Chapter 10
Clinical Studies of Bone Marrow-Derived Stem Cell Therapy in Stroke Patients

Francisco Moniche, Irene Escudero, Elena Zapata, Fernando Mancha, Ángela Vega-Salvatierra, Blanca Pardo, and Joan Montaner

Abstract Stroke is the leading cause of long-term disability in adults and the third cause of mortality worldwide. In the very acute phase of stroke, thrombolytics and endovascular thrombectomy can reduce stroke disability; however, only a small minority of patients receive these treatments. Once the neurological deficits are established, there are few options for recovery. In recent years, extensive cell therapy preclinical research has demonstrated a neurorestorative effect after cerebral ischemia. In cerebral ischemia animal models, bone marrow-derived stem cells improve neurological outcomes even in the long term, increasing brain plasticity and enhancing recovery mainly due to secretion of growth factors and cytokines.

In the bone marrow, different types of cells have been used for cell therapy in stroke. The first type of cells used for stroke and the most extensive studied in preclinical research are mesenchymal stem cells (MSCs). In recent years some other cells have been studied for stroke therapy with promising results, such as bone marrow mononuclear cells (BM-MNCs), hematopoietic stem cells (HSCs), and multipotent adult progenitor cells (MAPCs). Several phase I and II clinical trials have been published to date with these stem cells, which have already demonstrated the feasibility and safety of this therapy in the stroke setting. An increasing number of

F. Moniche (✉)
Stroke Unit, Neurology Department, Instituto de Biomedicina de Sevilla-IBiS, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot, s/n., 41013 Seville, Spain

Instituto de Biomedicina de Sevilla-IBiS, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot, s/n., 41013 Seville, Spain
e-mail: pmoniche@gmail.com

I. Escudero • E. Zapata • B. Pardo
Stroke Unit, Neurology Department, Instituto de Biomedicina de Sevilla-IBiS, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot, s/n., 41013 Seville, Spain

F. Mancha • Á. Vega-Salvatierra • J. Montaner
Instituto de Biomedicina de Sevilla-IBiS, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot, s/n., 41013 Seville, Spain

K. Jin et al. (eds.), *Bone Marrow Stem Cell Therapy for Stroke*,
DOI 10.1007/978-981-10-2929-5_10

clinical trials, mainly with bone marrow MSCs and BM-MNCs, are ongoing to further assess the best dose, route, and timing of this therapy and to elucidate the efficacy cell therapy in stroke.

Keywords Patients • Bone marrow-derived stem cells • Therapy • Stroke

10.1 Introduction

The World Health Organization (WHO) defined stroke as a "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 h or longer or leading to death, with no apparent cause other than of vascular origin" [1].

Annually, 15 million people worldwide suffer a stroke and, in the United States alone, a person dies every 3 min due to stroke. In high-income countries, stroke is the third most common cause of death, only after coronary heart disease and cancer. Stroke is also the main cause of acquired adult disability [2]. Of those who survive a stroke, five million people annually are left permanently disabled, placing a burden on family and society. Only 41 % are independent 6 months after a stroke.

The ischemic stroke represents 80–85 % of strokes and the incidence is 150–200 patients/100,000 per year [3]. Stroke prevalence is about 2 % of the population of >20 years; however, it increases to up to 6–7 % in older adults (>65 years). Projections show that by 2030, an additional 3.4 million people in the United States aged ≥18 years will have had a stroke, a 20.5 % increase in prevalence from 2012 [4]. In the EU, the cost attributed to stroke was €64.1 billion in 2010, mainly due to the high costs of long-term special care and rehabilitation [5]. Moreover, the socioeconomic burden of stroke is expected to increase due to aging of the population and the rise in diabetes and obesity, which are reaching an epidemic level.

The currently available therapies of acute stroke target rapid vessel recanalization, since, without restoration of cerebral blood flow, hypoperfused cerebral tissue in the penumbral region progresses to cellular death that ultimately expands the necrotic core lesion. Nowadays, thrombolytics (i.e., tissue plasminogen activator or tPA) and endovascular thrombectomy are the main therapies for restoring normal perfusion in acute ischemic stroke. However, tPA has important limitations, with a narrow therapeutic window of 4.5 h, which means that less than 5–10 % of ischemic stroke patients receive this treatment. Moreover, recanalization rates after the administration of tPA are low and prevent disability in only 55 patients per 1000 people treated, without reducing mortality [6]. Recently, endovascular thrombectomy has demonstrated efficacy in several clinical trials in those patients with a large vessel occlusion [7]. Although the treatment approach of acute ischemic stroke is rapidly evolving [8], recanalization therapies are only administered to a minority of acute stroke patients.

Another approach to improve outcomes in stroke is the administration of neuroprotective drugs. Neuroprotective treatment aims to reduce the damage of stroke, but as most of the injury occurs in the first 24–48 h, these therapies must be administrated soon after stroke onset, and to date, no drug has been demonstrated to ameliorate the disability or mortality after stroke [9, 10].

In recent years, many studies have shown that once the stroke is established, profound neurorestorative processes are induced in brain tissue in response to focal cerebral ischemia [11]. Although these processes are insufficient to restore neurological function, neurorestorative treatments with pharmacological or cell-based therapies could stimulate and amplify these endogenous mechanisms in stroke patients. This approach has the major advantage of a wider therapeutic window, as neurorestorative therapies can be instituted during the recovery phase of the stroke, and promotes the remodeling of brain tissue. This makes the treatment available to a much larger number of stroke patients.

Also, neurorestorative treatments target not only the ischemic and "penumbra" tissue (hypoperfused tissue) but also viable brain tissue with normal perfusion stimulating neuronal plasticity and neurological recovery [11]. However, until now, neurorestorative drugs targeting single steps in the cascade of cerebral ischemia have failed to improve neurological deficits, probably related to stroke complexity, with necrosis, apoptosis, inflammation, and remodeling occurring as a continuum.

Stem cell therapy, such as transplantation of bone marrow stem cells, represents one of the most exciting fields in regenerative medicine and has emerged as an attractive approach for the treatment of stroke. These stem cells are believed to exert multiple therapeutic actions. They might target simultaneously several processes by releasing different factors inducing neuroprotection and brain remodeling and modulating the post-ischemic inflammatory response [12–15]. Extensive basic research has been done during the last two decades in cell therapy and stroke animal models, but we are still in the first steps in the clinical research with stroke patients.

