International Journal of
Critical Care and Emergency Medicine
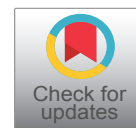
ORIGINAL ARTICLE

Stem Cell Therapy for Ischemic Stroke: From Bench to Bedside

Jolien De Meyer[1], Jolien De Pryck[1] and Said Hachimi-Idrissi[1,2*]

[1]Faculty of Medicine and Health Sciences, Ghent University, Belgium

[2]Department of Emergency Medicine, Ghent University Hospital, Belgium

***Corresponding author:** Saïd Hachimi-Idrissi MD, PhD, FCCM, Faculty of Medicine and Health Sciences and Department of Emergency Medicine, Ghent University Hospital, Corneel Heymanslaan 10, 9000 Gent, Belgium, Tel: +32-93324409, Fax: +3293324980*

Check for updates

Abstract

Introduction: Every year ischemic stroke takes many lives and leaves millions of people with neurological deficits. Currently the only approved therapy is recombinant tissue plasminogen activator, which should be administered within a narrow time window of 4.5 hours. Stem cell therapy was first initiated in several preclinical studies with promising results and lately in some clinical trials. Our research consists of 2 systematic reviews where preclinical and clinical studies were pooled. We provide a systemic review of the evidence of efficacy of cell-based therapy in both preclinical and clinical setting.

Materials and methodology: After screening of databases, 76 studies were included in our systematic review of studies in rodent stroke models and 4 randomized clinical trials were used for the systematic review of studies in humans. After data extraction and assessment of study quality, the pooled effects were calculated using Revman5.

Results: Stem cell therapy has a positive effect on behavior and histological outcome in rodent stroke models. These results are in line with previously conducted meta-analyses. This improvement in rodents is not translated into clinical trials in humans. Pooled study data of the randomized controlled clinical trials did show a significant improvement in neurologic outcome, but not in functional recovery.

Conclusion: Study quality is pointed out as one of the major reasons for failed translation of preclinical evidence to clinic. Large, well-designed preclinical trials are urgently needed. Good preclinical research is necessary to determine the optimal route of administration, the optimal cell dose and type and the most accurate administration time of the stem cells.

Keywords

Systematic review, Stroke, Stem cells therapy, Translation, Human, Rodents, Neurorestoration

Introduction

Stroke remains a major universal health problem. On yearly basis numbers of stroke patients who live with the consequences or subsequently died from it, are increasing. The ischemic stroke or infarction represents almost 70 percent of all strokes [1]. It is the second leading cause of global death [2].

Notwithstanding the advances in acute care and secondary preventive approaches, a stroke continues to be a major burden on the healthcare system worldwide. Currently the only approved medical therapy for patients with an acute ischemic stroke is recombinant tissue plasminogen activator (rtPA). International guidelines advise rtPA as a first-line treatment for eligible patients when administered within 4.5 hours after the onset of stroke [3,4]. Despite this recommendation, rtPA is widely underused [4]. Only a minority of patients receives thrombolysis, primarily because of delayed admission to a stroke center and the short time window for the use of rtPA [4]. Even when patients are fortunate enough to receive rtPA in the time frame, several neurological deficits persist [5]. After three hours neurological cells become irreversibly damaged [6]. Once stroke-induced cell damage has occurred, little can be done to improve the neurological function, except for rehabilitation therapy and pharmacological management of comorbidities [7].

It becomes clear that other approaches promoting recovery of neurological cells rather than neuroprotection need to be explored and developed. Observations from animal studies suggest that improved fun-

CLINMED
INTERNATIONAL LIBRARY

ctioning is associated with restorative processes [5]. Following a stroke, patients show some recovery trough plasticity and brain remodeling, and questions are raised if cellular therapy could be used to tap into these recovery processes and promote recovery of function [5]. The Stroke Progress review group has now identified neurorestoration as a major priority for stroke research [8]. Cell therapies account for a different biopharmaceutical approach than rtPA. Whereas rtPA only has a protective role and a narrow efficacy time window, cellular therapies have more neurorestorative mechanisms of action. Stem cell transplantation is highly convergent with rehabilitation and has a wider therapeutic time window [9].

