

## Randomized Controlled Trial

**Phase I / II randomized controlled trial of autologous bone marrow-derived mesenchymal stem cell therapy for chronic stroke**

Kam Sze Tsang, Chi Ping Stephanie Ng, Xian Lun Zhu, George Kwok Chu Wong, Gang Lu, Anil Tejbhan Ahuja, Ka Sing Lawrence Wong, Ho Keung Ng, Wai Sang Poon

Kam Sze Tsang, Chi Ping Stephanie Ng, Xian Lun Zhu, George Kwok Chu Wong, Gang Lu, Wai Sang Poon, Department of Surgery, the Chinese University of Hong Kong, Hong Kong, China

Kam Sze Tsang, Ho Keung Ng, Department of Anatomical and Cellular Pathology, the Chinese University of Hong Kong, Hong Kong, China

Anil Tejbhan Ahuja, Department of Imaging and Interventional Radiology, the Chinese University of Hong Kong, Hong Kong, China

Ka Sing Lawrence Wong, Department of Medicine and Therapeutics, the Chinese University of Hong Kong, Hong Kong, China

**Author contributions:** Tsang KS conducted experiments, analyzed data and wrote the manuscript; Ng CPS coordinated the trial and performed statistical analysis of data; Zhu XL and Wong GKC consulted and administered cells and placebos to patients; Lu G reviewed data; Ahuja AT analyzed radio-imaging; Wong KSL and Ng HK contributed intellectual content; Poon WS designed, supervised and monitored the trial, secured grant support, interpreted data and revised the manuscript.

**Institutional review board statement:** The randomized, controlled, double-blind, phase I / II clinical trial was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee of Hong Kong Hospital Authority in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonisation - Good Clinical Practice.

**Clinical trial registration statement:** The clinical trial CREC #2006.425-T was registered at <http://crec.cuhk.edu.hk>.

**Informed consent statement:** Written informed consent was obtained from all subjects - in the case of vegetative state, from their next of kin.

**Conflict-of-interest statement:** All authors declared that no conflict of interest exists.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Correspondence to:** Wai Sang Poon, MD, Division of Neurosurgery, Department of Surgery, the Chinese University of Hong Kong, Prince of Wales Hospital, 30-32, Ngan Shing Road, Shatin, Hong Kong, China. [wpoon@cuhk.edu.hk](mailto:wpoon@cuhk.edu.hk)  
Telephone: +852-26322624  
Fax: +852-26327974

Received: February 11, 2017  
Peer-review started: February 15, 2017  
First decision: March 8, 2017  
Revised: June 27, 2017  
Accepted: July 14, 2017  
Article in press: July 17, 2017  
Published online: August 26, 2017

**Abstract****AIM**

To examine the safety and efficacy of mesenchymal stem cell (MSC) therapy for intracerebral haemorrhage with neurological dysfunctions for a year.

## METHODS

MSC were *ex vivo* expanded from 29 mL (17-42 mL) autologous bone marrow. Patients were randomized to have two intravenous injections of autologous MSC or placebos in four weeks apart. Neurological functions and clinical outcomes were monitored before treatment and at 12<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week upon completion of the treatment.

## RESULTS

A mean of  $4.57 \times 10^7$  (range:  $1.43 \times 10^7$ - $8.40 \times 10^7$ ) MSC per infusion was administered accounting to  $8.54 \times 10^5$  ( $2.65 \times 10^5$ - $1.45 \times 10^6$ ) per kilogram body weight in two occasions. There was neither adverse event at time of administration nor sign of *de novo* tumour development among patients after monitoring for a year post MSC therapy. Neuro-restoration and clinical improvement in terms of modified Barthel index, functional independence measure and extended Glasgow Outcome Scale were evident among patients having MSC therapy compared to patients receiving placebos.

## CONCLUSION

Intravenous administration of autologous bone marrow-derived MSC is safe and has the potential of improving neurological functions in chronic stroke patients with severe disability.

**Key words:** Stroke; Intracerebral haemorrhage; Central nervous system; Mesenchymal stem cells; Cell therapy

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Contemporary treatments are ineffective in restoring lost neurological functions after stroke. Many stroke patients were noted to have lesions close to the sub-ventricular zone. The likely beneficial effects of mesenchymal stem cell (MSC) treatment might correlate with the spatial lesion, not part of the sub-ventricular zone where endogenous neurogenesis persists during adulthood, and indirect chaperon effects of MSC promote endogenous neuro-regeneration. We administered MSC intravenously to patients having severe neurological disability and presenting stable baseline scores one year after the onset of intracerebral haemorrhage to eliminate confounding attributes to the observation of MSC-mediated neurological recovery.

Tsang KS, Ng CPS, Zhu XL, Wong GKC, Lu G, Ahuja AT, Wong KSL, Ng HK, Poon WS. Phase I / II randomized controlled trial of autologous bone marrow-derived mesenchymal stem cell therapy for chronic stroke. *World J Stem Cells* 2017; 9(8): 133-143 Available from: URL: <http://www.wjgnet.com/1948-0210/full/v9/i8/133.htm> DOI: <http://dx.doi.org/10.4252/wjsc.v9.i8.133>