The potential of cell-based therapy relies on several *key properties*: (1) their capacity to differentiate into several cell lineages, (2) their immunomodulatory properties, (3) their ex vivo expansion potential, (4) their ability to secrete factors to regulate biological functions such as proliferation and differentiation over a broad target of cells, and (5) their ability to home to damaged tissues.

10.2 Bone Marrow Cell Therapy and Clinical Trials

To date, there are many different cells being investigated for stroke, both in preclinical studies and in clinical trials such as embryonic stem cells, neural stem cells, adipose-derived stem cells, induced pluripotent stem cells (iPS), and stem cells obtained from bone marrow, umbilical cord, and amniotic or placental tissue. However, in this chapter, we will focus on bone marrow cell therapy as most of preclinical and clinical studies have used bone marrow stem cells [16, 17].

There are several advantages of using bone marrow stem cells as a cell therapy for stroke. First, the efficacy and reproducible benefits of these cells have been demonstrated in several laboratories and in different animal stroke models. Second, bone marrow stem cells are adult cells and therefore do not have ethical problems, unlike fetal and embryonic cells. Third, it has been proven reliable to use bone marrow stem cells in different time periods of stroke, even in the acute stroke phase. Finally, although some studies have been done with allogenic cells, bone marrow stem cells allow autologous administration avoiding the possibility of rejection.

In the bone marrow, different types of cells have been used for cell therapy in stroke. The first type of cells used for stroke and the most extensive studied in preclinical research are mesenchymal stem cells (MSCs). In recent years, some other cells have been studied for stroke therapy with promising results, such as bone marrow mononuclear cells (BM-MNCs), hematopoietic stem cells (HSCs), and multipotent adult progenitor cells (MAPCs).

10.2.1 Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs or marrow stromal cells) are one of the first types of cells that have been studied for ischemic stroke. MSCs have a considerable therapeutic potential that has generated markedly increasing interest in a wide variety of biomedical disciplines. Extensive preclinical studies with MSCs have made this therapy a very promising cell-based approach for stroke.

MSCs are multipotent adult stem cells defined as those cells which have three characteristics: (a) must be plastic adherent when maintained in standard culture conditions; (b) must express CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14, or CD11b, CD79alpha, or CD19 and HLA-DR surface molecules; and (c) must at least differentiate to osteoblasts, adipocytes, and chondroblasts in vitro [18]. The lack of expression of HLA-DR (class II major histocompatibility complex) gives them an immunoprivileged status, and also their relative ease of isolation from bone marrow makes these cells a good candidate for cell therapy in different illness such as stroke.

The safety of MSCs has been analyzed in a recent meta-analysis of clinical trials with more than 1000 patients in different clinical conditions that included ischemic stroke, Crohn's disease, myocardial infarction, cardiomyopathy, and graft versus host disease. An excellent safety profile of MSCs was demonstrated, as there was no association between MSC treatment and acute infusional toxicity, organ system complications, infection, death, or malignancy [19]. However, larger controlled clinical trials are required before defining a definitive safety profile of MSCs.

Regarding efficacy outcomes, MSCs have demonstrated efficacy in clinical trials in other conditions such as graft versus host disease and are under study in autoimmune diseases (i.e., Crohn's disease, multiple sclerosis, and type 1 diabetes) and different models of ischemia (i.e., stroke, ischemic cardiac diseases, and limb ischemia) [20–22].

In animal stroke models, injection of MSCs resulted in very large and favorable effects on neurological outcomes. In a recent meta-analysis of preclinical studies, MSCs improved consistently multiple outcome measures with very large effect sizes. These data are robust across species studied, administration route, dose, and presence of comorbidities [23]. Furthermore, MSCs attenuated tissue damage and migrated into the ischemic boundary zone accompanied by reduced neuronal apoptosis and enhanced neoangiogenesis and synaptogenesis [24, 25].

The first clinical trial published with bone marrow stem cells in stroke patients was done by Bang et al. which used autologous MSCs [26] (Table 10.1). In this placebo-controlled phase I/II trial of 30 patients with chronic stroke, 5 patients were treated with MSCs and 25 were controls. Those patients in the treated group received two doses of IV autologous mesenchymal stromal cells at 4–5 weeks and 7–9 weeks from the onset of symptoms. This method was reportedly safe and feasible in the short term. In 2010 Lee et al. published the long-term follow-up, with 52 patients finally included (16 in MSC group and 36 in control group). No MSC-related adverse events were reported, with significant improved neurologic recovery in those patients receiving cellular therapy compared to controls (the proportion of patients with modified Rankin scale score 0–3 increased in the MSC group, $p = 0.046$) [27].

Other pilot trials have been published using MSCs in stroke. Honmou et al. reported IV MSCs transplantation in 12 patients with chronic ischemic stroke [28]. No adverse events were described from transplantation, and interestingly, a reduction of more than 20 % of infarction volume was observed in magnetic resonance imaging at 1 week after cell injection.

In another trial, Bhasin et al. published a trial including 40 chronic stroke patients [29]. Of these, 14 patients received intravenous BM-MNCs, 6 patients received intravenous MSCs, and 20 were control patients. During follow-up, stem cell transplantation was reported to be safe, and there was a significant improvement at 6 months in the Barthel index when the whole stem cell group was compared to the control group, although there was no difference in Rankin scale or Fugl-Meyer scale.

In spite of these previous experiences with MSCs, there are several disadvantages of using MSCs in stroke patients (Table 10.2):

1. MSCs require several weeks of cell culture to obtain sufficient quantity of cells for transplantation, not allowing the autologous injection of MSCs in the acute-subacute phase of stroke.
2. The large size of cells (13–19 μm) could also lead to pulmonary entrapment when administered by intravenous injection or even to microvascular occlusions and new cerebral infarctions in the intra-arterial (IA) route [30].