Cellular therapies comprise several cell types and different sources have been used in preclinical animal studies. These cell sources can be divided in three major groups: Embryonic or fetal cells, birth related cells and adult cells [9]. Although some cell types are more widely used in preclinical settings, there is still not enough evidence to withhold one particular cell type for clinical practice [8]. Several delivery routes can be used to administer these stem cells. Each delivery method has its safety issues, strengths and weaknesses. The stereotactic, intracerebral administration of stem cells relies on the tissue restoration hypothesis [10]. This delivery route has the advantage that more cells are implanted in the lesion site, but it may cause several safety issues and concomitantly brain secondary damage [8,10]. Intra-arterial administration of stem cells happens by an injection close to target which enhances the homing to the brain [11]. Following the chemoattractant gradient generated by the ischemic brain some of the stem cells penetrate the blood brain barrier. The number of cells reaching the lesion site is smaller than with intracerebral administration, but grafted cells do not necessarily have to be near the lesion site to have effect [8]. Intra-arterial delivery is an easy administration route with minimal invasiveness. It leads to a widespread cell distribution and secretion [8]. There is a lower risk of cell trapping, but large amounts of cells pose the hazard of secondary infarctions by microvascular plugging in the brain [9-11]. Intravenous administered cells do not reach the infarcted brain region in sufficient quantities to have a direct neurorestorative effect [9]. The cultured cells are indeed too big to pass the capillaries of the filtering systemic organs and are trapped in the lungs and spleen [10]. Cell homing is thus comprised, but intravenously administered stem cells do impact brain inflammation and repair through bystander mechanisms [8,9]. Intravenous injection is also considered as a safe, minimal invasive delivery route with wide distribution of cells [11]. The trapping in lungs and spleen however, poses the risk of splenic and pulmonary infarction [10,11]. Intracerebral administration is considered too invasive to be used in clinical practice, so intravascular injection definitely seems a more useable method [12]. Unfortunately, the-

re are no valid studies directly comparing the different vascular cell routes. Hence, it is too early to make clear statements about the optimal time, dose and cell delivery route. Route of administration thus poses another problem in the design of clinical trials [8].

Several studies in animal models of ischemic stroke have shown that stem cell transplantation can lead to structural and functional improvement [13,14]. The evidence for benefits in ischemic stroke patients however, is still lacking. In 2010 the Cochrane collaboration tried to establish a meta-analysis of randomized controlled trials (RCT) in humans. But with only one valid reference it became clear that more studies are desperately needed [7]. This review consists of 2 systematic reviews and provides a comparison between preclinical animal studies and clinical studies in humans about the use of stem cells as a therapy for a stroke.

Materials and Methods

Search strategy

Preclinical study: Animal studies of stem cell therapies for stroke were identified from electronic databases PUBMED and EMBASE. The following search strategy was used to screen PUBMED: ((((((cell transplantation) OR stem cell transplantation) OR cord blood stem cell transplantation) OR hematopoietic stem cell transplantation) OR mesenchymal stem cell transplantation) OR peripheral blood stem cell transplantation)) OR (((((((((((stem cells) OR adult stem cells) OR multipotent stem cells) OR totipotent stem cells) OR pluripotent stem cells) OR mesenchymal stem cells) OR fetal stem cells) OR embryonic stem cells) OR hematopoietic stem cells) OR tumor stem cells) OR myeloid progenitor cells) OR fibroblast))) AND (((((((((cerebrovascular disorders) OR basal ganglia cerebrovascular disease) OR brain ischemia) OR stroke) OR middle cerebral artery) OR MCA) OR anterior cerebral artery) OR ACA)))) AND ((((Rodent) OR Rat) OR mouse)) AND (((((controlled) OR control group) OR sham) OR placebo) OR comparison). The number of references were narrowed by using the filters 'Other Animals' and 'English'. This research strategy was used to screen EMBASE as well. Duplicates excluded, a total of 780 references were imported in Endnote for further screening. 514 references were excluded (Figure 1). The remaining 266 studies were subject to full reading of abstract and quick view on the full text. 97 studies were isolated for intensive reading of full text. Eligible studies were vehicle-controlled studies that reported histological (infarct size) or behavioural outcome of allogeneic or autologous stem cells, stem-like or progenitor-like cells in adult rodent models of focal cerebral ischemia. The ischemic stroke had to be induced trough transient or permanent anterior or middle cerebral artery occlusion. Studies were excluded