## INTRODUCTION

Stroke is a common neurological disorder and is a

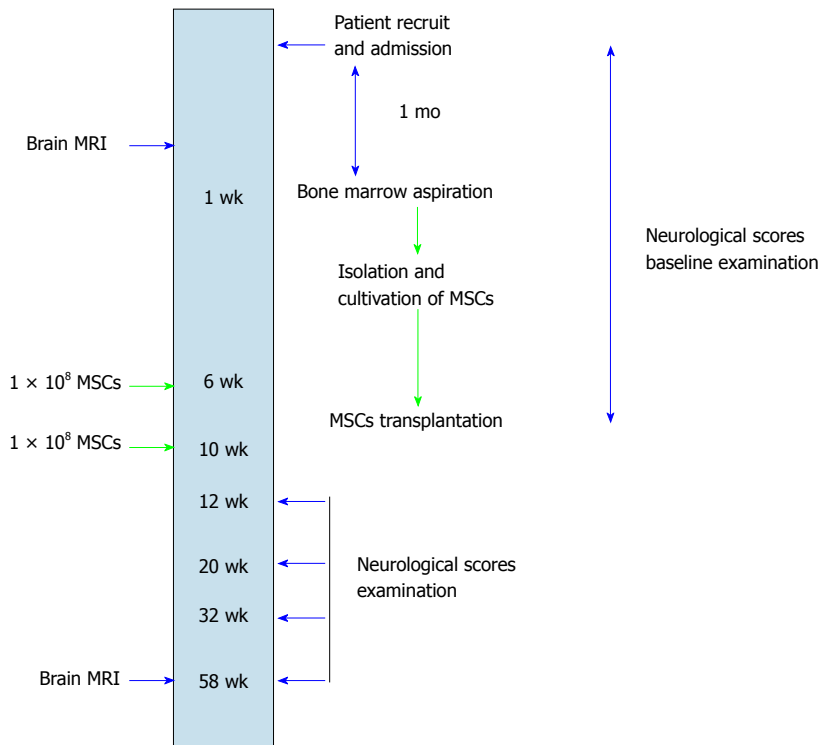
leading cause of death. More than six million cases of stroke are reported in the world annually<sup>[1]</sup>. Approximately 50% of the patients died and most of the survivors are left with various degree of neurological dysfunction. There is no effective treatment for restoring the neurological function of the patients to date. Recent studies in animal models of intra-cerebral haemorrhage demonstrated active neurogenesis in the sub-ventricular zone leading to new neurons of phenotypes of their dead counterparts<sup>[2,3]</sup>. Nevertheless, a majority of newly formed neurons die during the early weeks after stroke, and successful replacement only accounts for a small portion of the mature dead neurons<sup>[4]</sup>. The feasibility of using a variety of cell types including neural stem cells, embryonic stem cells, umbilical cord blood cells and mesenchymal stem cells (MSCs) to enhance re-innervation has been demonstrated in animal models<sup>[5-8]</sup>. The breakthrough opens cellular therapy for stroke. In clinical setting a reliable and accessible cell source is requisite. Bone marrow-derived MSCs, which were noted to generate trophic factors, growth stimulants, signalling regulators and cytokines, might help promote neuro-regeneration and neuro-restoration after stroke *via* neurogenesis, angiogenesis and synaptogenesis<sup>[9]</sup>. Cellular therapy employing large numbers of *ex-vivo* expanded viable MSC might be a potential treatment modality to patients after stroke.

An earlier study demonstrated the feasibility and safety of infusion of autologous MSC in patients nine weeks after stroke onset<sup>[10]</sup>. In the present study we conducted a phase I / II randomized controlled trial of autologous bone marrow-derived MSC therapy in patients one year after onset of stroke with the aim to study the long-term safety and functional efficacy of intravenous administration of MSC.

## MATERIALS AND METHODS

### Study design

This study is a randomized, controlled, double-blind, phase I / II clinical trial (CREC #2006.425-T) and was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee of Hong Kong Hospital Authority in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonisation - Good Clinical Practice. Inclusion criteria are that patient had the onset of stroke for one year ago with stable National Institutes of Health Stroke Scale scores  $\geq 7$  and Glasgow Outcome Scale score of severe disability and vegetative state at one year after onset of stroke<sup>[11,12]</sup>. Exclusion criteria are lacunar syndrome, malignant diseases, severe co-morbidity, hepatic/renal dysfunction and unwillingness to participate. Patients who presented stable baseline scores indicating severe neurological disability were recruited to the study. Informed consent was obtained from all subjects - in the case of vegetative state, from their next of kin. Eligible patients were randomly assigned to the



**Figure 1** Workup schema for patients recruited to the clinical trial of autologous mesenchymal stem cell therapy. MSC: Mesenchymal stem cell; MRI: Magnetic resonance imaging.

treatment group and control group for autologous MSC therapy (Figure 1). The study protocol was developed according to the guidelines of Consolidated Standards of Reporting Trials available on-line at <http://www.consort-statement.org/>.

### Radio-imaging

Computed tomography (CT) scan was conducted at onset of intracerebral haemorrhage. Haematoma volume in mL was computed by using the formula:  $\frac{1}{2} \times \text{maximal height (cm)} \times \text{width (cm)} \times \text{anterior-posterior diameter (cm)}$ . Magnetic resonance imaging (MRI, 1.5 Tesla) of the brain were performed on the day before the first injection of either MSC or placebos. A follow-up procedure was conducted on patients at 60th week upon completion of the study (Figure 1).

### Ex vivo expansion and infusion of MSC

The procedure of MSC expansion described by Le Blanc *et al.*<sup>[13]</sup> was adopted with minor modifications. In brief, bone marrow aspirates from the superior iliac crest of patients under local anaesthesia were anticoagulated in 10 IU/mL preservative-free heparin (DBL, Hospira, Melbourne, Australia). Mononuclear cells were enriched by using density-gradient centrifugation in ficoll-hypaque with specific gravity of 1.077 g/mL (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and cultured in low-glucose Dulbecco modified eagles' medium (Invitrogen, Life Technologies, Carlsbad, CA, United States) supplemented with 10% foetal bovine serum (Invitrogen). MSC cultures of approximately 80% in confluence were passaged using 0.05% trypsin (Invitrogen) and *ex vivo* expanded by subcultures in 175 cm<sup>2</sup> flasks.

On the day of infusion, MSC cultures were enzymatically segregated and dislodged from culture flasks by trypsin digestion, washed with phosphate-buffered saline, sieved to remove cell aggregates *via* 40- $\mu\text{m}$  filter and re-suspended in 10 mL 5% normal human albumin (Hong Kong Red Cross Blood Transfusion Service) for intravenous injection in five to ten minutes. Another booster bolus of autologous MSC was prepared and administered to patient four weeks thereafter. A placebo of an equal volume of 5% normal human albumin was administered to patients being allotted to the control group. Cultures and cell processing were conducted under conditions meeting the requirements of good manufacturing practices.

### Characterisation of MSC

Immunophenotyping of MSC by flow cytometry was reported elsewhere<sup>[14]</sup>. Unless stated otherwise, fluorescence-conjugated monoclonal antibodies from Beckman Coulter were used. They were IgG1-FITC, IgG1-PE, HLA-DR-FITC, CD45-FITC, CD3-FITC, CD19-PE, CD16-FITC, CD33-FITC, CD38-FITC, CD34-PE, CD133-PE (Miltenyi Biotec GmbH, Germany), CD29-PE, CD44-FITC, CD73-FITC, CD90-PE, CD105-PE (Serotec, United Kingdom) and CD166-PE were used. At least 10000 events were acquired and signals were analysed by using the Coulter Epic XL MCL flow cytometer (Coulter, Miami, FL, United States).

