

Although stroke stimulates the proliferation of NPCs, most of these cells die after injury. Alleviating apoptosis is a major way to improve the survival of stem cells. A number of anti-apoptotic drugs were found to improve the survival of neurons via a variety of signaling pathways.

The tolerance of HWJ-MSC-derived neural-like cells was improved when they are preconditioned with deferoxamine (DFO). The tolerance may be due to the increase of HIF-1, BDNF, pAkt-1 and decrease of Bax/Bcl-2 ratio<sup>[131]</sup>.

Pifithrin-a (PFT-a), a p53 inhibitor, starting from day 6 after MCAO, was found to enhance the survival of endogenous

NPCs in the SVZ. These data suggest that inhibition of p53 may extend the survival of endogenous NPCs after stroke.

Ginsenoside Rg1 prevents NSCs from oxygen-glucose deprivation (OGD) insult through inhibiting oxidative stress and the activity of p38/JNK2 signaling way in NSCs<sup>[132]</sup>.

#### **Relieve neuronal morphological damage**

Noggin is a signaling molecule involved in embryonic development. Grafting NSCs modified by noggin gene reduced the percentage of apoptotic neurons and relieved neuronal morphological damage, which was accompanied by the decrease of the MDA levels, the SOD activity, and downregulation of the bone morphogenesis protein 4 (BMP4), VEGF, and bFGF proteins<sup>[133]</sup>.

#### **Promote the functional connection**

Establishment of cell-cell interaction is considered to be crucial after stem cell transplantation. However, in recent studies, the functional connection, which is the key step for successful neurogenesis and was regarded as important for the repair of host brain architecture, is seldom considered. In order to promote the functional communication of neuron, the following methods may be useful in future studies. First, use of cell sheet as opposed to cell suspension. Previous transplant approaches have utilized injection of the cells in a cell suspension; however, these cells cannot establish a connection to the damaged tissues. However, the cell sheet was supposed to maintain cell-cell interactions and improve neurological functions<sup>[134]</sup>. Second, sensory stimuli promoted pluripotent stem cell-derived cortical neurons to incorporate into injured cortical circuitry and contribute to functional recovery in stroke<sup>[135]</sup>.

#### **Multiple functions on neurogenesis**

Some pharmacological agents serve multiple functions in neurogenesis. For instance, after cerebral ischemia, IL-1Ra was found to increase stem cell proliferation, enhance neuroblast migration and promote the survival of newly born neurons<sup>[136]</sup>. IL-17A, secreted by astrocytes, augments survival of SVZ neural precursor cells (NPCs), neuronal differentiation and synaptogenesis via p38 MAPK/calpain 1 signaling pathway after ischemic stroke<sup>[137]</sup>. Indomethacin, a modulator of microglia activation, contributed to increased neuroblast proliferation in the SVZ and migration to the ischemic striatum following stroke<sup>[138]</sup>.

#### **Herbal medicine**

Epigallocatechin-3-gallate (EGCG), the predominant constituent of green tea, was found to increase proliferation of SVZ NPCs and migration of SVZ neuroblasts, improve functional recovery, and attribute to the M2 phenotype induction in microglia<sup>[84]</sup>.

Huang-Lian-Jie-Du-Decoction (HLJDD) is broadly used in Traditional Chinese Medicine (TCM) and shown to enhance neurogenesis. The main ingredients of HLJDD are alkaloids and iridoids. Alkaloids and iridoids enhance the level of VEGF, Ang-1, Ang-2, phosphorylation of AKT, and GSK-

3beta, increasing the number of BrdU-positive cells<sup>[139]</sup>.

Huatuo Zaizao pill (HT), a widely used TCM in clinic for the treatment of cerebrovascular disease, was also found to effectively enhance neurogenesis. HT treatment for 3 days increased neurogenesis in cerebral ischemia reperfusion animal models, and its effects may be associated with the increase of BDNF mRNA, PKA, and phosphorylated CREB<sup>[140]</sup>.

Tongxinluo was shown to enhance neurogenesis and angiogenesis in the peri-infarct area and SVZ, which partly contributes to the amelioration of the neurological function deficit<sup>[141]</sup>.

Danggui-Shaoyao-San (DSS) treatment significantly activated vascular endothelial growth factor, enhance microvessel density in the perifocal region, increased the numbers of BrdU<sup>+</sup>/DCX<sup>+</sup> cells in the SVZ and improved neurobehavioral outcomes<sup>[142]</sup>.

Buyang Huanwu Decoction (BYHWD) could markedly facilitated stem cell migration by increasing the expression of neurotrophic factors, such as stromal cell-derived factor-1, vascular endothelial growth factor, reelin, and BDNF in the ipsilateral infarct area after MCAO<sup>[143]</sup>.

#### **Enhance neurogenesis with GCSF**

Stimulating the proliferation of neural stem/progenitor cells is another method to be used to improve neurobehavioral functions. Granulocyte colony-stimulating factor (GCSF), a glycoprotein that stimulates the bone marrow to produce and release granulocytes and stem cells into the bloodstream, has been considered as a promising cytokine to promote neurogenesis in ischemic stroke mice. A pre-clinical trial performed by Kawada in 2006 determined the role of GCSF in stimulating the proliferation of intrinsic neural stem/progenitor cells<sup>[144]</sup>. A randomized, blinded controlled trial enrolled 10 patients (7 for GCSF therapy) found that GCSF improved neurologic functioning (NIHSS, ESS, EMS, and BI) and fluorodeoxyglucose in the area surrounding the core<sup>[145]</sup>. To further determine the efficacy of GCSF on ischemic stroke, in 2016, a randomized controlled multicenter phase II trial enrolled more patients (49 patients, among them, 40 patients received GCSF therapy). However, this study found that GCSF neither improved functional recovery (NIHSS, ESS, EMS, and BI) nor reduced infarct volume<sup>[146]</sup> (Table 3). These results indicate that successful neurogenesis includes multiple steps, besides proliferation, other steps such as migration, differentiation, survival of mature neurons and functional connections are also critical. Pharmacological drugs targeting multiple steps of neurogenesis are potential ways to improve neurogenesis and neurological outcomes of ischemic stroke patients.