Figure 1: Flow diagram of the preclinical studies.
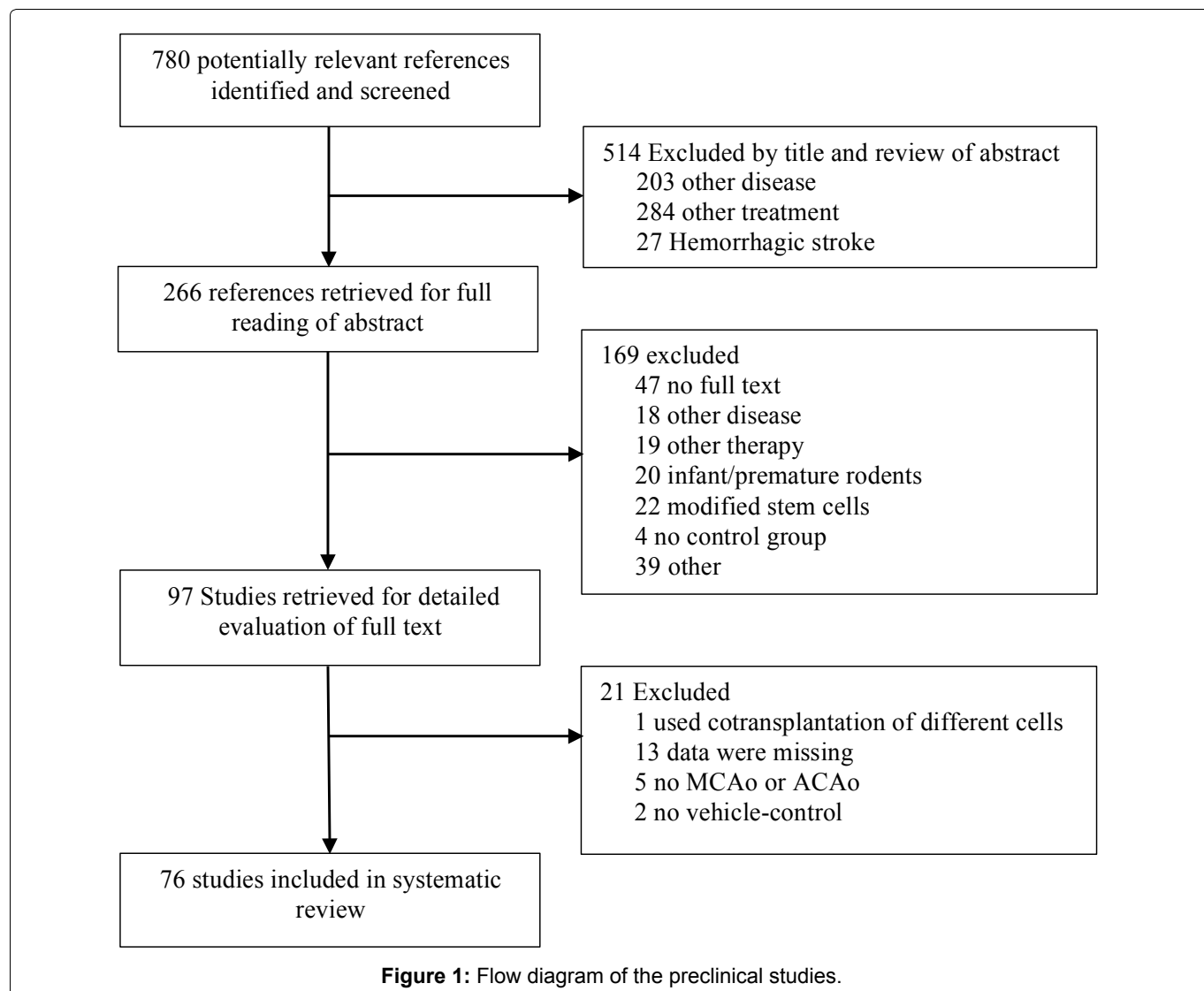
if the therapy involved additional active components such as bioscaffolding or gene modification other than labelling or tracing markers or if the studies involved co-transplantation of different stem cells. Finally, 76 references, with 101 treatment arms fulfilled all proposed criteria and were included in the systematic review.