To date, there is no published data of allogenic transplantation of bone marrow MSCs or with a different route than intravenous but a very recent interim report of an open-label single-arm study of surgical transplantation of modified bone marrow-derived mesenchymal stem cells [31]. In this interim analysis of 16 patients that have completed 12 months of follow-up, authors describe significant improvement in NIHSS (National Institute of Health Stroke Scale) (mean decrease 2.00 [95 %

Table 10.1 Published clinical trials with bone marrow-derived stem cell therapy in stroke patients

Cell population	Author	No. of patient treated (controls)	Design	Route	Cell dose	Time window	Follow-up (month)	Adverse events
MSCs								
Autologous	Bang (2005) [26]	5 (25)	Randomized, observer-blinded phase I/II	Intravenous	5×10^7	4–5 weeks	12	None reported
Autologous	Lee (2010) [27]	16 (36)	Observer-blinded phase II	Intravenous	5×10^7	5 weeks	60	None study-related reported
Autologous	Honmou (2011) [28]	12 (0)	Open-label phase I	Intravenous	$6–16\times10^7$	36–133 days	12	Mild fever, nausea, appetite loss
Autologous	Bhasin (2013) [29]	6 (6)	Open-label phase I/II	Intravenous	$5–6\times10^7$	3–24 months	6	None study-related reported
Allogenic	Steinberg (2016) [31]	18 (0)	Open-label phase I/II	Intraparenchymal	$2.5–10\times10^6$	6–60 months	12	Headache, nausea, subdural hematoma (2 of 18)
BM-MNCs								
Autologous	Suarez-Monteagudo (2009) [41]	5 (0)	Open-label phase I	Intraparenchymal	$1.4–5.5\times10^7$	1–10 years	12	Headache, drowsiness, nausea, fever
Autologous	Barbosa (2010) [60]	6 (0)	Open-label phase I	Intra-arterial	$12.5–50\times10^7$	<90 days	6	None study-related reported
Autologous	Battistella (2011) [43]	6 (0)	Open-label phase I	Intra-arterial	$10–50\times10^7$	2–3 months	6	Seizures (2 of 6)
Autologous	Savitz (2011) [46]	10 (0)	Open-label phase I	Intravenous	$7–10\times10^6$/kg	24–72 h	6	None study-related reported

Autologous	Friedrich (2012) [44]	20 (0)	Open-label phase I	Intra-arterial	2.2×10^7	3–7 days	6	None study-related reported
Autologous	Moniche (2012) [47]	10 (10)	Observer-blinded phase I/II	Intra-arterial	16×10^7	5–9 days	6	Seizures (2 of 10)
Autologous	Prasad (2012) [49]	11 (0)	Open-label phase I	Intravenous	$0.2–18\times10^7$	7–30 days	6	One reinfarction
Autologous	Li (2013) [42]	60 (40)	Observer-blinded phase I/II (hemorrhagic stroke)	Intraparenchymal	$0.2–2\times10^7$	5–7 days	6	Fever, one unspecified pulmonary tumor
Autologous	Rosado-de-Castro (2013) [45]	12 (0)	Open-label phase I	Intravenous ($n=5$), intra-arterial ($n=7$)	$10–50\times10^7$	19–89 days	6	Seizures (7 of 12)
Autologous	Prasad (2014) [50]	85 (35)	Blinded randomized phase II	Intravenous	28×10^7	18.5 days	12	None reported
Autologous	Sharma (2014) [59]	24 (0)	Open-label phase I/II	Intrathecal	1×10^6/kg	40 months	30	None reported
CD34+								
Autologous	Banerjee (2014) [16]	5 (0)	Open-label phase I	Intra-arterial	$1.2–2.5\times10^6$	<7 days	6	None study-related reported
MAPC								
Allogenic	Hess (public presentation)	65 (61)	Blinded phase II	Intravenous	40×10^7	24–48 h	6	None study-related reported

Table 10.2 Comparative of bone marrow mononuclear cells and bone marrow mesenchymal cells

Type of cell	Advantages	Disadvantages
BM-MNCs	Consistent beneficial effect and an excellent safety profile in animal models	Not allow allogenic transplantation without immunosuppressive drugs
	Excellent safety profile in pilot clinical trials in stroke	Variability in the number of cells obtained after bone marrow harvest
	Prepared for administration within hours	
	No tumor formation	
BM-MSCs	Very large and favorable effects in stroke models	Require cell culture (several weeks)
	Immunoprivileged status, allow allogenic transplantation	No allow autologous administration in acute stroke patients
	Excellent safety profile in other clinical conditions and pilot stroke trials	Large size of cells that could lead to microvascular occlusions or pulmonary entrapment
	No tumor formation	

confidence interval, −2.7 to −1.3; $P<0.001$]) and Fugl-Meyer scale (mean increase 19.20 [95 % confidence interval, 11.4–27.0; $P<0.001$]). Patients included had a chronic ischemic stroke (mean 22 months from stroke onset) and received a stereotactic injection of allogenic MSCs (*SB623 cells*) in the peri-infarct area. Serious adverse events were unrelated or unlikely to be related to cell treatment. Postsurgery headache was the most common adverse event that was probably or definitely related to the procedure, experienced by 77.8 % of patients, and subdural hematoma and epileptic seizure were detected in two patients (11 %).

Although the immunoprivileged status of MSCs makes a rejection of allogenic transplantation very unlikely, its safety has to be proven in stroke patients. However, previous reports of allogenic use of MSCs in other conditions described no acute infusional toxicity [19]. Regarding the route, the probably main reason for the absence of MSC clinical trials using intra-arterial route is due to the potential of arterial embolism that has been described with animal stroke models, even in mammalians. Lu et al. [30] described an intra-arterial MSC transplantation in a canine stroke model with the development of new infarctions 24 h after transplantation in 16 % of dogs, probably due to impeded cerebral blood flow [32].

Due to the large and favorable effects in preclinical studies and in spite of the disadvantages described, MSCs are still one of the best candidates for cell therapy in stroke patients, and several clinical trials are currently ongoing.

10.2.2 Hematopoietic Stem Cells

Other bone marrow stem cells that have been investigated in animal stroke models are hematopoietic stem cells (HSCs). These cells express CD34 (CD34+ cell) and can be also found in peripheral blood and umbilical cord blood. Preclinical studies of CD34+ cells have shown significant benefits in animal stroke models, with

evidence of functional improvement as well as reduced infarct volume [33]. In a preclinical study, intravenous CD34+ cell transplantation resulted in increased perilesional angiogenesis and subsequent neurogenesis in mice at 48 h post-stroke [34]. There are also other evidences of neurogenesis and angiogenesis induced by CD34+ cells in subacute stroke, with cells transplanted expressing neuronal, glial, and vascular endothelial cell markers [35]. There are some preliminary clinical trials demonstrating the safety of autologous CD34+ peripheral blood stem cells [36, 37]. Bone marrow hematopoietic stem cells have only been used in a pilot open-label clinical trial of five stroke patients [38]. CD34+ cells were collected from the bone marrow of the subjects before being delivered by catheter angiography into the ipsilateral middle cerebral artery within 7 days from stroke onset. No safety issues were described and all patients show improvement of neurological deficit during follow-up, although no comparison was done with a control group. Authors found a nonsignificant reduction in the mean lesion volume from inclusion to day 180, with no new lesions in MRI (edema, hemorrhage, or tumor). To date, very few clinical trials are currently ongoing testing bone marrow CD34+ cells in stroke patients.