#### **Conclusion and future directions**

Neurogenesis after stroke has been considered as an important mechanism for functional recovery. Numerous studies of animal experiments, prospective or pilot clinical trials and single arm trails showed that stem cell transplantation therapy improved neurological function. However, no clear evidence has validated the role of cell-transplantation therapy in improving stroke outcome from multicenter, large sample,



**Table 3.** Pre-clinical or clinical trials for endogenous neurogenesis.

Author Country	Trial	Design	Patients No. Total (Therapy)	Time (post-stroke)	Dosage Route	Follow-Up (month)	Efficacy	Adverse Effects
Shyu [145] 2006 Canada	Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial	Random-ized, blinded controlled trial	n=10 (n=7)	≤7 d	15 mg/kg per day*5 d, SC	12 m	Improve neuro-logic function-ing.	Safe
Mizuma [146] 2016 Japan	Intravenous Low-Dose Granulocyte Colony-Stimulating Factor in Acute Ischemic Stroke	Random-ized Controlled Trial	n=49 (n=40)	≤24 h	150,300 mg/d * 5 d, SC.	3 m	Did not improve functional re-covery	Safe

MCA: middle cerebral artery; G-CSF, Granulocyte colony-stimulating factor; SC, Subcutaneous.

randomizes, controlled, and blinded clinical trials. Pharmacological drugs may enhance the efficacy of cell-transplantation therapy by promoting the proliferation, migration, differentiation, survival of newborn neuron and the function connection. Thus, using pharmacological drugs in combination with cell-based therapy can be a potential strategy to improve the post-stroke outcomes in clinical trials. Many drugs and herbal compounds that have been tested in animal models. Future studies could consider to selectively test these drugs and herbal compounds in well-designed randomized clinical trials.

### Acknowledgements

This work was supported by Canadian Institutes of Health Research (CIHR) China-Canada Joint Health Research Initiative to Hong-shuo SUN (CIHR, FRN #132571), and Canadian Institutes of Health Research to Zhong-ping FENG (PJT-153155). Vivian SZETO holds a Canadian Graduate Scholarship from Natural Sciences and Engineering Research Council of Canada (NSERC-CGS-M).

### References

- Feigin VL, Norrving B, Mensah GA. Global burden of stroke. *Circ Res* 2017; 120: 439–48.
- Hawkins KE, DeMars KM, Alexander JC, de Leon LG, Pacheco SC, Graves C, *et al*. Targeting resolution of neuroinflammation after ischemic stroke with a lipoxin A4 analog: protective mechanisms and long-term effects on neurological recovery. *Brain Behav* 2017; 7: e00688.
- Yu G, Wu F, Wang ES. BQ-869, a novel NMDA receptor antagonist, protects against excitotoxicity and attenuates cerebral ischemic injury in stroke. *Int J Clin Exp Pathol* 2015; 8: 1213–25.
- Sun HS, Xu B, Chen W, Xiao A, Turlova E, Alibraham A, *et al*. Neuronal  $K_{ATP}$  channels mediate hypoxic preconditioning and reduce subsequent neonatal hypoxic-ischemic brain injury. *Exp Neurol* 2015; 263: 161–71.
- Sun HS, Jackson MF, Martin LJ, Jansen K, Teves L, Cui H, *et al*. Suppression of hippocampal TRPM7 protein prevents delayed neuronal death in brain ischemia. *Nat Neurosci* 2009; 12: 1300–7.
- Sun HS, Doucette TA, Liu Y, Fang Y, Teves L, Aarts M, *et al*. Effectiveness of PSD95 inhibitors in permanent and transient focal ischemia in the rat. *Stroke* 2008; 39: 2544–53.
- Tan Z, Li X, Turner RC, Logsdon AF, Lucke-Wold B, DiPasquale K, *et al*. Combination treatment of r-tPA and an optimized human apyrase reduces mortality rate and hemorrhagic transformation 6 h after ischemic stroke in aged female rats. *Eur J Pharmacol* 2014; 738: 368–73.
- Alia C, Spalletti C, Lai S, Panarese A, Lamola G, Bertolucci F, *et al*. Neuroplastic changes following brain ischemia and their contribution to stroke recovery: novel approaches in neurorehabilitation. *Front Cell Neurosci* 2017; 11: 76.
- Cocks G, Carta MG, Arias-Carrion O, Nardi AE. Neural plasticity and neurogenesis in mental disorders. *Neural Plast* 2016; 2016: 3738015.
- Cheatwood JL, Emerick AJ, Kartje GL. Neuronal plasticity and functional recovery after ischemic stroke. *Topics Stroke Rehabil* 2008; 15: 42–50.
- Wu J, Sun Z, Sun HS, Wu J, Weisel RD, Keating A, *et al*. Intravenously administered bone marrow cells migrate to damaged brain tissue and improve neural function in ischemic rats. *Cell Transplant* 2008; 16: 993–1005.
- Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992; 255: 1707–10.
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, *et al*. Neurogenesis in the adult human hippocampus. *Nat Med* 1998; 4: 1313–7.
- Pincus DW, Harrison-Restelli C, Barry J, Goodman RR, Fraser RA, Nedergaard M, *et al*. *In vitro* neurogenesis by adult human epileptic temporal neocortex. *Clin Neurosurg* 1997; 44: 17–25.
- Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subventricular zone. *J Neurosci* 2002; 22: 629–34.
- Inta D, Gass P. Is forebrain neurogenesis a potential repair mechanism after stroke? *J Cereb Blood Flow Metab* 2015; 35: 1220–1.
- Boonstra R, Galea L, Matthews S, Wojtowicz JM. Adult neurogenesis in natural populations. *Can J Physiol Pharmacol* 2001; 79: 297–302.
- Goritz C, Frisen J. Neural stem cells and neurogenesis in the adult. *Cell Stem Cell* 2012; 10: 657–9.
- Curtis MA, Low VF, Faull RL. Neurogenesis and progenitor cells in the adult human brain: a comparison between hippocampal and subventricular progenitor proliferation. *Dev Neurobiol* 2012; 72:

- 990–1005.
- 20 Liu S, Li C, Xing Y, Tao F. Effect of transplantation of human embryonic stem cell-derived neural progenitor cells on adult neurogenesis in aged hippocampus. *Am J Stem Cells* 2014; 3: 21–6.
- 21 Qu HL, Zhao M, Zhao SS, Xiao T, Song CG, Cao YP, et al. Forced limb-use enhanced neurogenesis and behavioral recovery after stroke in the aged rats. *Neuroscience* 2015; 286: 316–24.
- 22 Minger SL, Ekonomou A, Carta EM, Chinoy A, Perry RH, Ballard CG. Endogenous neurogenesis in the human brain following cerebral infarction. *Regener Med* 2007; 2: 69–74.
- 23 Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, et al. Evidence for stroke-induced neurogenesis in the human brain. *Proc Natl Acad Sci U S A* 2006; 103: 13198–202.
- 24 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: 13114–9.
- 25 Lindvall O, Kokaia Z. Neurogenesis following stroke affecting the adult brain. *Cold Spring Harbor Perspect Biol* 2015; 7.
- 26 Nakayama D, Matsuyama T, Ishibashi-Ueda H, Nakagomi T, Kasahara Y, Hirose H, et al. Injury-induced neural stem/progenitor cells in post-stroke human cerebral cortex. *Eur J Neurosci* 2010; 31: 90–8.
- 27 Arvidsson A, Kokaia Z, Lindvall O. *N*-methyl-*D*-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. *Eur J Neurosci* 2001; 14: 10–8.
- 28 Jiang W, Gu W, Brannstrom T, Rosqvist R, Wester P. Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. *Stroke* 2001; 32: 1201–7.
- 29 Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann Neurol* 2002; 52: 802–13.
- 30 Yu TS, Washington PM, Kernie SG. Injury-induced neurogenesis: mechanisms and relevance. *Neuroscientist* 2016; 22: 61–71.
- 31 Magnusson JP, Goritz C, Tatarishvili J, Dias DO, Smith EM, Lindvall O, et al. A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. *Science* 2014; 346: 237–41.
- 32 Duan CL, Liu CW, Shen SW, Yu Z, Mo JL, Chen XH, et al. Striatal astrocytes transdifferentiate into functional mature neurons following ischemic brain injury. *Glia* 2015; 63: 1660–70.
- 33 Shen SW, Duan CL, Chen XH, Wang YQ, Sun X, Zhang QW, et al. Neurogenic effect of VEGF is related to increase of astrocytes transdifferentiation into new mature neurons in rat brains after stroke. *Neuropharmacology* 2016; 108: 451–61.
- 34 Becerra-Calixto A, Cardona-Gomez GP. The role of astrocytes in neuroprotection after brain stroke: potential in cell therapy. *Front Mol Neurosci* 2017; 10: 88.
- 35 Cabezas R, Avila-Rodriguez M, Vega-Vela NE, Echeverria V, Gonzalez J, Hidalgo OA, et al. Growth factors and astrocytes metabolism: possible roles for platelet derived growth factor. *Med Chem* 2016; 12: 204–10.
- 36 Sato Y, Chin Y, Kato T, Tanaka Y, Tozuka Y, Mase M, et al. White matter activated glial cells produce BDNF in a stroke model of monkeys. *Neurosci Res* 2009; 65: 71–8.
- 37 Ahmed S, Reynolds BA, Weiss S. BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronal precursors. *J Neurosci* 1995; 15: 5765–78.
- 38 Rosenblum S, Smith TN, Wang N, Chua JY, Westbroek E, Wang K, et al. BDNF pretreatment of human embryonic-derived neural stem cells improves cell survival and functional recovery after transplantation in hypoxic-ischemic stroke. *Cell Transplant* 2015; 24: 2449–61.
- 39 Nojiri Y, Takeda S, Enomoto M, Nishitsuji H, Masuda T, Sotome S, et al. Co-overexpression of GDNF and GFRalpha1 induces neural differentiation in neural progenitor cells in comparison to bone marrow stromal cells. *J Med Dent Sci* 2008; 55: 121–8.
- 40 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: 2361–7.
- 41 Lee TH, Kato H, Chen ST, Kogure K, Itoyama Y. Expression of nerve growth factor and TRKA after transient focal cerebral ischemia in rats. *Stroke* 1998; 29: 1687–96; discussion 97.
- 42 Zhu W, Cheng S, Xu G, Ma M, Zhou Z, Liu D, et al. Intranasal nerve growth factor enhances striatal neurogenesis in adult rats with focal cerebral ischemia. *Drug Deliv* 2011; 18: 338–43.
- 43 Crouch EE, Liu C, Silva-Vargas V, Doetsch F. Regional and stage-specific effects of prospectively purified vascular cells on the adult V-SVZ neural stem cell lineage. *J Neurosci* 2015; 35: 4528–39.
- 44 Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* 2002; 99: 11946–50.
- 45 Sun Y, Jin K, Childs JT, Xie L, Mao XO, Greenberg DA. Vascular endothelial growth factor-B (VEGFB) stimulates neurogenesis: evidence from knockout mice and growth factor administration. *Develop Biol* 2006; 289: 329–35.
- 46 Quittet MS, Touzani O, Sindji L, Cayon J, Fillesoye F, Toutain J, et al. Effects of mesenchymal stem cell therapy, in association with pharmacologically active microcarriers releasing VEGF, in an ischaemic stroke model in the rat. *Acta Biomater* 2015; 15: 77–88.
- 47 Gomez-Gaviro MV, Scott CE, Sesay AK, Matheu A, Booth S, Galichet C, et al. Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. *Proc Natl Acad Sci U S A* 2012; 109: 1317–22.
- 48 Lavado A, Oliver G. Jagged1 is necessary for postnatal and adult neurogenesis in the dentate gyrus. *Develop Biol* 2014; 388: 11–21.
- 49 Silva-Vargas V, Doetsch F. A new twist for neurotrophins: endothelial-derived NT-3 mediates adult neural stem cell quiescence. *Neuron* 2014; 83: 507–9.
- 50 Shimazu K, Zhao M, Sakata K, Akbarian S, Bates B, Jaenisch R, et al. NT-3 facilitates hippocampal plasticity and learning and memory by regulating neurogenesis. *Learn Memory* 2006; 13: 307–15.
- 51 Yan YH, Li SH, Gao Z, Zou SF, Li HY, Tao ZY, et al. Neurotrophin-3 promotes proliferation and cholinergic neuronal differentiation of bone marrow-derived neural stem cells via notch signaling pathway. *Life Sci* 2016; 166: 131–8.
- 52 Chojnacki A, Weiss S. Pigment epithelium-derived growth factor: modulating adult neural stem cell self-renewal. *Nat Neurosci* 2009; 12: 1481–3.
- 53 Ramirez-Castillejo C, Sanchez-Sanchez F, Andreu-Agullo C, Ferron SR, Aroca-Aguilar JD, Sanchez P, et al. Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* 2006; 9: 331–9.
- 54 Nakata M, Nakagomi T, Maeda M, Nakano-Doi A, Momota Y, Matsuyama T. Induction of perivascular neural stem cells and possible contribution to neurogenesis following transient brain ischemia/reperfusion injury. *Transl Stroke Res* 2017; 8: 131–43.
- 55 Zou J, Chen Z, Wei X, Chen Z, Fu Y, Yang X, et al. Cystatin C as a potential therapeutic mediator against Parkinson's disease via VEGF-induced angiogenesis and enhanced neuronal autophagy in neurovascular units. *Cell Death Dis* 2017; 8: e2854.
- 56 Kim DH, Lee HE, Kwon KJ, Park SJ, Heo H, Lee Y, et al. Early immature neuronal death initiates cerebral ischemia-induced neurogenesis in the dentate gyrus. *Neuroscience* 2015; 284: 42–