Procedural details of immunofluorescence staining were described previously<sup>[15]</sup>. IgM anti-stage-specific embryonic antigen-4 (SSEA-4, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, United States), IgG<sub>2b</sub> anti-octamer-binding transcription factor-4 (Oct-4; 1:100, Santa Cruz Biotechnology), IgG<sub>1</sub> anti-Nestin (1:400;

BD Biosciences, San Francisco, CA, United States) were employed.

Cell viability was evaluated by using trypan blue dye exclusion test. Sterility check against microbial contamination was conducted at each MSC passage.

### Neurological functional assessments

Patient safety and efficacy of cell therapy were evaluated during the first and second MSC infusion and thereafter at 16<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week of the study (Figure 1). The safety of intravenous infusion of either MSC or placebos was assessed in terms of the development of immediate or delayed adverse reactions. Immediate reactions included allergic responses (tachycardia, fever, skin eruption and leucocytosis), local complications (hematoma, local infection at the injection site), vascular obstructions (tachypnea, oliguria, peripheral vascular insufficiency, recurrence of stroke), and systemic complications (systemic infections, increased aspartate aminotransferase, alanine aminotransferase and/or blood urea nitrogen/creatinine levels). Presence of delayed adverse reaction of tumour formation was evaluated by physical examination of skin and oral mucosa and followed up with magnetic resonance imaging (MRI), if necessary. Modified Barthel Index and Functional Independence Measure were monitored by a neurologist being blinded to group allocation and radiological data<sup>[16,17]</sup>. Scores of Extended Glasgow Outcome Scale were also used to track the progress of disability of patients over time<sup>[12]</sup>. Stroke scale scores, vascular risk factors, medical history and demographic details were recorded.

### Statistical analysis

Means, ranges and standard deviations of continuous variables of years of age, kilogram in body weight, milliliter of hematoma and bone marrow, percentages of cell counts and viability were calculated. Assuming that data were normally distributed, non-parametric Wilcoxon's rank sum test was used to compare variables derived from the treatment and control groups in the study. Paired *t*-test with one-sided testing was used to analyse scores of modified Barthel Index, Functional Independence Measure and Extended Glasgow Outcome Scale of patients at time of assessments. Fisher's exact test was applied to examine the incidence of clinical neurological improvement between the treatment and control groups of patients. Differences between groups were regarded as significant if  $P \leq 0.05$ .

## RESULTS

### Patient characteristics

We conducted a double-blind, randomized, controlled phase I/II trial to examine the safety and efficacy of autologous MSC therapy in a small cohort of nine patients (four females and five males) with a mean age of 52 years (range: 41-59 years) who had undergone intracerebral haemorrhage (ICH) for a year. CT scan at

time of the onset demonstrated cerebral haematoma of 52 mL (12-75 mL) located in the basal ganglion region of the brains of the nine patients in the study cohort. The sizes of the lesion areas were comparable between the treatment and control groups.

MSC were *ex vivo* expanded from a mean volume of 29 mL (17-42 mL) autologous bone marrow. Patients were randomized to have two intravenous injections of autologous MSC (treatment group of MSC:  $n = 5$  or control group of placebos:  $n = 4$ ) four weeks apart. The body weight of patients in the treatment group were statistically lighter than those of the control group [treatment vs control; 54.2 kg (42-60 kg) vs 67.2 kg (64-72.7 kg),  $P = 0.03$ ], however the years of age of both groups were comparable [treatment vs control; 53.4 (48-56) vs 51.5 (41-59);  $P = 0.64$ ]. There was no difference between the severities of disability in terms of neurologic scores of patients assigned to both groups (data not shown).

### MSC autograft

*Ex vivo* expanded MSC at a mean of 4 passages (1-8) were used for infusion. MSC up to passage-8 displayed longitudinal, bi-polar, spindle-shaped and fibroblast-like morphology. Immunofluorescence staining demonstrated expressions of embryonic stem cell marker SSEA-4, transcription factor Oct-4 and neural stem cell marker Nestin; suggesting the pluripotency and neurogenesis of MSC (Figure 2). Flow cytometry demonstrated that they were immunophenotypically positive for CD29, CD44, CD73, CD90, CD105 and CD166 (Figure 3A), but negative for haematopoietic stem cell markers (CD34 and CD133), myeloid progenitor cell markers (CD33 and CD38), leucocyte markers (HLA-DR and CD45), T-cell marker CD3, NK cell marker CD16 and B-cell marker CD19 (Figure 3B).

### MSC infusions

Table 1 shows numbers and doses of MSC in 11 episodes of infusion into five patients (three females and two males). A mean of  $4.57 \times 10^7$  ( $1.43 \times 10^7$ - $8.40 \times 10^7$ ) MSC per infusion was administered accounting to  $8.54 \times 10^5$  ( $2.65 \times 10^5$ - $1.45 \times 10^6$ ) per kilogram body weight in two occasions except Patient NSCT02 underwent three infusions. Infused cells were immunophenotypically homogenous; HLA-DR-, CD45-, CD3-, CD19-, CD16-, CD33-, CD38-, CD34- and CD133-positive cells were less than 1% on average, whereas CD29-, CD44-, CD73-, CD90-, CD105- and CD166-positive cells were more than 96% (Supplementary Tables 1 and 2). Cell viability was 94.4% (88.5%-99.0%). There was no microbial growth as evident by aerobic and anaerobic cultures of 11 infusates. The control group of four patients (one female and three males) received placebos in an identical manner. No adverse reaction of acute infusion-related toxicity, transient fever, complication in organs or infection was experienced by both groups of patients at time of and a day following MSC administration. There was no sign of tumour development among patients in