10.2.3 Bone Marrow Mononuclear Cells (BM-MNCs)

BM-MNCs are one of the most studied types of cells for use as stroke therapy. BM-MNCs are composed of a mixture of myeloid, lymphoid, erythroid, and stem cell populations, which includes HSCs, MSCs, and endothelial progenitor cells. The main advantage over other types of cell therapy is that autologous transplantation is feasible, even in the acute phase of stroke, as they are isolated from bone marrow and prepared for administration within hours. As MSCs, BM-MNCs have been extensively studied in animal models demonstrating a consistent beneficial effect and an excellent safety profile. Several biological effects such as attenuation of neuronal death, modulating microglia, reducing pro-inflammatory responses, increasing neoangiogenesis, and promoting proliferation of endogenous neural stem cells have been invoked [12, 39, 40]. However, few clinical studies have assessed the safety and efficacy of BM-MNC transplantation in stroke patients.

The first trial published with BM-MNCs was an open-label trial with five stroke patients treated with intraparenchymal route by Suarez-Monteagudo et al. [41]. Patients included had a chronic stroke from 1 to 10 years from onset and authors describe an excellent tolerance of procedure and with no important adverse events derived from surgery or transplant. After this study, only Li et al. have published a clinical trial using intraparenchymal route, although not including ischemic stroke but intracerebral hemorrhage (ICH) [42]. In this study, autologous BM-MNCs were injected to the perihemorrhage area in the base ganglia through an intracranial drainage tube 6 days after ICH. Surgical drainage of ICH was performed in every patient within the first day from ICH onset, and after 5 days those patients who consent to be treated with BM-MNCs were included in the study group ($n=60$), and those who rejected cell therapy were the control group ($n=40$). Both groups had

similar baseline characteristics and similar NIHSS (National Institute of Health Stroke Scale) after surgery, but authors describe a significant improvement in Barthel and NIHSS scores in the study group 6 months after inclusion (57.39 ± 23.51 in study group vs 46.90 ± 20.29 in control group, $P < 0.01$ in Barthel scale and 10.09 ± 8.86 vs 14.35 ± 10.14, $P < 0.01$ in NIHSS).

Since the trial published by Suarez-Monteagudo et al. in 2009, several clinical trials have been published with BM-MNCs. Most of them used less invasive routes as intravenous or intra-arterial injection.

A Brazilian trial published by Battistella et al. included six patients treated with intra-arterial BM-MNCs with a time window of 2–3 months from stroke onset [43]. There was no worsening immediately after the procedure or during follow-up period. At the 180-day follow-up evaluation, there was a slight improvement in NIHSS (range −1 to −8 points). Although BM-MNC transplantation was safe in these patients, there is less evidence from the animal studies to suggest that BM-MNCs could be effective in this time window. The same group later published another trial, including 20 patients with moderate to severe middle cerebral artery (MCA) ischemic stroke in a time window of 3–7 days [44], showing no procedure-related adverse events, with 40 % of good clinical outcomes at 6 months.

In 2013, this group (Rosado de Castro et al.) compared IV vs. IA routes in BM-MNC transplantation in 12 stroke patients, demonstrating that with the IV route more cells were trapped in lungs after injection than IA injection. However, they found similar rates of brain homing between both routes [45]. Remarkably, all of the intravenous patients suffered seizures during the follow-up period. Authors hypothesize that the infused cells could modify excitability in the perilesional regions, generating seizures, which should be evaluated further in future clinical trials.

In another trial evaluating the test, feasibility, and safety of autologous BM-MNC infusion in patients with acute ischemic stroke, Savitz et al. [46] included ten patients with a time window of 24–72 h after stroke onset treated with intravenous BM-MNCs. This methodology is supported by a preclinical study in which rats with middle cerebral artery occlusion performed better on neurologic tests with IV mononuclear cells infused up to 72 h, compared with 1 week from stroke onset. There were no study-related severe adverse events. However, of the ten patients included, two of them required hemicraniectomy due to malignant middle cerebral artery infarction after transplantation. In the efficacy analysis, there was a trend toward better outcomes in BM-MNC patients when compared to 79 historical controls who met the NIHSS inclusion criteria.

Our group performed a pilot single-blind (outcomes assessor) phase I/II controlled clinical trial in patients with subacute MCA ischemic stroke [47]. The aim was to assess the safety, feasibility, and clinical effects of autologous intra-arterial BM-MNC transplantation. Twenty patients (ten cases and ten controls) with severe ischemic stroke in the middle cerebral artery territory within 5–9 days from stroke onset were included. The primary outcome was safety and feasibility of the procedure. Secondary outcomes were the improvement in neurological function assessed by modified Rankin scale, Barthel index, and NIHSS.

All were severely disabled at inclusion (mean NIHSS score of 15.6 in BM-MNC group vs. 15.0 in control group, $p=0.82$). BM-MNC transplantation was done at 6.4 ± 1.3 days after stroke onset. A mean 1.59×10^8 BM-MNCs ($\pm1.21\times10^8$) were intra-arterially injected. Rate of infusion through microcatheter was 0.5–1 mL/min, as rates of up to 2 mL/min do not seem to produce cell damage nor the use of heparin or iodine contrast [48].

There were no adverse events related to BM-MNC transplantation. No significant hemodynamic or respiratory changes occurred during the bone marrow harvest or the intra-arterial BM-MNC injection. DWI-MRI did not show new ischemic lesions in the active group after transplantation. During follow-up, two BM-MNC-treated patients had an isolated partial seizure. No deaths or stroke recurrence were observed during the follow-up period, and the 6-month MRI also showed no tumor formation in either group. There were no significant differences in neurological function compared to the control group. At 6 months, a greater nonsignificant proportion of BM-MNC-treated patients had an mRankin (modified Rankin) ≤2 (20 %) than the control group (0 %) ($p=0.47$). No differences were found in the Barthel index ($p=0.80$) or in NIHSS scores compared to the control group ($p=0.43$).