- 54.
- 57 Lu J, Bradley RA, Zhang SC. Turning reactive glia into functional neurons in the brain. *Cell Stem Cell* 2014; 14: 133–4.
- 58 Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, *et al*. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci* 2005; 8: 752–8.
- 59 Raivich G. Like cops on the beat: the active role of resting microglia. *Trends Neurosci* 2005; 28: 571–3.
- 60 Cacci E, Claassen JH, Kokaia Z. Microglia-derived tumor necrosis factor- $\alpha$  exaggerates death of newborn hippocampal progenitor cells *in vitro*. *J Neurosci Res* 2005; 80: 789–97.
- 61 Cacci E, Ajmone-Cat MA, Anelli T, Biagioni S, Minghetti L. *In vitro* neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* 2008; 56: 412–25.
- 62 Ramanan S, Kooshki M, Zhao W, Hsu FC, Robbins ME. PPAR $\alpha$  ligands inhibit radiation-induced microglial inflammatory responses by negatively regulating NF- $\kappa$ B and AP-1 pathways. *Free Radic Biol Med* 2008; 45: 1695–704.
- 63 Lull ME, Block ML. Microglial activation and chronic neurodegeneration. *Neurotherapeutics* 2010; 7: 354–65.
- 64 Parajuli B, Horiuchi H, Mizuno T, Takeuchi H, Suzumura A. CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. *Glia* 2015; 63: 2274–84.
- 65 Sato K. Effects of microglia on neurogenesis. *Glia* 2015; 63: 1394–405.
- 66 Jakubs K, Bonde S, Iosif RE, Ekdahl CT, Kokaia Z, Kokaia M, *et al*. Inflammation regulates functional integration of neurons born in adult brain. *J Neurosci* 2008; 28: 12477–88.
- 67 Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC. Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. *J Neurochem* 2009; 108: 1343–59.
- 68 Keohane A, Ryan S, Maloney E, Sullivan AM, Nolan YM. Tumor necrosis factor- $\alpha$  impairs neuronal differentiation but not proliferation of hippocampal neural precursor cells: role of Hes1. *Mol Cell Neurosci* 2010; 43: 127–35.
- 69 Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J, *et al*. Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* 2009; 57: 835–49.
- 70 Jin Q, Cheng J, Liu Y, Wu J, Wang X, Wei S, *et al*. Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. *Brain Behav Immunity* 2014; 40: 131–42.
- 71 Lively S, Hutchings S, Schlichter LC. Molecular and cellular responses to interleukin-4 treatment in a rat model of transient ischemia. *J Neuropathol Exp Neurol* 2016. pii: nlw081.
- 72 Ziv Y, Schwartz M. Orchestrating brain-cell renewal: the role of immune cells in adult neurogenesis in health and disease. *Trends Mol Med* 2008; 14: 471–8.
- 73 Paolicelli RC, Gross CT. Microglia in development: linking brain wiring to brain environment. *Neuron Glia Biol* 2011; 7: 77–83.
- 74 Zeng W, Ju R, Mao M. Therapeutic potential of hepatocyte growth factor against cerebral ischemia (Review). *Exp Ther Med* 2015; 9: 283–8.
- 75 Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med* 2002; 8: 963–70.
- 76 Bonato M. Unveiling residual, spontaneous recovery from subtle hemispatial neglect three years after stroke. *Front Human Neurosci* 2015; 9: 413.
- 77 Delavaran H, Aked J, Sjunnesson H, Lindvall O, Norrving B, Kokaia Z, *et al*. Spontaneous recovery of upper extremity motor impairment after ischemic stroke: implications for stem cell-based therapeutic approaches. *Transl Stroke Res* 2017; 8: 351–61.
- 78 Jin K, Wang X, Xie L, Mao XO, Greenberg DA. Transgenic ablation of doublecortin-expressing cells suppresses adult neurogenesis and worsens stroke outcome in mice. *Proc Natl Acad Sci U S A* 2010; 107: 7993–8.
- 79 Sun C, Sun H, Wu S, Lee CC, Akamatsu Y, Wang RK, *et al*. Conditional ablation of neuroprogenitor cells in adult mice impedes recovery of poststroke cognitive function and reduces synaptic connectivity in the perforant pathway. *J Neurosci* 2013; 33: 17314–25.
- 80 Daynac M, Morizur L, Chicheportiche A, Mouthon MA, Boussin FD. Age-related neurogenesis decline in the subventricular zone is associated with specific cell cycle regulation changes in activated neural stem cells. *Sci Rep* 2016; 6: 21505.
- 81 Nada SE, Tulsulkar J, Shah ZA. Heme oxygenase 1-mediated neurogenesis is enhanced by *Ginkgo biloba* (EGb 761(R)) after permanent ischemic stroke in mice. *Mol Neurobiol* 2014; 49: 945–56.
- 82 Tang Y, Wang J, Lin X, Wang L, Shao B, Jin K, *et al*. Neural stem cell protects aged rat brain from ischemia-reperfusion injury through neurogenesis and angiogenesis. *J Cereb Blood Flow Metab* 2014; 34: 1138–47.
- 83 Niv F, Keiner S, Krishna, Witte OW, Lie DC, Redecker C. Aberrant neurogenesis after stroke: a retroviral cell labeling study. *Stroke* 2012; 43: 2468–75.
- 84 Zhang JC, Xu H, Yuan Y, Chen JY, Zhang YJ, Lin Y, *et al*. Delayed treatment with green tea polyphenol egcg promotes neurogenesis after ischemic stroke in adult mice. *Mol Neurobiol* 2017; 54: 3652–64.
- 85 Wu KJ, Yu S, Lee JY, Hoffer B, Wang Y. Improving neurorepair in stroke brain through endogenous neurogenesis-enhancing drugs. *Cell Transplant* 2017; 26: 1596–600.
- 86 Yuan M, Wen SJ, Yang CX, Pang YG, Gao XQ, Liu XQ, *et al*. Transplantation of neural stem cells overexpressing glial cell line-derived neurotrophic factor enhances Akt and Erk1/2 signaling and neurogenesis in rats after stroke. *Chin Med J* 2013; 126: 1302–9.
- 87 Zhao Y, Lai W, Xu Y, Li L, Chen Z, Wu W. Exogenous and endogenous therapeutic effects of combination sodium ferulate and bone marrow stromal cells (BMSCs) treatment enhance neurogenesis after rat focal cerebral ischemia. *Metab Brain Dis* 2013; 28: 655–66.
- 88 Stonesifer C, Corey S, Ghanekar S, Diamandis Z, Acosta SA, Borlongan CV. Stem cell therapy for abrogating stroke-induced neuroinflammation and relevant secondary cell death mechanisms. *Prog Neurobiol* 2017; 158: 94–131.
- 89 Nakazaki M, Sasaki M, Kataoka-Sasaki Y, Oka S, Namioka T, Namioka A, *et al*. Intravenous infusion of mesenchymal stem cells inhibits intracranial hemorrhage after recombinant tissue plasminogen activator therapy for transient middle cerebral artery occlusion in rats. *J Neurosurg* 2017; 127: 917–26.
- 90 Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY, *et al*. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells* 2010; 28: 1099–106.
- 91 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: 1003–8.
- 92 Bhasin A, Kumaran SS, Bhatia R, Mohanty S, Srivastava MVP. Safety