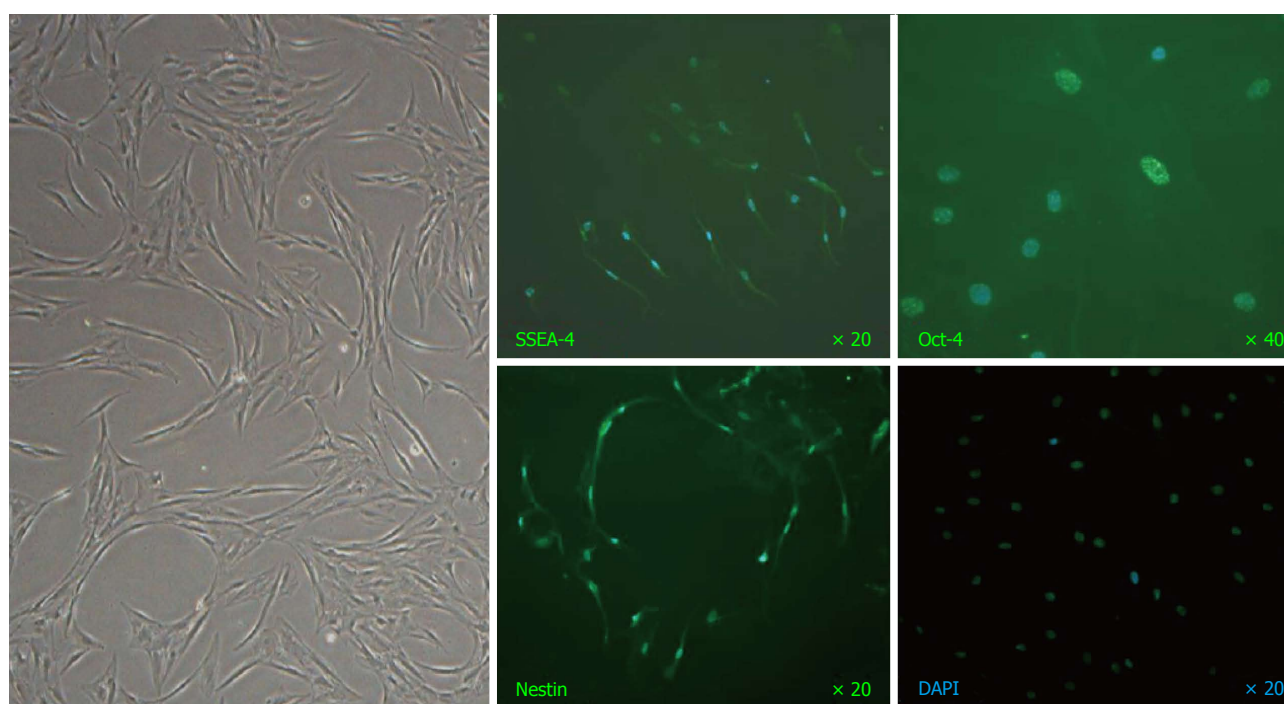


Figure 2 A representative image of mesenchymal stem cells at passage-8 captured under phase-contrast microscopy (left panel) and immunofluorescence staining of stage-specific embryonic antigen SSEA-4, transcription factor Oct-4 and neural stem cell marker Nestin (green fluorescence) with nuclei counterstained by DAPI (blue fluorescence).

Table 1 Characteristics of intracerebral haemorrhage patients and infuses for autologous bone marrow-derived mesenchymal stem cell therapy

Patients	Sex/age in years	Location of haemorrhage	CT readout (cm)	Hemorrhage volume (mL)	MSC passage numbers	Viability (%)	MSC ( $\times 10^7$ )	MSC/kg ( $\times 10^5$ )
UPN02	F/50	Basal ganglia, left	4.4 × 5.8 × 5.9	75	4, 7	96.8	3.1	5.44
					3-5	92.1	3.2	5.61
					3-6	92.6	3.1	5.44
UPN08	F/48	Basal ganglia, left	4.0 × 2.0 × 3.0	12	3, 4	95.8	1.43	2.65
					3, 4	96.5	1.43	2.65
UPN09	M/56	Basal ganglia, right	6.5 × 5.0 × 4.0	65	3-8	98.5	7.6	13.1
					2-6	96.8	4	6.84
UPN10	F/55	Frontal lobe, right	5.6 × 4.5 × 5.0	63	1-6	88.5	5.5	13.1
					1-6	98	5.6	12.4
UPN11	M/55	Basal ganglia, left	2.4 × 5.0 × 3.5	21	1-6	90.5	5.4	9
					1-7	99	8.4	14.5
UPN05	F/59	Basal ganglia, left	6.0 × 4.0 × 4.0	48				
UPN06	M/56	Basal ganglia, frontoparietal temporal lobe, right	7.3 × 3.5 × 5.0	64				
UPN07	M/41	Basal ganglia, right	4.3 × 6.4 × 5.0	69				
UPN12	M/50	Basal ganglia, putaminal, right	NA	55				

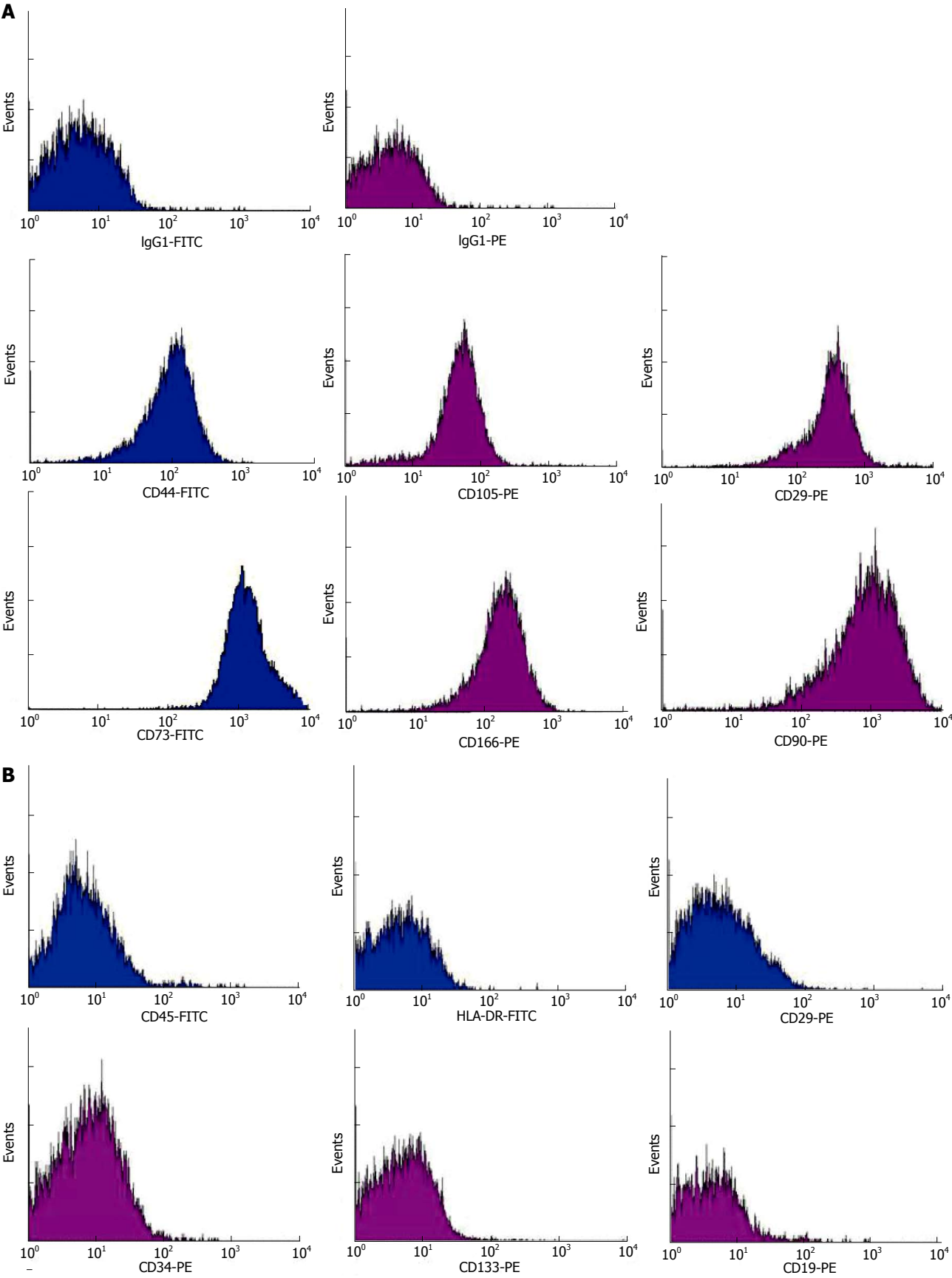
MSC: Mesenchymal stem cell; CT: Computed tomography; NA: Not available.