Prasad et al. also reported a trial with 11 stroke patients within 7–30 days from stroke onset [49]. Patients received IV BM-MNC transplantation and were followed up for a year, with no detection of tumor formation or other adverse events related to cell therapy.

The same group published in 2014 the biggest trial to date with BM-MNCs using intravenous route, including 120 patients in a phase II trial [50]. Fifty-eight patients were treated with BM-MNCs and 60 patients were controls. Patients with subacute ischemic stroke between 7 and 30 days were included in the study. A randomization was done in a 1:1 ratio and a single intravenous infusion of autologous BM-MNCs was performed in experimental group with a mean of 280.75 million BM-MNCs at median of 18.5 days after stroke onset. During follow-up, 8.4 % patients died and Kaplan-Meier survival curve showed no differences between both groups. Adverse events and serious adverse events were also comparable between the two arms.

In the efficacy analysis, there were no significant differences between BM-MNC arm and control arm in the Barthel index score (63.1 versus 63.6; $p=0.92$), modified Rankin scale shift analysis ($p=0.53$) or score >3 (47.5 % versus 49.2 %; $p=0.85$), NIHSS score (6.3 versus 7.0; $p=0.53$), or change in infarct volume (-11.1 versus -7.36; $P=0.63$) at day 180. Authors concluded that with the methods and timing used, the intravenous injection of BM-MNCs is safe, but there is no beneficial effect on stroke outcome.

Several other trials are ongoing testing different time windows, doses, and routes, which will give more light about the possible efficacy of BM-MNCs in stroke.

One of the *disadvantages* of BM-MNCs is that the mixture of cells (i.e., myeloid, erythroid, lymphoid, and stem cell populations) makes not possible to perform an allogenic BM-MNC transplantation without immunosuppressive drugs due to rejection. Another issue is the variability in the number of cells obtained after a bone marrow harvest, with a variability in final dose of cells injected when a standardized volume of bone marrow is harvested. In our previous trial, a volume of 50 mL of bone marrow leads to doses as different as $0\cdot33$ and $4\cdot96\times10^6$/kg.

On the other hand, the presence of different populations of cells within the mononuclear fraction of bone marrow could be an *advantage* and seems to be beneficial, as not only stem cells contribute to improved outcomes after stroke [51]. In a recent paper, Yang et al. showed that, in a mouse stroke model, both myeloid cells and stem cell populations are important cell types that reduce inflammation and subsequent infarct maturation. The stem cell subpopulation within BM-MNCs is critical for the therapeutic effect in post-stroke recovery. However, myeloid cells (granulocytes and monocytes) seem to modify also pro-inflammatory cytokines and regulate the microglia decreasing the neurotoxic effect and improving neuron survival rates leading to improve stroke outcomes [52].

10.2.4 Human Multipotent Adult Progenitor Cells (MAPCs®)

Recently, multipotent adult progenitor cells (MAPCs), a subpopulation of stem cells isolated from bone marrow, have been described and characterized. Human MAPCs are multipotent stem cells that have been shown to differentiate into various mesodermal cell types, with a remarkable proliferative capacity in culture. In particular, their vascular potential in vitro and in vivo has been demonstrated which make them an attractive candidate for novel cell-based treatment of ischemic diseases. Moreover MAPCs are also immunoprivileged. In a recent preclinical study comparing MSC and MAPC, the latter compared favorably with hMSC and provides a greater beneficial effect as indicated by the increase in angiogenesis, SVZ cell proliferation, and decreased inflammatory response providing an attractive new source of allogenic source of cells for stroke [53]. With data not yet published, Hess et al. (Table 10.1) have communicated the safety and feasibility of intravenous MAPC therapy in acute stroke patients.

10.3 Timing, Route, and Dose of Bone Marrow Stem Cell Transplantation

10.3.1 Time Window

The *optimal time window* for stem cell therapy is not well known. In the stroke rat model, this time window seems to be wide, even up to 1 month after cerebral infarction, but only rats receiving bone marrow stem cells 7 days after MCA occlusion exhibit decreased ischemic lesion volume [54]. However, some groups have demonstrated that an earlier transplantation results in better neurological recovery, especially when MSC or BM-MNC injection is performed during the first week after stroke or even in the first 72 h [55, 56]. Therefore, it is plausible that an earlier treatment could produce a greater effect on inflammation, apoptosis, and remodeling after stroke. In line with this preclinical evidence, our group described that when

BM-MNCs are administered intra-arterially in subacute MCA stroke patients, they seem to induce changes in serum levels of cytokines and growth factors (i.e., GM-CSF, PDGF-BB, and MMP-2) even 3 months after transplantation, which seem to be associated with better functional outcomes in stroke patients [57].

On the other hand, stem cell transplantation in the acute stroke phase (i.e., within 72 h) could be challenging, as these patients are usually neurologically unstable and prone to deteriorate. Also, this short window needs extensive logistical efforts to perform an autologous bone marrow cell injection in a hospitalized stroke patient. An allogenic transplantation would probably be more feasible in this setting although the usual complications of patients in this early stage of stroke could make more difficult to evaluate safety issues of transplantation.

Although there is no much evidence from preclinical studies to perform a clinical trial with stem cells in the chronic phase of stroke, several trials are treating patients with MSCs or BM-MNCs and stable deficit from chronic strokes [31, 41].

10.3.2 Route of Delivery

Based on animal models of stroke, it is not clear which route of delivery is preferable. Although intravenous (IV) stem cell delivery is increasingly used in clinical trials, IV injection leads to an initial random dispersion of cells throughout the body, and recent data suggest that the majority of the stem cells administered are trapped in filter organs such as the lungs, liver, and spleen, with a therapeutically questionable number of cells reaching the ischemic brain [58]. In contrast, intra-arterial cell delivery provides the opportunity to target the entire ischemic lesion enabling exposure of cells to chemoattractant signals (originating from the lesion). Other routes are being tested such as the intrathecal route [59] or the report of Steinberg et al. [31] using intraparenchymal route with exciting preliminary results, but with some serious adverse events (i.e., subdural hematoma and pneumocephalus).