- and feasibility of autologous mesenchymal stem cell transplantation in chronic stroke in indian patients. A four-year follow up. *J Stem Cells Regener Med* 2017; 13: 14–9.
- 93 Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 2005; 57: 874–82.
- 94 Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S, *et al*. Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial. *Stroke* 2014; 45: 3618–24.
- 95 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.
- 96 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: 2242–4.
- 97 Taguchi A, Sakai C, Soma T, Kasahara Y, Stern DM, Kajimoto K, *et al*. Intravenous autologous bone marrow mononuclear cell transplantation for stroke: phase 1/2a clinical trial in a homogeneous group of stroke patients. *Stem Cells Develop* 2015; 24: 2207–18.
- 98 Moniche F, Escudero I, Zapata-Arriaza E, Usero-Ruiz M, Prieto-Leon M, de la Torre J, *et al*. Intra-arterial bone marrow mononuclear cells (BM-MNCs) transplantation in acute ischemic stroke (IBIS trial): protocol of a phase II, randomized, dose-finding, controlled multicenter trial. *Int J Stroke* 2015; 10: 1149–52.
- 99 Kalladka D, Sinden J, Pollock K, Haig C, McLean J, Smith W, *et al*. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. *Lancet* 2016; 388: 787–96.
- 100 Bliss TM, Andres RH, Steinberg GK. Optimizing the success of cell transplantation therapy for stroke. *Neurobiol Dis* 2010; 37: 275–83.
- 101 Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G 2nd, Jiang L, *et al*. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nat Med* 2003; 9: 439–47.
- 102 Arsenijevic Y, Villemure JG, Brunet JF, Bloch JJ, Deglon N, Kostic C, *et al*. Isolation of multipotent neural precursors residing in the cortex of the adult human brain. *Exp Neurol* 2001; 170: 48–62.
- 103 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: 1757–63.
- 104 Chekhonin VP, Lebedev SV, Volkov AI, Pavlov KA, Ter-Arutyunants AA, Volgina NE, *et al*. Activation of expression of brain-derived neurotrophic factor at the site of implantation of allogenic and xenogenic neural stem (progenitor) cells in rats with ischemic cortical stroke. *Bull Exp Biol Med* 2011; 150: 515–8.
- 105 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: 274–85.
- 106 Hwang DH, Lee HJ, Park IH, Seok JI, Kim BG, Joo IS, *et al*. Intrathecal transplantation of human neural stem cells overexpressing VEGF provide behavioral improvement, disease onset delay and survival extension in transgenic ALS mice. *Gene Ther* 2009; 16: 1234–44.
- 107 Chen B, Gao XQ, Yang CX, Tan SK, Sun ZL, Yan NH, *et al*. Neuroprotective effect of grafting GDNF gene-modified neural stem cells on cerebral ischemia in rats. *Brain Res* 2009; 1284: 1–11.
- 108 Tsupykov O, Kanemitsu M, Smozhanik E, Skibo G, Dayer AG, Kiss JZ. Relationship of grafted FGF-2-overexpressing neural stem/progenitor cells with the vasculature in the cerebral cortex. *Cell Transplant* 2016; 25: 1359–69.
- 109 Naruse M, Shibasaki K, Ishizaki Y. FGF-2 signal promotes proliferation of cerebellar progenitor cells and their oligodendrocytic differentiation at early postnatal stage. *Biochem Biophys Res Commun* 2015; 463: 1091–6.
- 110 Hess DC, Wechsler LR, Clark WM, Savitz SI, Ford GA, Chiu D, *et al*. Safety and efficacy of multipotent adult progenitor cells in acute ischaemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Neurol* 2017; 16: 360–8.
- 111 Buhnemann C, Scholz A, Bernreuther C, Malik CY, Braun H, Schachner M, *et al*. Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain* 2006; 129: 3238–48.
- 112 Jin K, Xie L, Mao X, Greenberg MB, Moore A, Peng B, *et al*. Effect of human neural precursor cell transplantation on endogenous neurogenesis after focal cerebral ischemia in the rat. *Brain Res* 2011; 1374: 56–62.
- 113 Drury-Stewart D, Song M, Mohamad O, Guo Y, Gu X, Chen D, *et al*. Highly efficient differentiation of neural precursors from human embryonic stem cells and benefits of transplantation after ischemic stroke in mice. *Stem Cell Res Ther* 2013; 4: 93.
- 114 Peng H, Wang Q, Lou T, Qin J, Jung S, Shetty V, *et al*. Myokine mediated muscle-kidney crosstalk suppresses metabolic reprogramming and fibrosis in damaged kidneys. *Nat Commun* 2017; 8: 1493.
- 115 Klein R, Mahlberg N, Ohren M, Ladwig A, Neumaier B, Graf R, *et al*. The Neural Cell Adhesion Molecule-Derived (NCAM)-Peptide FG Loop (FGL) mobilizes endogenous neural stem cells and promotes endogenous regenerative capacity after stroke. *J Neuroimmunol Pharmacol* 2016; 11: 708–20.
- 116 Meng C, Zhang JC, Shi RL, Zhang SH, Yuan SY. Inhibition of interleukin-6 abolishes the promoting effects of pair housing on post-stroke neurogenesis. *Neuroscience* 2015; 307: 160–70.
- 117 Zhang Y, Zhang X, Cui L, Chen R, Zhang C, Li Y, *et al*. Salvianolic Acids for Injection (SAFI) promotes functional recovery and neurogenesis via sonic hedgehog pathway after stroke in mice. *Neurochem Int* 2017; 110: 38–48.
- 118 Li PC, Jiao Y, Ding J, Chen YC, Cui Y, Qian C, *et al*. Cystamine improves functional recovery via axon remodeling and neuroprotection after stroke in mice. *CNS Neurosci Ther* 2015; 21: 231–40.
- 119 Yang LC, Li J, Xu SF, Cai J, Lei H, Liu DM, *et al*. L-3-n-butylphthalide promotes neurogenesis and neuroplasticity in cerebral ischemic rats. *CNS Neurosci Ther* 2015; 21: 733–41.
- 120 Zhang Q, Zhao Y, Xu Y, Chen Z, Liu N, Ke C, *et al*. Sodium ferulate and n-butylidene-phthalate combined with bone marrow stromal cells (BMSCs) improve the therapeutic effects of angiogenesis and neurogenesis after rat focal cerebral ischemia. *J Transl Med* 2016; 14: 223.
- 121 Wang Y, Zhao Z, Rege SV, Wang M, Si G, Zhou Y, *et al*. 3K3A-activated protein C stimulates postischemic neuronal repair by human neural stem cells in mice. *Nat Med* 2016; 22: 1050–5.
- 122 Liu QS, Li SR, Li K, Li X, Yin X, Pang Z. Ellagic acid improves endogenous neural stem cells proliferation and neurorestoration through Wnt/beta-catenin signaling *in vivo* and *in vitro*. *Mol Nutr Food Res* 2017; 61. doi: 10.1002/mnfr.201600587.
- 123 Zhou HH, Tang Y, Zhang XY, Luo CX, Gao LY, Wu HY, *et al*. Delayed administration of Tat-HA-NR2B9c promotes recovery after stroke in rats. *Stroke* 2015; 46: 1352–8.
- 124 Chen J, Zhang C, Jiang H, Li Y, Zhang L, Robin A, *et al*. Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. *J Cereb Blood Flow Metab* 2005; 25: 281–90.