the study cohort having monitored for a year.

### Functional outcomes

Neurological functions and clinical outcomes were monitored before and at 12<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week upon completion of the treatment. In terms of the scores of modified Barthel Index and Functional Independence Measure, the magnitudes of physical and cognitive disability were comparable between the treatment and control groups. Improvements of motor

disability and cognitive impairment were observed over the course of a year among patients undergoing MSC therapy (Tables 2 and 3). Similar progresses were not apparent in the control group receiving placebos (Supplementary Tables 3 and 5). Scores of Extended Glasgow Outcome Scale demonstrated a trend of improvement of clinical outcomes of patients at 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week upon completion of the MSC therapy (Table 4). Evident clinical improvement in patients of both groups were comparable (Patients with higher



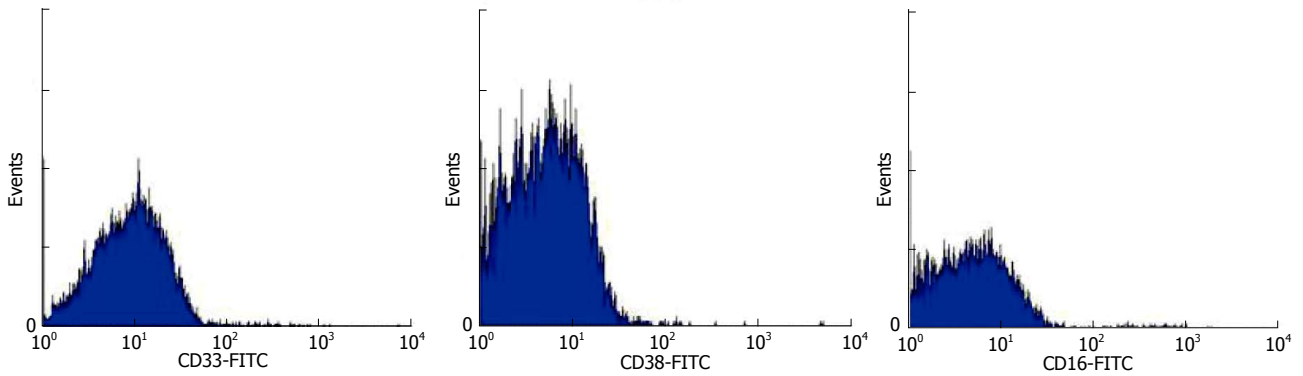


Figure 3 Representative histograms derived from infusates by flow cytometric analyses of mesenchymal stem cell markers CD29, CD44, CD73, CD90, CD105 and CD166 (A) and haemic markers HLA-DR, CD45, CD3, CD19, CD16, CD33, CD38, CD34 and CD133 (B). FITC conjugation in blue and PE conjugation in purple.

**Table 2 Modified Barthel indices of the treatment group (*n* = 5) having mesenchymal stem cell therapy at the 1<sup>st</sup>, 12<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week upon completion of the study**

Study duration (wk)	Modified barthel indices					P value
	UPN02	UPN08	UPN09	UPN10	UPN11	
1 <sup>st</sup>	19	66	0	4	69	
12 <sup>th</sup>	19	73	0	5	70	0.12
16 <sup>th</sup>	30	76	0	5	77	0.03
24 <sup>th</sup>	32	76	0	5	77	0.03
36 <sup>th</sup>	32	78	1	6	77	0.02
60 <sup>th</sup>	32	78	1	-	77	0.03

Data derived from different time points were compared to the baseline values established at the first week of the study.

**Table 3 Functional independence measure of the treatment group (*n* = 5) having mesenchymal stem cell therapy at the 1<sup>st</sup>, 12<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week upon completion of the study**

Study duration (wk)	Scores of functional independence measure					P value
	UPN02	UPN08	UPN09	UPN10	UPN11	
1 <sup>st</sup>	36	72	20	83	70	
12 <sup>th</sup>	35	73	21	88	74	0.07
16 <sup>th</sup>	40	79	21	89	80	0.01
24 <sup>th</sup>	41	85	21	89	84	0.02
36 <sup>th</sup>	44	87	22	102	84	0.01
60 <sup>th</sup>	42	88	22	-	85	0.03

Data derived from different time points were compared to the baseline values established at the first week of the study.

scores of Extended Glasgow Outcome Scale: MSC vs placebo; 3/5 vs 1/4; *P* = 0.52). There was no re-occurrence of ICH among patients in the study.

**Radio-imaging**

Comparing MRI brain before MSC injection and at completion of the study, there was no interval change in morphology.

**DISCUSSION**

In the study we demonstrated the safety, feasibility and

improvement of neurological outcomes of intravenous administration of autologous bone marrow-derived MSC in a small cohort of nine chronic stroke patients one year after intracerebral haemorrhage through a randomized controlled double-blinded phase I / II clinical trial.