However, similar to prior animal experiments, clinical trials with IV or IA injection of bone marrow stem cells also have found cells sequestered in the spleen, lung, liver, and kidney [55, 60]. This fact raises the question of whether cells need brain homing to produce the beneficial effects or the cytokine and growth factor secretion is enough to improve stroke outcomes. Although paracrine mechanisms are now the leading hypotheses to explain how cell therapies may enhance stroke recovery [16], it seems critical to expose cells to the ischemic environment to stimulate growth factor production [61].

10.3.3 Cell Dose

A wide range of number of cells has been used for transplantation in animal stroke models and in clinical trials. While in preclinical studies there is strong evidence that a higher dose of cells increases the probability of a good neurological outcome

[23, 55], the optimal number of cells to be transplanted for ischemic stroke is unknown. This raises the question of whether a higher dose of stem cells would produce a greater effect in recovery in stroke patients, but to date clinical data regarding dose is scarce.

Our group [47] found that although no significant correlation between the functional status and the amount of transplanted BM-MNCs was detected, there was a trend toward a better outcome when higher numbers of CD34+ cells were injected. In the three follow-up evaluations, a trend to positive correlation with Barthel index and negative correlations with mRankin scale and NIHSS was found, especially in the Barthel index at 1 month after transplantation ($r=0.57$, $p=0.09$). These data may support the hypothesis that a higher number of cells could lead to better outcomes.

Taguchi et al. [62] evaluated in a clinical trial two different doses of BM-MNCs administered intravenously in stroke patients after 7–10 days of stroke onset (250×10^6 and 340×10^6 cells in the lower and higher dose groups, respectively), and although it was a phase I/IIa clinical trial not designed to test efficacy, authors described a trend toward improved neurological outcomes in those patients receiving the higher dose of bone marrow cells.

On the other side, Prasad et al. [50] published a phase II trial including 120 stroke patients with 58 of them being treated with intravenous injection of BM-MNCs, showing no relationship between cell dose and outcomes.

Also, in a meta-analysis of cell-based therapies for treating stroke patients [63], authors found that stem cell therapy was more effective with higher dose of cells and also when intra-arterial route was used.

In a recent pooling data of two different clinical trials with BM-MNCs [64], a higher dose of autologous BM-MNC was related to better outcome in stroke patients. In this paper, 22 patients were analyzed and intra-arterial route was used in 77.3 % and intravenous in 22.7 % of patients. A higher number of cells injected were associated with better outcomes at 6 months ($p=0.015$). Also, a strong negative correlation was found between cell dose and disability when intravenous patients were excluded from analysis ($r=-0.63$, $p=0.006$), pointing to the hypothesis that the combination of higher number of cells and intra-arterial route could be a key factor to improve neurological outcomes in stroke patients. This pooling data showed that the optimal threshold of transplanted cells is probably around 310×10^6 BM-MNCs in order to obtain good functional outcome with high probability among treated stroke patients. However, further clinical data is needed and dose-finding clinical trials are ongoing in ischemic stroke patients [31, 65].

10.4 Conclusions

As no effective neuroprotective or neurorestorative drug has demonstrated efficacy for ischemic stroke, new therapeutic strategies such as cell therapies to enhance neurological recovery after stroke are urgently needed.

Data from preclinical and clinical studies with stem cells in stroke strengthens the notion that stem cells could increase brain plasticity and improve stroke recovery. Extensive preclinical studies have demonstrated large and favorable effects of different types of bone marrow stem cells in stroke.

Several phase I and II clinical trials have been published to date with bone marrow stem cells that have already demonstrated the feasibility and safety of this therapy in the stroke setting. An increasing number of clinical trials, mainly with bone marrow MSCs and BM-MNCs, are ongoing to further assess the best dose, route, and timing of this therapy and to elucidate the efficacy of cell therapy in stroke.

Funding No disclosure information. This study is funded by grants from Junta de Andalucía [TCRM 0001/2006], Fundación Mutua Madrileña, and Instituto de Salud Carlos III [PI15/01197].

References

1. WHO MONICA Project Investigators. The World Health Organization MONICA Project (Monitoring trends and determinants in cardiovascular disease). J Clin Epidemiol. 1988;41:105–14.
2. World Health Organization. The Atlas of disease cardiovascular and stroke. http://www.who.int/cardiovascular_diseases/resources/atlas/en/
3. Sudlow CLM, Warlow CP, for the International stroke incidence collaboration. Comparable studies of the incidence of stroke and its pathological types. Results from a International collaboration. Stroke. 1997;28:491–9.
4. Ovbiagele B, Goldstein LB, Higashida RT, Howard VJ, Johnston SC, Khavjou OA, Lackland DT, Lichtman JH, Mohl S, Sacco RL, Saver JL, Trogdon JG, on behalf of the American Heart Association Advocacy Coordinating Committee and Stroke Council. Forecasting the future of stroke in the United States: a policy statement from the American Heart Association and American Stroke Association. Stroke. 2013;44:2361–75.
5. Gustavsson A, Svensson M, et al. Cost of disorders of the brain in Europe 2010. Eur Neuropsychopharmacol. 2011;21:718–79.
6. Wardlaw JM, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, Cohen G. Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. Lancet. 2012;379:2364–72.
7. Goyal M, Menon BK, van Zwam WH, et al, for the HERMES collaborators. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. Lancet 2016; published online Feb 18. http://dx.doi.org/10.1016/S0140-6736(16)00163-X.
8. Muir KW, White P. HERMES: messenger for stroke interventional treatment. Lancet. 2016 Feb 18. [Epub ahead of print].
9. Lees KR, Zivin JA, Ashwood T, et al. NXY-059 for acute ischemic stroke. N Engl J Med. 2006;354:588–600.
10. Dávalos A, Alvarez-Sabín J, Castillo J, et al. Citicoline in the treatment of acute ischaemic stroke: an international, randomised, multicentre, placebo-controlled study (ICTUS trial). Lancet. 2012;380:349–57.
11. Hermann DM, Chopp M. Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol. 2012;11:369–80.
12. Brenneman M, Sharma S, Harting M, Strong R, Cox Jr CS, Aronowski J, Grotta JC, Savitz SI. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow Metab. 2010;30:140–9.