Clinical study: Studies of stem cell therapies for stroke in humans were identified from electronic databases PUBMED, EMBASE and the Cochrane Central Register of Controlled Trials (CENTRAL). The following search strategy was used to screen PUBMED: (((((((cell transplantation) OR stem cell transplantation) OR cord blood stem cell transplantation) OR hematopoietic stem cell transplantation) OR mesenchymal stem cell transplantation) OR peripheral blood stem cell transplantation)) OR ((((((((((((stem cells) OR adult stem cells) OR multipotent stem cells) OR totipotent stem cells) OR pluripotent stem cells) OR mesenchymal stem cells) OR fetal stem cells) OR embryonic stem cells) OR hematopoietic stem cells) OR tumor stem cells) OR myeloid progenitor cells) OR fibroblast))) AND (((((((cerebrovascular disorders) OR basal ganglia cerebrovascular disease) OR brain ischemia) OR stroke)

OR middle cerebral artery) OR MCA) OR anterior cerebral artery) OR ACA). This search strategy was modified to search the other databases. In PUBMED the filters 'humans' and 'clinical trial' were activated. In EMBASE the search was limited to 'humans' only. Duplicates excluded, a total of 806 references was found. 532 were excluded. The remaining 274 studies were subject to full reading of abstract and quick view of the full text. 16 articles were isolated for reading of full text. Finally, 4 randomized controlled clinical trials (RCTs) were used in the systematic review (Figure 2). All the RCTs involved patients with ischemic stroke, a minimum of six-month follow-up and efficacy as primary or secondary outcome.

Data extraction and statistical analysis

We withdrew data from all available sources in each article, including text and graph. When solely graphic presentation was accessible, values for mean and standard deviation (SD) were manually obtained. When outcomes were reported at numerous time points, only the final assessments were accepted. We used RevMan version 5.3 [15] for all data and analysis. A P-value of < 0.05 was considered significant.
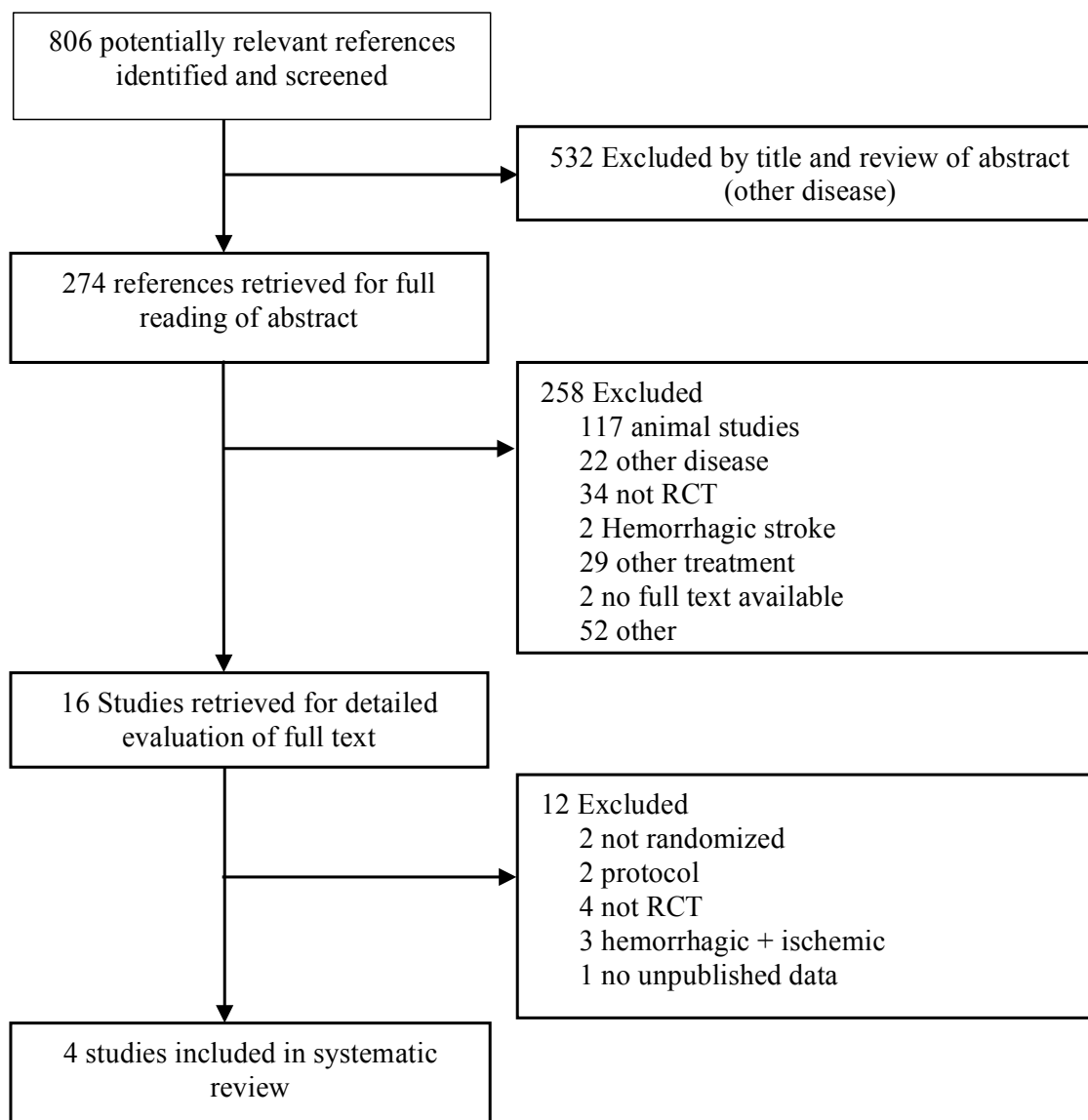
De Meyer et al. Int J Crit Care Emerg Med 2018, 4:058