Despite advances in neurosurgery and contemporary medical regimes, survivors of intracerebral haemorrhage often suffer long-term to permanent severe disabilities in terms of cognitive impairment and motor dysfunction. Neurological restoration remains poor. There is an imperative to develop therapeutic

**Table 4** Scores of extended Glasgow Outcome Scale of the treatment group ( $n = 5$ ) having mesenchymal stem cell therapy at the 1<sup>st</sup>, 12<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup>, 36<sup>th</sup> and 60<sup>th</sup> week upon completion of the study

Study duration (wk)	Scores of extended glasgow outcome scale					P value
	UPN02	UPN08	UPN09	UPN10	UPN11	
1 <sup>st</sup>	3	4	3	3	4	
12 <sup>th</sup>	3	4	3	3	4	1
16 <sup>th</sup>	3	4	3	3	4	1
24 <sup>th</sup>	3	4	3	3	5	0.19
36 <sup>th</sup>	3	5	3	3	5	0.09
60 <sup>th</sup>	4	6	3	-	5	0.05

Data derived from different time points were compared to the baseline values established at the first week of the study.

modalities to promote neurological recovery. Stem cell-based therapy has drawn a lot of attention recently and the therapeutic efficacies of various cell types were studied<sup>[18]</sup>. Some cell types are deemed difficult for a wide application. Human neural stem cells may be the prototype, however they are not easily harvestable for transplantation unless collected from aborted fetuses or during necropsy<sup>[19,20]</sup>. Embryonic stem cells are capable to give rise to all cell lineages, but the application to brain therapy is hindered by the risk of teratoma development and not to mention the ethical controversy<sup>[21]</sup>. Therapeutic potentials of human stem and progenitor cells from other sources; including bone marrow mononuclear cells, umbilical cord blood CD34<sup>+</sup> cells, dental pulp stem cells, adipose-derived stem cells and bone marrow-derived MSC, have also been widely investigated<sup>[22-26]</sup>. Previous studies indicated that only a limited number of extraneous cells had eventually implanted and integrated into neural networks of recipients. The numbers of successfully engrafted cells were far less than those lost and died to facilitate neuro-restoration. Nonetheless, the implanted cells elicited the neurological recovery *via* indirect chaperon mechanisms of paracrine signalling of cytokines, chemokines, growth factors, trophic factors, signalling regulators and immuno-modulators, which ultimately stimulated endogenous neurogenesis, angiogenesis and synaptogenesis. It is essential to investigate MSC transplantation as a cell therapy for stroke.

Shortly after the first report on the clinical trial of MSC therapy for stroke<sup>[10]</sup>, many issues arose to be resolved before MSC therapy can be safely and effectively administered to stroke patients. A plethora of clinical trials of MSC therapy for stroke, including the present study, were conducted in small cohorts of patients rendering the statistical power of safety and efficacy less valid<sup>[10,27-30]</sup>. In parallel with studies in large patient cohorts, clinical trials in small cohorts of patients would be more easily manageable and feasible to provide data for meta-analysis. The study serves the goal.

Autologous serum and platelet lysate were used to replace foetal calf serum in the supplement of

basal culture media for MSC propagation in fear of the likelihood of zoonosis<sup>[28,31]</sup>. Likewise, animal serum-free chemically modified culture media are feasible alternatives to override the likely hurdle<sup>[27]</sup>. There were concerns of loss of stemness, change of functions, senescence and transformation of prolonged cultures of human MSC, nonetheless little report on the clinical trial of human MSC at high passages is available. Honmou *et al.*<sup>[28]</sup> reported no side effect on the administration of autologous MSC at passages  $\leq 3$  in stroke patients during one year of follow-up. More pronounced neurogenesis was observed in a rat stroke model receiving human MSC at earlier passage 2 than counterparts having human MSC at later passage 6<sup>[32]</sup>. Bernardo and co-workers reported that long-term *in vitro* cultures of human bone marrow-derived MSC up to passage 25 are not susceptible to malignant transformation<sup>[33]</sup>. In the study we investigated MSC at passages up to 8. There was neither morphological changes, phenotypic alterations nor growth senescence. No infusion-related toxicity and complications were experienced by patients at time and upon completion of MSC infusion. Data of the study suggest that MSC up to passage 8 are applicable to clinical use without a compromise of safety over an observation period of a year.

It is intuitive that MSC should rest precisely in the locality of interest in the brain in order to achieve the optimal therapeutic effects. Data of clinical trials of MSC therapy demonstrate that intravenous administration is a feasible approach<sup>[10,27,28,30]</sup>. However, the homing of MSC into the brain was shown to be limited and many cells were trapped into the peripheral organs especially the lungs. Alternate means of intra-arterial delivery and intracranial injection using stereotactic device were also reported to be safe and feasible in human<sup>[29,34,35]</sup>. Nonetheless, intra-arterial administration was found not superior to intravenous delivery of bone marrow mononuclear cells in a rat stroke model<sup>[36]</sup>. Both modes of cell delivery achieve comparable structural and functional outcomes in stroke animals after stem cell therapy despite the low homing efficiency.

The optimal dose of MSC applied to human is largely



unknown. The empirical cell numbers of  $0.5 - 5 \times 10^8$  in human are extrapolated from the effective dose of  $0.1 - 3 \times 10^5$  cells per rat in rat stroke model<sup>[10,28,37]</sup>. Cells of  $5 \times 10^7$  were administered twice in the first report on the clinical trial of MSC therapy for stroke and better outcome in Barthel index was noted one year post-treatment<sup>[10]</sup>. Bhasin *et al.*<sup>[27]</sup> transplanted a mean of  $5-6 \times 10^7$  MSC and reported neural plasticity. Honmou *et al.*<sup>[28]</sup> administered intravenously  $0.6-1.6 \times 10^8$  cells per patient and observed reduction of lesion size by  $> 20\%$  after one week. In the study a mean of  $4.6 \times 10^7$  MSC was administered twice and improvements of motor disability and cognitive impairment were noted.

At present, available reports on clinical trials of MSC therapy suggest neuro-restoration, increase of neural plasticity and reduction of lesion volume<sup>[10,27-30,38]</sup>. Many stroke patients were noted to have lesions close to the sub-ventricular zone<sup>[39]</sup>. The likely beneficial effects of MSC treatment might correlate with the spatial lesion not part of the sub-ventricular zone where endogenous neurogenesis persists during adulthood and indirect chaperon effects of MSC promote endogenous neuro-regeneration<sup>[40]</sup>. In the study we administered MSC to patients having severe neurological disability and presenting stable baseline scores one year after the onset of intracerebral haemorrhage to eliminate confounding attributes to the observation of MSC-mediated neurological recovery.