- 125 Yu SJ, Tseng KY, Shen H, Harvey BK, Airavaara M, Wang Y. Local administration of AAV-BDNF to subventricular zone induces functional recovery in stroke rats. *PLoS One* 2013; 8: e81750.
- 126 Kong X, Zhong M, Su X, Qin Q, Su H, Wan H, *et al*. Tetramethylpyrazine promotes migration of neural precursor cells via activating the phosphatidylinositol 3-kinase pathway. *Mol Neurobiol* 2016; 53: 6526–39.
- 127 Mo SF, Liao GY, Yang J, Wang MY, Hu Y, Lian GN, *et al*. Protection of neuronal cells from excitotoxicity by disrupting nNOS-PSD95 interaction with a small molecule SCR-4026. *Brain Res* 2016; 1648: 250–6.
- 128 Stokowska A, Atkins AL, Moran J, Pekny T, Bulmer L, Pascoe MC, *et al*. Complement peptide C3a stimulates neural plasticity after experimental brain ischaemia. *Brain* 2017; 140: 353–69.
- 129 Li Y, Chen CH, Yin Y, Mao WW, Hua XM, Cheng J. Neuroprotection by intravenous transplantation of bone marrow mononuclear cells from 5-fluorouracil pre-treated rats in a model of ischemic stroke. *Neurol Res* 2016; 38: 921–8.
- 130 Zhang LL, Zhang HT, Cai YQ, Han YJ, Yao F, Yuan ZH, *et al*. Anti-inflammatory effect of mesenchymal stromal cell transplantation and quercetin treatment in a rat model of experimental cerebral ischemia. *Cell Mol Neurobiol* 2016; 36: 1023–34.
- 131 Nouri F, Salehinejad P, Nematollahi-Mahani SN, Kamarul T, Zarrindast MR, Sharifi AM. Deferoxamine preconditioning of neural-like cells derived from human wharton's jelly mesenchymal stem cells as a strategy to promote their tolerance and therapeutic potential: an *in vitro* study. *Cell Mol Neurobiol* 2016; 36: 689–700.
- 132 Li Y, Suo L, Liu Y, Li H, Xue W. Protective effects of ginsenoside Rg1 against oxygen-glucose-deprivation-induced apoptosis in neural stem cells. *J Neurol Sci* 2017; 373: 107–12.
- 133 Zhu JD, Wang JJ, Ge G, Kang CS. Effects of Noggin-transfected neural stem cells on neural functional recovery and underlying mechanism in rats with cerebral ischemia reperfusion injury. *J Stroke Cerebrovasc Dis* 2017; 26: 1547–59.
- 134 Suzuki N, Arimitsu N, Shimizu J, Takai K, Hirotsu C, Takada E, *et al*. Neuronal cell sheet of cortical motor neuron phenotype derived from human iPS cells. *Cell Transplant* 2017; 26: 1355–64.
- 135 Tornero D, Tsupykov O, Granmo M, Rodriguez C, Gronning-Hansen M, Thelin J, *et al*. Synaptic inputs from stroke-injured brain to grafted human stem cell-derived neurons activated by sensory stimuli. *Brain* 2017; 140: 692–706.
- 136 Pradillo JM, Murray KN, Coutts GA, Moraga A, Oroz-Gonjar F, Boutin H, *et al*. Reparative effects of interleukin-1 receptor antagonist in young and aged/co-morbid rodents after cerebral ischemia. *Brain Behav Immun* 2017; 61: 117–26.
- 137 Lin Y, Zhang JC, Yao CY, Wu Y, Abdelgawad AF, Yao SL, *et al*. Critical role of astrocytic interleukin-17 A in post-stroke survival and neuronal differentiation of neural precursor cells in adult mice. *Cell Death Dis* 2016; 7: e2273.
- 138 Lopes RS, Cardoso MM, Sampaio AO, Barbosa MS, Souza CC, da Silva MC, *et al*. Indomethacin treatment reduces microglia activation and increases numbers of neuroblasts in the subventricular zone and ischaemic striatum after focal ischaemia. *J Biosci* 2016; 41: 381–94.
- 139 Zou H, Long J, Zhang Q, Zhao H, Bian B, Wang Y, *et al*. Induced cortical neurogenesis after focal cerebral ischemia—three active components from Huang-Lian-Jie-Du decoction. *J Ethnopharmacol* 2016; 178: 115–24.
- 140 Duan S, Wang T, Zhang J, Li M, Lu C, Wang L, *et al*. Huatuo Zaizao pill promotes functional recovery and neurogenesis after cerebral ischemia-reperfusion in rats. *BMC Complement Alternat Med* 2017; 17: 19.