• Page 3 of 19 •

Figure 2: Flow diagram of the clinical studies.

Preclinical study: Efficacy of stem cell transplantation in animal models was assessed trough two outcomes: Behavior and histology. These outcomes were analyzed separately and so when both were reported, we extracted both. Our statistical methods were based on previous meta-analyses [13,14].

For the assessment of the effect on behavior, the nine most frequently applied behavioral tests were incorporated in the analysis: Modified neurologic severity score (mNSS), cylinder test, rotarod test, beam walk, treadmill stress test, forelimb reaching, body swing, corner test and adhesive removal test. Infarct volume was used as the representation of the histologic outcome. All functional and histologic data were inserted in RevMan as continuous variables with mean and standard deviation. If the standard error (SE) or 95% confidence interval (95% CI) was given, the standard deviation was calculated using these data before entered in RevMan. The inverse-variance method was used as a statistical method to examine the mean effect size, 95% confidence intervals, forest plots,

and significance. The effect measure was set as standard mean difference because infarct volume was measured in a variety of ways [15]. Substantial heterogeneity was present across the functional (I^2 = 82%) and the histologic endpoint [I^2 = 72%], so random effects models were used as analysis model.

To estimate the methodologic quality for each pre-clinical study the Quality score for methodologic quality of Lees, et al. which defines 10 criteria based on STAIR guidelines was used [13,14]. The checklist comprises 10 criteria by which methodologic quality can be assessed. For the calculation of a mean study quality score one point was attributed for each criterion reported. The scores range from 0 to 10, with higher scores indicating greater methodologic accuracy [13,14].

Clinical study: To evaluate the efficacy of stem cell transplantation on functional and neurological outcome in clinical setting, we incorporated the three most frequently used tests [the modified Rankin scale (mRS) and Barthel index (BI) for functional evaluation and the National Institute of Health stroke scale (NIHSS) score

Table 1: Study characteristics of preclinical studies.

Clinical measure	Studies
No. of publications	76
No. of treatment arms	101
Source of stem cell	
• Rat	30
• Human	40
• Mouse	6
• Porcine	1
Species receiving stem cell	
• Rat	71
• Mouse	4
• Both	1
Route of administration*	
• IA	34
• IV	13
• IC	33
• IT	1
Range of stem cell doses	1500-20000000
Time of administration	
• 0-14 h	21
• 24 h	26
• > 24 h to 1 wk	26
• > 1 wk to 30 d	5
Stem cell immunogenicity	
• Autologous	7
• Allogeneic	26
• Xenogeneic	44
Outcome measure	
• Behavioural	23
• Histologic	6
• Both	47
Artery occlusion	
• Permanent	24
• Transient	52

*Route of administration: IA: Intra-arterial; IV: Intravenous; IC: Intracerebral; IT: Intrathecal.

Table 2: Quality score of preclinical studies.

Quality score criterion	% studies meeting the criterion
Publication in a peer-reviewed journal	100.00
Temperature control	71.05
Avoided neuroprotective anesthetics	21.05
Compliance with animal welfare	85.53
Random assignment	53.95
Blinded assessment of outcomes	55.26
Allocation concealment	5.26
Conflicts of interest statement	32.89
Animals with comorbidities	5.26
Sample size calculation	1.32

reported by 69 of the 101 treatment arms. Infarct volume was described in either absolute or relative terms. The absolute lesion volume was given as mm^3, while the relative was given as a percentage compared to the volume of contralateral (non-infarcted) brain hemisphere or of the total brain volume.