13. Fujita Y, Ihara M, Ushiki T, Hirai H, Kizaka-Kondoh S, Hiraoka M, Ito H, Takahashi R. Early protective effect of bone marrow mononuclear cells against ischemic white matter damage through augmentation of cerebral blood flow. Stroke. 2010;41:2938–43.
14. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. Lancet Neurol. 2009;8:491–500.
15. Yoo SW, Chang DY, Lee HS, Kim GH, Park JS, Ryu BY, et al. Immune suppression following mesenchymal stem cell transplantation in the ischemic brain is mediated by TGF-β. Neurobiol Dis. 2013;58:249–57.
16. Banerjee S, Williamson DA, Habib N, Chataway J. The potential benefit of stem cell therapy after stroke: an update. Vasc Health Risk Manag. 2012;8:569–80.
17. Rosado-de-Castro PH, Pimentel-Coelho PM, da Fonseca LM, de Freitas GR, Mendez-Otero R. The rise of cell therapy trials for stroke: review of published and registered studies. Stem Cells Dev. 2013;22:2095–111.
18. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells: the International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315–7.
19. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. PLoS One. 2012;7:e47559.
20. Gupta PK, Chullikana A, Parakh R, Desai S, Das A, Gottipamula S, Krishnamurthy S, Anthony N, Pherwani A, Majumdar AS. A double blind randomized placebo controlled phase I/II study assessing the safety and efficacy of allogeneic bone marrow derived mesenchymal stem cell in critical limb ischemia. J Transl Med. 2013;11:143.
21. Chou SH, Lin SZ, Kuo WW, Pai P, Lin JY, Lai CH, Kuo CH, Lin KH, Tsai FJ, Huang CY. Mesenchymal stem cell insights: prospects in cardiovascular therapy. Cell Transplant. 2014;23:513–29.
22. De Miguel MP, Fuentes-Julián S, Blázquez-Martínez A, Pascual CY, Aller MA, Arias J, Arnalich-Montiel F. Immunosuppressive properties of mesenchymal stem cells: advances and applications. Curr Mol Med. 2012;12:574–91.
23. Vu Q, Xie K, Eckert M, Zhao W, Cramer SC. Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. Neurology. 2014;82:1277–86.
24. Chen J, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. J Neurosci Res. 2003;73:778–86.
25. Chen J, Zhang ZG, Li Y, Wang L, Xu YX, Gautam SC, Lu M, Zhu Z, Chopp M. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res. 2003;92:692–9.
26. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol. 2005;57:874–82.
27. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH. OY Bang and STARTING collaborators. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010;28:1099–106.
28. Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, Waxman SG, Kocsis JD. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain. 2011;134:1790–807.
29. 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.
30. Lu SS, Liu S, Zu QQ, Xu XQ, Yu J, Wang JW, Zhang Y, Shi HB. In vivo MR imaging of intraarterially delivered magnetically labeled mesenchymal stem cells in a canine stroke model. PLoS One. 2013;8:e54963.
31. Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, Kim AS, Johnson JN, Bates D, King B, Case C, McGrogan M, Yankee EW, Schwartz NE. Clinical outcomes of transplanted modified bone marrow-derived mesenchymal stem cells in stroke: a phase 1/2a study. Stroke. 2016;47(7):1817–24.

32. Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG, Pittenger MF, van Zijl PC, Huang J, Bulte JW. Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. Stroke. 2008;39:1569–74.
33. Schwarting S, Litwak S, Hao W, Bähr M, Weise J, Neumann H. Hematopoietic stem cells reduce postischemic inflammation and ameliorate ischemic brain injury. Stroke. 2008;39:2867–75.
34. Taguchi A, Soma T, Tanaka H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest. 2004;114:330–8.
35. Shyu WC, Lin SZ, Chiang MF, Su CY, Li H. Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. J Neurosci. 2006;26:3444–53.
36. Chen DC, Lin SZ, Fan JR, Lin CH, Lee W, Lin CC, Liu YJ, Tsai CH, Chen JC, Cho DY, Lee CC, Shyu WC. Intracerebral implantation of autologous peripheral blood stem cells in stroke patients: a randomized phase II study. Cell Transplant. 2014;23:1599.
37. England TJ, Abaei M, Auer DP, Lowe J, Jones DR, Sare G, Walker M, Bath PM. Granulocyte-colony stimulating factor for mobilizing bone marrow stem cells in subacute stroke: the stem cell trial of recovery enhancement after stroke 2 randomized controlled trial. Stroke. 2012;43:405–11.
38. Banerjee S, Bentley P, Hamady M, Marley S, Davis J, Shlebak A, Nicholls J, Williamson DA, Jensen SL, Gordon M, Habib N, Chataway J. Intra-arterial immunoselected CD34+ stem cells for acute ischemic stroke. Stem Cells Transl Med. 2014;3:1322–30.
39. Nakano-Doi A, Nakagomi T, Fujikawa M, Nakagomi N, Kubo S, Lu S, et al. Bone marrow mononuclear cells promote proliferation of endogenous neural stem cells through vascular niches after cerebral infarction. Stem Cells. 2010;28:1292–302.
40. Sharma S, Yang B, Strong R, Xi X, Brenneman M, Grotta JC, et al. Bone marrow mononuclear cells protect neurons and modulate microglia in cell culture models of ischemic stroke. J Neurosci Res. 2010;88:2869–76.
41. Suárez-Monteagudo C, Hernández-Ramírez P, Alvarez-González L, García-Maeso I, de la Cuétara-Bernal K, Castillo-Díaz L, et al. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study. Restor Neurol Neurosci. 2009;27:151–61.
42. Li ZM, Zhang ZT, Guo CJ, Geng FY, Qiang F, Wang LX. Autologous bone marrow mononuclear cell implantation for intracerebral hemorrhage-a prospective clinical observation. Clin Neurol Neurosurg. 2013;115:72–6.
43. Battistella V, de Freitas GR, da Fonseca LM, Mercante D, Gutfilen B, Goldenberg RC, Dias JV, Kasai-Brunswick TH, Wajnberg E, Rosado-de-Castro PH, Alves-Leon SV, Mendez-Otero R, Andre C. Safety of autologous bone marrow mononuclear cell transplantation in patients with nonacute ischemic stroke. Regen Med. 2011;6:45–52.
44. Friedrich MA, Martins MP, Araújo MD, Klamt C, Vedolin L, Garicochea B, Raupp EF, Sartori El Ammar J, Machado DC, Costa JC, Nogueira RG, Rosado-de-Castro PH, Mendez-Otero R, Freitas GR. 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.
45. Rosado de Castro PH, Schmidt FR, Battistella V, Lopes de Souza SA, Gutfilen B, Goldenberg RC, Kasai-Brunswick TH, Vairo L, Silva RM, et al. Biodistribution of bone marrow mononuclear cells after intra-arterial or intravenous transplantation in subacute stroke patients. Regen Med. 2013;8:145–55.
46. Savitz SI, Misra V, Kasam M, Juneja H, Cox Jr CS, Alderman S, Aisiku I, Kar S, Gee A, Grotta JC. Intravenous autologous bone marrow mononuclear cells for ischemic stroke. Ann Neurol. 2011;70:59–69.
47. Moniche F, Gonzalez A, Gonzalez-Marcos JR, Carmona M, Piñero P, Espigado I, Garcia-Solis D, Cayuela A, Montaner J, Boada C, Rosell A, Jimenez MD, Mayol A, Gil-Peralta A. Intra-arterial bone marrow mononuclear cells in ischemic stroke. A pilot clinical trial. Stroke. 2012;43:2242–4.