- 141 Chen L, Wang X, Zhang J, Dang C, Liu G, Liang Z, *et al*. Tongxinluo enhances neurogenesis and angiogenesis in peri-infarct area and subventricular zone and promotes functional recovery after focal cerebral ischemic infarction in hypertensive rats. *Evid-Based Complement Alternat Med* 2016; 2016: 8549590.
- 142 Ren C, Wang B, Li N, Jin K, Ji X. Herbal formula Danggui-Shaoyao-San promotes neurogenesis and angiogenesis in rat following middle cerebral artery occlusion. *Aging Dis* 2015; 6: 245–53.
- 143 Kong X, Su X, Zhu J, Wang J, Wan H, Zhong M, *et al*. Neuroprotective effect of Buyang Huanwu decoction on rat ischemic/reperfusion brain damage by promoting migration of neural precursor cells. *Rejuvenat Res* 2014; 17: 264–75.
- 144 Kawada H, Takizawa S, Takanashi T, Morita Y, Fujita J, Fukuda K, *et al*. Administration of hematopoietic cytokines in the subacute phase after cerebral infarction is effective for functional recovery facilitating proliferation of intrinsic neural stem/progenitor cells and transition of bone marrow-derived neuronal cells. *Circulation* 2006; 113: 701–10.
- 145 Shyu WC, Lin SZ, Lee CC, Liu DD, Li H. Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. *CMAJ* 2006; 174: 927–33.
- 146 Mizuma A, Yamashita T, Kono S, Nakayama T, Baba Y, Itoh S, *et al*. Phase II trial of intravenous low-dose granulocyte colony-stimulating factor in acute ischemic stroke. *J Stroke Cerebrovasc Dis* 2016; 25: 1451–7.
- 147 Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, *et al*. Clinical outcomes of transplanted modified bone marrow-derived mesenchymal stem cells in stroke: a phase 1/2a study. *Stroke* 2016; 47: 1817–24.
- 148 Ohshima M, Taguchi A, Tsuda H, Sato Y, Yamahara K, Harada-Shiba M, *et al*. Intraperitoneal and intravenous deliveries are not comparable in terms of drug efficacy and cell distribution in neonatal mice with hypoxia-ischemia. *Brain Develop* 2015; 37: 376–86.
- 149 Qiao LY, Huang FJ, Zhao M, Xie JH, Shi J, Wang J, *et al*. A two-year follow-up study of cotransplantation with neural stem/progenitor cells and mesenchymal stromal cells in ischemic stroke patients. *Cell Transplant* 2014; 23 Suppl 1: S65–72.
- 150 Karlupia N, Manley NC, Prasad K, Schafer R, Steinberg GK. Intraarterial transplantation of human umbilical cord blood mononuclear cells is more efficacious and safer compared with umbilical cord mesenchymal stromal cells in a rodent stroke model. *Stem Cell Res Ther* 2014; 5: 45.
- 151 Moniche F, Montaner J, Gonzalez-Marcos JR, Carmona M, Pinero P, Espigado I, *et al*. Intra-arterial bone marrow mononuclear cell transplantation correlates with GM-CSF, PDGF-BB, and MMP-2 serum levels in stroke patients: results from a clinical trial. *Cell Transplant* 2014; 23 Suppl 1: S57–64.
- 152 Diez-Tejedor E, Gutierrez-Fernandez M, Martinez-Sanchez P, Rodriguez-Frutos B, Ruiz-Ares G, Lara ML, *et al*. Reparative therapy for acute ischemic stroke with allogeneic mesenchymal stem cells from adipose tissue: a safety assessment: a phase II randomized, double-blind, placebo-controlled, single-center, pilot clinical trial. *J Stroke Cerebrovasc Dis* 2014; 23: 2694–700.
- 153 Chen DC, Lin SZ, Fan JR, Lin CH, Lee W, Lin CC, *et al*. Intracerebral implantation of autologous peripheral blood stem cells in stroke patients: a randomized phase II study. *Cell Transplant* 2014; 23: 1599–612.
- 154 Banerjee S, Bentley P, Hamady M, Marley S, Davis J, Shlebak A, *et al*. Intra-arterial immunoselected CD34<sup>+</sup> stem cells for acute ischemic stroke. *Stem Cells Transl Med* 2014; 3: 1322–30.