Quality index: The median (Q1, Q3) quality score of the included studies was 4 (3-5), with a range from 1 to 7. Sample size calculation, allocation concealment and the use of animals with relevant comorbidities were the quality criteria that were least addressed in the incorporated studies. The proportions of studies meeting each Quality Score criterion is given in Table 2. There was also a substantial degree of heterogeneity for the histologic outcome analyses: I^2 = 72%.

Treatment effect: The overall effect size, that is the improvement in functional outcome for stem cell-treated rodents relative to the control group, is 1.83 (Figure 3). Cell therapy thus has a positive effect on the behavior of post-stroke animals. Concerning the infarct size, the overall effect size for cell-treated animals is -1.17 (Figure 4). These values represent a significant effect. When assessing the infarct size, it is important to correct for brain edema, as this can result in an overestimation of lesion volume. The swelling of the brain may vary in degree from brain to brain and can introduce major defaults in volume determination [16]. We can correct for brain edema by using the indirect measure method [16,17]. The corrected effect size is -0.95 (P < 0.00001), which still represents a substantial and significant improvement.

Systematic review of clinical studies

Study characteristics: The 4 RCTs all used autologous cell transplants in severe or moderately severe models of ischemic stroke. No sham control of bone marrow aspiration was performed in the control groups. All transplant and control groups received conventional treatment. One study excluded patients that had received rtPA [18]. All studies had a long-term follow-up of at least 1 year.

Risk of bias: We evaluate the methodological quality of the clinical trials (Table 3) using criteria based on

as neurological outcome]. Data were administered as continuous variables with mean and SD. If necessary, the SD was calculated using the 95% CI. The mean effect size, 95% CI, forest plots and significance of stem cell therapy on functional and neurological outcomes were calculated in Revman5 using inverse-variance method. The effect measure was set as mean difference. Substantial heterogeneity occurred, so random effects were set as analysis model.

We evaluated the risk of bias in each study by verifying the presence of allocation concealment, outcome blinding, intention-to-treat analysis and addressment of incomplete outcome data (losses to follow-up) [7].

Results

Systematic review of preclinical studies in rodents

Study characteristics: In total 76 publications comprising 101 stem cell treatment arms were included (Table 1). 94 of these treatment arms reported behavioral outcomes trough a total of 134 results of 9 different behavioral tests. Histologic outcome was

Study or Subgroup	Std. Mean Difference IV, Random, 95% CI	Std. Mean Difference IV, Random, 95% CI
1.3.1 mNSS score		
Chen B 2009 (88)	0.38 [−0.18, 0.94]	
Chen J 2001 Early (90)	1.13 [−0.03, 2.30]	
Chen J 2004 (92)	0.69 [−0.21, 1.60]	
Cui X 2012 (94)	1.34 [0.22, 2.45]	
Dai J 2013 (69)	3.10 [1.52, 4.68]	
Du G 2014 IA (67)	2.09 [0.38, 3.81]	
Du G 2014 IC (67)	1.88 [0.25, 3.51]	
Du G 2014 IV (67)	1.36 [−0.09, 2.82]	
Gutiérrez−Fernandéz M 2011 IA (118)	1.81 [0.73, 2.88]	
Gutiérrez−Fernandéz M 2011 IV (118)	2.48 [1.26, 3.71]	
Gutiérrez−Fernandéz M 2015 hADMSCs (119)	3.33 [1.88, 4.78]	
He XY 2011 BMSCs (96)	1.68 [0.63, 2.73]	
He XY 2011 EPCs (96)	1.37 [0.37, 2.37]	
Heo JS 2013 (97)	1.41 [0.41, 2.41]	
Jiang C 2013 (100)	0.81 [−0.11, 1.73]	
Kaengkan P 2013 (102)	0.75 [−1.01, 2.50]	
Kranz A 2010 fpMSCs (35)	−2.92 [−4.45, −1.40]	
Kranz A 2010 fpMSCs(2x) (35)	1.99 [0.73, 3.25]	
Kranz A 2010 mpMSCs (35)	0.52 [−0.49, 1.52]	
Kranz A 2010 mpMSCs(2x) (35)	3.45 [1.76, 5.14]	
Li D 2015 (64)	1.00 [0.14, 1.86]	
Li F 2012 (76)	1.86 [0.88, 2.85]	
Li N 2014 (105)	2.69 [1.41, 3.97]	
Liu XL 2014 (43)	4.70 [3.04, 6.35]	
Liu Z 2010 (107)	1.13 [0.05, 2.21]	
Nam HS 2015 (61)	2.26 [0.35, 4.18]	
Shen LH 2007 (70)	2.12 [0.83, 3.41]	
Sugiyama M 2011 (114)	8.52 [5.45, 11.60]	
Tang Y 2014 Aged (40)	2.70 [1.12, 4.27]	
Tang Y 2014 Young (40)	1.34 [0.56, 2.11]	
Toyoshima A 2015 (65)	0.60 [−0.20, 1.39]	
Wang J 2013 ESCs (116)	3.29 [1.65, 4.92]	
Wang J 2013 iPSCs (116)	3.91 [2.07, 5.75]	
Yan T 2015 (38)	0.94 [−0.11, 1.98]	
Yavagal DR 2014 (44)	2.49 [0.95, 4.03]	
Yu H 2010 (57)	5.00 [3.46, 6.55]	
Zhang L 2013 (41)	1.23 [0.14, 2.33]	
Zhang P, Zhao J 2009 (58)	6.54 [4.11, 8.96]	
Zhang ZH 2008 (79)	2.97 [1.75, 4.19]	
Zhao MZ 2006 Early (87)	1.15 [−0.12, 2.41]	
Zhao MZ 2006 Late (87)	1.47 [0.13, 2.81]	
Subtotal (95% CI)	**1.90 [1.50, 2.30]**	