Data of the study suggest that intravenous administration of autologous bone marrow-derived MSC is safe and facilitate the recovery of neurological functions in patients with severe disability long after the onset of intracerebral haemorrhage. Clinical neurological improvement of patients having MSC therapy was evident compared to patients receiving placebos. MSC therapy is effective independently of other treatment courses. The work may help define criteria for future phase III studies.

## ACKNOWLEDGMENTS

The GMP facility of Stem Cell Laboratory located at Li Ka Shing Institute of Health Sciences, Faculty of Medicine, the Chinese University of Hong Kong was utilised to *ex vivo* expand bone marrow-derived mesenchymal stem cells. We are thankful to the patients participating in the study. We are also grateful to the occupational therapists and physiotherapists of Prince of Wales Hospital in conducting the functional assessments.

## COMMENTS

### Background

This study was initiated from the randomised Phase II from the Korean Neurologist, suggesting efficacy. Collaborating with the authors Bone Marrow Transplant Unit, the authors were able to be using autologous bone marrow mesenchymal stem cells (MSCs) of sufficient number for two interval infusion.

### Research frontiers

To generate efficacy data post-stroke is important. At present there has been no

effective treatment for improving neurological deficits after any stroke illnesses.

### Innovations and breakthroughs

There were more data on ischaemic stroke. For brain haemorrhage, clinical data were scarce. This study showed a trend towards improvement for intracerebral haemorrhage.

### Applications

The report of this work demonstrated a trend of clinical improvement on two intravenous infusions of autologous bone marrow MSC in four weeks apart.

### Terminology

The work provided supportive evidence for phase III randomized controlled trial in the future.

### Peer-review

This manuscript is worth publishing, reporting the result of the phase I / II clinical trial of autologous BM-MSC transplantation therapy in a small cohort of patients with chronic brain haemorrhage.

## REFERENCES

- 1 **WHO.** Global status report on noncommunicable diseases 2014. Available from: URL: <http://www.who.int/nmh/publications/ncd-status-report-2014/en/>
- 2 **Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM.** Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann Neurol* 2002; **52**: 802-813 [PMID: 12447935 DOI: 10.1002/ana.10393]
- 3 **Jin K, Sun Y, Xie L, Peel A, Mao XO, Betteur S, Greenberg DA.** Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. *Mol Cell Neurosci* 2003; **24**: 171-189 [PMID: 14550778 DOI: 10.1016/S1044-7431(03)00159-3]
- 4 **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**: 963-970 [PMID: 12161747 DOI: 10.1038/nm747]
- 5 **Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK.** Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proc Natl Acad Sci USA* 2004; **101**: 11839-11844 [PMID: 15280535 DOI: 10.1073/pnas.0404474101]
- 6 **Yoshizaki T, Inaji M, Kouike H, Shimazaki T, Sawamoto K, Ando K, Date I, Kobayashi K, Suhara T, Uchiyama Y, Okano H.** Isolation and transplantation of dopaminergic neurons generated from mouse embryonic stem cells. *Neurosci Lett* 2004; **363**: 33-37 [PMID: 15157991 DOI: 10.1016/j.neulet.2004.03.074]
- 7 **Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T.** Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* 2004; **114**: 330-338 [PMID: 15286799 DOI: 10.1172/JCI20622]
- 8 **Shen LH, Li Y, Chen J, Cui Y, Zhang C, Kapke A, Lu M, Savant-Bhonsale S, Chopp M.** One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. *Stroke* 2007; **38**: 2150-2156 [PMID: 17525391 DOI: 10.1161/STROKEAHA.106.481218]
- 9 **Chopp M, Li Y.** Treatment of neural injury with marrow stromal cells. *Lancet Neurol* 2002; **1**: 92-100 [PMID: 12849513 DOI: 10.1016/S1474-4422(02)00040-6]
- 10 **Bang OY, Lee JS, Lee PH, Lee G.** Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 2005; **57**: 874-882 [PMID: 15929052 DOI: 10.1002/ana.20501]
- 11 **NIH.** National Institute of Neurological Disorders and Stroke. Stroke Scale. Available from: URL: [http://www.ninds.nih.gov/doctors/NIH\\_Stroke\\_Scale.pdf](http://www.ninds.nih.gov/doctors/NIH_Stroke_Scale.pdf)
- 12 **Jennett B, Snoek J, Bond MR, Brooks N.** Disability after severe head injury: observations on the use of the Glasgow Outcome