48. El Khoury R, Misra V, Sharma S, Cox CS, Walker P, Grotta JC, et al. The effect of transcatheter injections on cell viability and cytokine release of mononuclear cells. AJNR Am J Neuroradiol. 2010;31:1488–92.
49. Prasad K, Mohanty S, Bhatia R, Srivastava MV, Garg A, Srivastava A, Goyal V, Tripathi M, Kumar A, Bal C, Vij A, Mishra NK. Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischaemic stroke: a pilot study. Indian J Med Res. 2012;136:221–8.
50. Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S, Singh KK, Nair V, Sarkar RS, Gorthi SP, Hassan KM, Prabhakar S, Marwaha N, Khandelwal N, Misra UK, Kalita J, Nityanand S, InveST Study Group. Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial. Stroke. 2014;45:3618–24.
51. Yang B, Parsha K, Schaar K, Xi X, Aronowski J, Savitz SI. Various cell populations within the mononuclear fraction of bone marrow contribute to the beneficial effects of autologous bone marrow cell therapy in a rodent stroke model. Transl Stroke Res. 2016 Mar 20. [Epub ahead of print].
52. Womble TA, Green S, Shahaduzzaman M, Grieco J, Sanberg PR, Pennypacker KR, Willing AE. Monocytes are essential for the neuroprotective effect of human cord blood cells following middle cerebral artery occlusion in rat. Mol Cell Neurosci. 2014;59:76–84.
53. Mora-Lee S, Sirerol-Piquer MS, Gutiérrez-Pérez M, Gomez-Pinedo U, Roobrouck VD, López T, Casado-Nieto M, Abizanda G, Rabena MT, Verfaille C, Prósper F, García-Verdugo JM. Therapeutic effects of hMAPC and hMSC transplantation after stroke in mice. PLoS One. 2012;7(8):e43683.
54. Komatsu K, Honmou O, Suzuki J, Houkin K, Hamada H, Kocsis JD. Therapeutic time window of mesenchymal stem cells derived from bone marrow after cerebral ischemia. Brain Res. 2010;1334:84–92.
55. Yang B, Strong R, Sharma S, Brenneman M, Mallikarjunarao K, Xi X, Grotta JC, Aronowski J, Savitz SI. Therapeutic time window and dose response of autologous bone marrow mononuclear cells for ischemic stroke. J Neurosci Res. 2011;89:833–9.
56. de Vasconcelos Dos Santos A, da Costa RJ, Diaz Paredes B, Moraes L, Jasmin, Giraldi-Guimarães A, Mendez-Otero R. Therapeutic window for treatment of cortical ischemia with bone marrow-derived cells in rats. Brain Res. 2010;1306:149–58.
57. Moniche F, Montaner J, Gonzalez-Marcos JR, Carmona M, Piñero P, Espigado I, Cayuela A, Escudero I, de la Torre-Laviana FJ, Boada C, Rosell A, Mayol A, Jimenez MD, Gil-Peralta A, Gonzalez A. 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.
58. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev. 2009;18:683–92.
59. Sharma A, Sane H, Gokulchandran N, Khopkar D, Paranjape A, Sundaram J, Gandhi S, Badhe P. Autologous bone marrow mononuclear cells intrathecal transplantation in chronic stroke. Stroke Res Treat. 2014;2014:234095.
60. Barbosa da Fonseca LM, Gutfilen B, Rosado de Castro PH, Battistella V, Goldenberg RC, Kasai-Brunswick T, Chagas CL, Wajnberg E, Maiolino A, Salles Xavier S, Andre C, Mendez-Otero R, de Freitas GR. Migration and homing of bone-marrow mononuclear cells in chronic ischemic stroke after intra-arterial injection. Exp Neurol. 2010;221:122–8.
61. Chen X, Li Y, Wang L, Katakowski M, Zhang L, Chen J, Xu Y, Gautam SC, Chopp M. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. Neuropathology. 2002;22:275–9.
62. Taguchi A, Sakai C, Soma T, Kasahara Y, Stern DM, Kajimoto K, Ihara M, Daimon T, Yamahara K, Doi K, Kohara N, Nishimura H, Matsuyama T, Naritomi H, Sakai N, Nagatsuka K. Intravenous autologous bone marrow mononuclear cell transplantation for stroke: phase1/2a clinical trial in a homogeneous group of stroke patients. Stem Cells Dev. 2015;24:2207–18.

63. Jeong H, Yim HW, Cho YS, Kim YI, Jeong SN, Kim HB, Oh IH. Efficacy and safety of stem cell therapies for patients with stroke: a systematic review and single arm meta-analysis. Int J Stem Cells. 2014;7:63–9.
64. Moniche F, Rosado-de-Castro PH, Escudero I, Zapata E, de la Torre Laviana FJ, Mendez-Otero R, Carmona M, Piñero P, Bustamante A, Lebrato L, Cabezas JA, Gonzalez A, de Freitas GR, Montaner J. Increasing dose of autologous bone marrow mononuclear cells (BM-MNCs) transplantation is related with stroke outcome. Results from a pooled analysis of two clinical trials. Stem Cells Int. 2016;2016:8657173.
65. Moniche F, Escudero I, Zapata-Arriaza E, Usero-Ruiz M, Prieto-León M, de la Torre J, Gamero MA, Tamayo JA, Ochoa-Sepúlveda JJ, Maestre J, Carmona M, Piñero P, Calderón-Cabrera C, Jimenez MD, Gonzalez A, Montaner J. 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.