Heterogeneity: Tau^2 = 1.26; Chi^2 = 182.19, df = 40 (P < 0.00001); I^2 = 78%
Test for overall effect: Z = 9.27 (P < 0.00001)

Study or Subgroup	Std. Mean Difference IV, Random, 95% CI	
1.3.2 Cylinder test		
Brenneman M 2010 Old (39)	5.52 [2.17, 8.86]	
Brenneman M 2010 Young (39)	3.27 [1.50, 5.05]	
Hicks AU 2009 (75)	0.20 [−0.68, 1.08]	
Ishizaka S 2013 Early (98)	1.96 [0.30, 3.62]	
Lin YC 2011 (106)	1.03 [0.44, 1.62]	
Mitkari B 2014 Early (62)	0.02 [−0.84, 0.87]	
Mitkari B 2014 Late (62)	0.25 [−0.72, 1.22]	
Mäkinen S 2006 (108)	0.01 [−0.94, 0.97]	
Patkar S 2012 (47)	1.92 [0.67, 3.16]	
Yang B 2013 IV High (50)	2.08 [1.11, 3.06]	
Yang B 2013 IV Low (50)	0.27 [−0.54, 1.07]	
Subtotal (95% CI)	**1.08 [0.46, 1.69]**	

Heterogeneity: Tau^2 = 0.72; Chi^2 = 37.89, df = 10 (P < 0.0001); I^2 = 74%
Test for overall effect: Z = 3.44 (P = 0.0006)

Study or Subgroup	Std. Mean Difference IV, Random, 95% CI	
1.3.3 Rotarod test		
Chen J 2000 (89)	0.35 [−0.86, 1.57]	
Chen J 2001 Early (90)	1.32 [0.11, 2.52]	
Chen J 2002 (91)	0.66 [−0.30, 1.62]	
Chu K 2004 (93)	1.49 [0.67, 2.31]	
Daadi MM 2009 (55)	1.05 [0.07, 2.04]	
Kamiya N 2008 IA (66)	1.56 [0.62, 2.49]	
Karlupia N 2014 cbMNCs High (53)	1.37 [0.59, 2.15]	
Karlupia N 2014 cbMSCs Low (53)	1.47 [0.65, 2.29]	
Karlupia N 2014 cmMSCs Low (53)	1.44 [0.65, 2.23]	
Lim JY 2011 IT (51)	1.01 [−0.13, 2.15]	
Lim JY 2011 IV (51)	1.44 [0.22, 2.66]	
Lin YC 2011 (106)	1.48 [0.85, 2.11]	
Liu YP 2009 (36)	8.24 [4.06, 12.43]	
Nam HS 2015 (61)	1.74 [0.04, 3.44]	
Park HW 2015 (113)	2.32 [0.92, 3.73]	
Tang Y 2014 Aged (40)	2.58 [1.04, 4.12]	
Tang Y 2014 Young (40)	2.60 [1.63, 3.57]	