- Scale. *J Neurol Neurosurg Psychiatry* 1981; **44**: 285-293 [PMID: 6453957]
- 13 **Le Blanc K**, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; **363**: 1439-1441 [PMID: 15121408 DOI: 10.1016/S0140-6736(04)16104-7]
  - 14 **Li G**, Zhang XA, Wang H, Wang X, Meng CL, Chan CY, Yew DT, Tsang KS, Li K, Tsai SN, Ngai SM, Han ZC, Lin MC, He ML, Kung HF. Comparative proteomic analysis of mesenchymal stem cells derived from human bone marrow, umbilical cord, and placenta: implication in the migration. *Proteomics* 2009; **9**: 20-30 [PMID: 19116983 DOI: 10.1002/pmic.200701195]
  - 15 **Fong SP**, Tsang KS, Chan AB, Lu G, Poon WS, Li K, Baum LW, Ng HK. Trophism of neural progenitor cells to embryonic stem cells: neural induction and transplantation in a mouse ischemic stroke model. *J Neurosci Res* 2007; **85**: 1851-1862 [PMID: 17492787 DOI: 10.1002/jnr.21319]
  - 16 **Shah S**, Vanclay F, Cooper B. Improving the sensitivity of the Barthel Index for stroke rehabilitation. *J Clin Epidemiol* 1989; **42**: 703-709 [PMID: 2760661]
  - 17 **Keith RA**, Granger CV, Hamilton BB, Sherwin FS. The functional independence measure: a new tool for rehabilitation. *Adv Clin Rehabil* 1987; **1**: 6-18 [PMID: 3503663]
  - 18 **Doepfner TR**, Hermann DM. Stem cell-based treatments against stroke: observations from human proof-of-concept studies and considerations regarding clinical applicability. *Front Cell Neurosci* 2014; **8**: 357 [PMID: 25400548 DOI: 10.3389/fncel.2014.00357]
  - 19 **Amariglio N**, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, Trakhtenbrot L, Paz N, Koren-Michowitz M, Waldman D, Leider-Trejo L, Toren A, Constantini S, Rechavi G. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med* 2009; **6**: e1000029 [PMID: 19226183 DOI: 10.1371/journal.pmed.1000029]
  - 20 **Sanai N**, Tramontin AD, Quiñones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S, Lawton MT, McDermott MW, Parsa AT, Manuel-García Verdugo J, Berger MS, Alvarez-Buylla A. Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* 2004; **427**: 740-744 [PMID: 14973487 DOI: 10.1038/nature02301]
  - 21 **Blum B**, Benvenisty N. The tumorigenicity of human embryonic stem cells. *Adv Cancer Res* 2008; **100**: 133-158 [PMID: 18620095 DOI: 10.1016/S0065-230X(08)00005-5]
  - 22 **Savitz SI**, Misra V, Kasam M, Juneja H, Cox CS Jr, 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 [PMID: 21786299 DOI: 10.1002/ana.22458]
  - 23 **Tsuji M**, Taguchi A, Ohshima M, Kasahara Y, Sato Y, Tsuda H, Otani K, Yamahara K, Ihara M, Harada-Shiba M, Ikeda T, Matsuyama T. Effects of intravenous administration of umbilical cord blood CD34(+) cells in a mouse model of neonatal stroke. *Neuroscience* 2014; **263**: 148-158 [PMID: 24444827 DOI: 10.1016/j.neuroscience.2014.01.018]
  - 24 **Leong WK**, Henshall TL, Arthur A, Kremer KL, Lewis MD, Helps SC, Field J, Hamilton-Bruce MA, Warming S, Manavis J, Vink R, Gronthos S, Koblar SA. Human adult dental pulp stem cells enhance poststroke functional recovery through non-neural replacement mechanisms. *Stem Cells Transl Med* 2012; **1**: 177-187 [PMID: 23197777 DOI: 10.5966/sctm.2011-0039]
  - 25 **Yang KL**, Lee JT, Pang CY, Lee TY, Chen SP, Liew HK, Chen SY, Chen TY, Lin PY. Human adipose-derived stem cells for the treatment of intracerebral hemorrhage in rats via femoral intravenous injection. *Cell Mol Biol Lett* 2012; **17**: 376-392 [PMID: 22544763 DOI: 10.2478/s11658-012-0016-5]
  - 26 **Zheng W**, Honmou O, Miyata K, Harada K, Suzuki J, Liu H, Houkin K, Hamada H, Kocsis JD. Therapeutic benefits of human mesenchymal stem cells derived from bone marrow after global cerebral ischemia. *Brain Res* 2010; **1310**: 8-16 [PMID: 19913518 DOI: 10.1016/j.brainres.2009.11.012]
  - 27 **Bhasin A**, Srivastava MV, Kumaran SS, Mohanty S, Bhatia R, Bose S, Gaikwad S, Garg A, Airan B. Autologous mesenchymal stem cells in chronic stroke. *Cerebrovasc Dis Extra* 2011; **1**: 93-104 [PMID: 22566987 DOI: 10.1159/000333381]
  - 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**: 1790-1807 [PMID: 21493695 DOI: 10.1093/brain/awr063]
  - 29 **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**: 2291-2298 [PMID: 23127560 DOI: 10.3727/096368912X658818]
  - 30 **Diez-Tejedor E**, Gutiérrez-Fernández M, Martínez-Sánchez P, Rodríguez-Frutos B, Ruiz-Ares G, Lara ML, Gimeno BF. 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**: 2694-2700 [PMID: 25304723 DOI: 10.1016/j.jstrokecerebrovasdis.2014.06.011]
  - 31 **Shih DT**, Burnouf T. Preparation, quality criteria, and properties of human blood platelet lysate supplements for ex vivo stem cell expansion. *N Biotechnol* 2015; **32**: 199-211 [PMID: 24929129 DOI: 10.1016/j.nbt.2014.06.001]
  - 32 **Li WY**, Choi YJ, Lee PH, Huh K, Kang YM, Kim HS, Ahn YH, Lee G, Bang OY. Mesenchymal stem cells for ischemic stroke: changes in effects after ex vivo culturing. *Cell Transplant* 2008; **17**: 1045-1059 [PMID: 19177841 DOI: 10.3727/096368908786991551]
  - 33 **Bernardo ME**, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 2007; **67**: 9142-9149 [PMID: 17909019 DOI: 10.1158/0008-5472.CAN-06-4690]
  - 34 **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**: 2242-2244 [PMID: 22764211 DOI: 10.1161/STROKEAHA.112.659409]
  - 35 **Suárez-Monteagudo C**, Hernández-Ramírez P, Alvarez-González L, García-Maeso I, de la Cuétara-Bernal K, Castillo-Díaz L, Bringas-Vega ML, Martínez-Aching G, Morales-Chacón LM, Báez-Martín MM, Sánchez-Catasús C, Carballo-Barreda M, Rodríguez-Rojas R, Gómez-Fernández L, Alberti-Amador E, Macías-Abraham C, Balea ED, Rosales LC, Del Valle Pérez L, Ferrer BB, González RM, Bergado JA. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study. *Restor Neurol Neurosci* 2009; **27**: 151-161 [PMID: 19531871 DOI: 10.3233/RNN-2009-0483]
  - 36 **Yang B**, Migliati E, Parsha K, Schaar K, Xi X, Aronowski J, Savitz SI. Intra-arterial delivery is not superior to intravenous delivery of autologous bone marrow mononuclear cells in acute ischemic stroke. *Stroke* 2013; **44**: 3463-3472 [PMID: 24114454 DOI: 10.1161/STROKEAHA.111.000821]
  - 37 **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**: 778-786 [PMID: 12949903 DOI: 10.1002/jnr.10691]
  - 38 **Lee JS**, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY; STARTING collaborators. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells* 2010; **28**: 1099-1106 [PMID: 20506226 DOI: 10.1002/stem.430]
  - 39 **Delavaran H**, Sjunnesson H, Arvidsson A, Lindvall O, Norrving B, van Westen D, Kokaia Z, Lindgren A. Proximity of brain infarcts to

regions of endogenous neurogenesis and involvement of striatum in ischaemic stroke. *Eur J Neurol* 2013; **20**: 473-479 [PMID: 23057628 DOI: 10.1111/j.1468-1331.2012.03877.x]

40 **Jessberger S**, Gage FH. Adult neurogenesis: bridging the gap between mice and humans. *Trends Cell Biol* 2014; **24**: 558-563 [PMID: 25124338 DOI: 10.1016/j.tcb.2014.07.003]

**P- Reviewer:** Jun Y, Saeki K **S- Editor:** Ji FF **L- Editor:** A  
**E- Editor:** Lu YJ





Published by **Baishideng Publishing Group Inc**  
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <http://www.f6publishing.com/helpdesk>  
<http://www.wjgnet.com>