- 155 Kim DS, Lee MW, Noh YH, Jang MC, Lee SH, Son MH, *et al.* Engraftment efficacy of human hematopoietic stem cells transplanted into NOD/SCID mice using two methods: intra-bone marrow transplantation of hematopoietic stem cells and intravenous co-transplantation with mesenchymal stem cells. *Acta Haematol* 2014; 131: 179–82.
- 156 Jiang Y, Zhu W, Zhu J, Wu L, Xu G, Liu X. Feasibility of delivering mesenchymal stem cells via catheter to the proximal end of the lesion artery in patients with stroke in the territory of the middle cerebral artery. *Cell Transplant* 2013; 22: 2291–8.
- 157 Chen L, Xi H, Huang H, Zhang F, Liu Y, Chen D, *et al.* Multiple cell transplantation based on an intraparenchymal approach for patients with chronic phase stroke. *Cell Transplant* 2013; 22 Suppl 1: S83–91.
- 158 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: 221–8.
- 159 Laterza C, Wattananit S, Uoshima N, Ge R, Tornero D, Monni E, *et al.* Monocyte depletion early after stroke promotes neurogenesis from endogenous neural stem cells in adult brain. *Exp Neurol* 2017; 297: 129–37.
- 160 Xu W, Mu X, Wang H, Song C, Ma W, Jolkkonen J, *et al.* Chloride Co-transporter NKCC1 inhibitor bumetanide enhances neurogenesis and behavioral recovery in rats after experimental stroke. *Mol Neurobiol* 2017; 54: 2406–14.
- 161 Wang LL, Li J, Gu X, Wei L, Yu SP. Delayed treatment of 6-bromoindirubin-3'-oxime stimulates neurogenesis and functional recovery after focal ischemic stroke in mice. *Int J Develop Neurosci* 2017; 57: 77–84.
- 162 Deng G, Qiu Z, Li D, Fang Y, Zhang S. Delayed administration of guanosine improves longterm functional recovery and enhances neurogenesis and angiogenesis in a mouse model of photothrombotic stroke. *Mol Med Rep* 2017; 15: 3999–4004.
- 163 Chen CK, Hsu PY, Wang TM, Miao ZF, Lin RT, Juo SH. TRPV4 activation contributes functional recovery from ischemic stroke via angiogenesis and neurogenesis. *Mol Neurobiol* 2018; 55: 4127–35.
- 164 Sun X, Zhou Z, Liu T, Zhao M, Zhao S, Xiao T, *et al.* Fluoxetine enhances neurogenesis in aged rats with cortical infarcts, but this is not reflected in a behavioral recovery. *J Mol Neurosci* 2016; 58: 233–42.
- 165 Sun X, Sun X, Liu T, Zhao M, Zhao S, Xiao T, *et al.* Fluoxetine enhanced neurogenesis is not translated to functional outcome in stroke rats. *Neurosci Lett* 2015; 603: 31–6.
- 166 Ling L, Zhang S, Ji Z, Huang H, Yao G, Wang M, *et al.* Therapeutic effects of lipo-prostaglandin E1 on angiogenesis and neurogenesis after ischemic stroke in rats. *Int J Neurosci* 2016; 126: 469–77.
- 167 Li YC, Tsai LK, Young TH. Intraventricular infusion of a low fraction of serum enhances neurogenesis and improves recovery in a rodent stroke model. *Neurosci Lett* 2016; 611: 14–20.
- 168 Wang ZL, Cheng SM, Ma MM, Ma YP, Yang JP, Xu GL, *et al.* Intranasally delivered bFGF enhances neurogenesis in adult rats following cerebral ischemia. *Neurosci Lett* 2008; 446: 30–5.
- 169 Kim H, Wei Y, Lee JY, Wu Y, Zheng Y, Moskowitz MA, *et al.* Myeloperoxidase inhibition increases neurogenesis after ischemic stroke. *J Pharmacol Exp Ther* 2016; 359: 262–72.
- 170 Jiang C, Zuo F, Wang Y, Lu H, Yang Q, Wang J. Progesterone changes VEGF and BDNF expression and promotes neurogenesis after ischemic stroke. *Mol Neurobiol* 2016. doi: 10.1007/s12035-015-9651-y.
- 171 Ahmed ME, Tucker D, Dong Y, Lu Y, Zhao N, Wang R, *et al.* Methylene Blue promotes cortical neurogenesis and ameliorates behavioral deficit after photothrombotic stroke in rats. *Neuroscience* 2016; 336: 39–48.
- 172 Yang J, Shi QD, Yang YB, Qian YH, Feng GF, Chang L, *et al.* Vasoactive intestinal peptide administration after stroke in rats enhances neurogenesis and improves neurological function. *Brain Res* 2015; 1625: 189–97.
- 173 Giuliani D, Zaffe D, Ottani A, Spaccapelo L, Galantucci M, Minutoli L, *et al.* Treatment of cerebral ischemia with melanocortins acting at MC4 receptors induces marked neurogenesis and long-lasting functional recovery. *Acta Neuropathol* 2011; 122: 443–53.
- 174 Liu XY, Zhou XY, Hou JC, Zhu H, Wang Z, Liu JX, *et al.* Ginsenoside Rd promotes neurogenesis in rat brain after transient focal cerebral ischemia via activation of PI3K/Akt pathway. *Acta Pharmacol Sin* 2015; 36: 421–8.
- 175 Cruz Y, Lorea J, Mestre H, Kim-Lee JH, Herrera J, Mellado R, *et al.* Copolymer-1 promotes neurogenesis and improves functional recovery after acute ischemic stroke in rats. *PLoS One* 2015; 10: e0121854.
- 176 Chen L, Wang X, Chen X, Xing S, Zhang J, Li J, *et al.* Tongxinluo attenuates neuronal loss and enhances neurogenesis and angiogenesis in the ipsilateral thalamus and improves neurological outcome after focal cortical infarction in hypertensive rats. *Restor Neurol Neurosci* 2014; 32: 533–46.