Wu Y 2015 MSCs (117)   0.07 [−0.92, 1.05]
Wu Y 2015 RMNE6 (117)   −0.98 [−2.03, 0.08]
Subtotal (95% CI)   1.31 [0.90, 1.71]
Heterogeneity: $Tau^2 = 0.50$; $Chi^2 = 52.12$, df = 18 (P < 0.0001); $I^2 = 65\%$
Test for overall effect: Z = 6.29 (P < 0.00001)

1.3.4 Beam walk
Heo JS 2013 (97)   0.24 [−0.64, 1.12]
Kranz A 2010 fpMSCs (35)   −4.73 [−6.85, −2.60]
Kranz A 2010 fpMSCs(2x) (35)   5.56 [3.14, 7.99]
Kranz A 2010 mpMSCs (35)   −1.33 [−2.45, −0.22]
Kranz A 2010 mpMSCs(2x) (35)   4.45 [2.42, 6.48]
Li F 2012 (76)   2.57 [1.44, 3.70]
Yang Z 2015 (54)   2.10 [1.07, 3.13]
Yuan T 2013 (48)   0.74 [−0.56, 2.05]
Subtotal (95% CI)   1.15 [−0.47, 2.76]
Heterogeneity: $Tau^2 = 4.79$; $Chi^2 = 84.09$, df = 7 (P < 0.00001); $I^2 = 92\%$
Test for overall effect: Z = 1.39 (P = 0.16)

1.3.5 Treadmill test
Horita Y 2006 (85)   5.21 [1.37, 9.06]
Jang DK 2011 (99)   2.07 [0.37, 3.77]
Komatsu K 2010 Early (68)   1.28 [0.35, 2.21]
Liu H 2006 (86)   3.22 [1.47, 4.98]
Nomura T 2005 (111)   16.43 [9.18, 23.69]
Omori Y 2008 High (52)   11.95 [7.97, 15.94]
Omori Y 2008 Low (52)   7.06 [4.67, 9.44]
Onda T 2008 (112)   2.74 [1.94, 3.55]
Ukai R 2007 BMSCs (115)   4.90 [1.88, 7.93]
Ukai R 2007 PMSCs (115)   4.68 [1.77, 7.60]
Subtotal (95% CI)   4.76 [3.15, 6.37]
Heterogeneity: $Tau^2 = 4.79$; $Chi^2 = 62.09$, df = 9 (P < 0.00001); $I^2 = 86\%$
Test for overall effect: Z = 5.80 (P < 0.00001)

1.3.6 Body swing test
Daadi MM 2009 (55)   2.40 [1.15, 3.65]
Ding DC 2007 (74)   1.36 [0.37, 2.36]
Heo JS 2013 (97)   5.11 [3.14, 7.08]
Li F 2012 (76)   0.86 [0.02, 1.71]
Ma S 2013 (56)   3.10 [1.85, 4.35]
Shyu WC 2006 (45)   1.73 [0.67, 2.79]
Tang Y 2014 Aged (40)   1.96 [0.61, 3.31]
Tang Y 2014 Young (40)   5.36 [3.79, 6.92]
Subtotal (95% CI)   2.59 [1.58, 3.60]
Heterogeneity: $Tau^2 = 1.70$; $Chi^2 = 39.13$, df = 7 (P < 0.00001); $I^2 = 82\%$
Test for overall effect: Z = 5.01 (P < 0.00001)

1.3.7 Forelimb reaching task
Andrews EM 2008 (72)   3.81 [2.31, 5.31]
Braun RG 2012 (73)   3.55 [1.48, 5.62]
Liu H 2006 (86)   1.86 [0.53, 3.18]
Mäkinen S 2006 (108)   −0.26 [−1.22, 0.70]
Park HW 2015 (113)   2.24 [0.86, 3.62]
Subtotal (95% CI)   2.14 [0.54, 3.75]
Heterogeneity: $Tau^2 = 2.81$; $Chi^2 = 27.24$, df = 4 (P < 0.0001); $I^2 = 85\%$
Test for overall effect: Z = 2.61 (P = 0.009)

1.3.8 Corner test
Brenneman M 2010 Old (39)   3.59 [1.22, 5.96]
Brenneman M 2010 Young (39)   6.78 [3.64, 9.92]
Hattori H 2012 (78)   1.33 [0.70, 1.96]
Leu S 2010 (103)   1.21 [0.42, 1.99]
Wu Y 2015 iNSCs (117)   0.02 [−0.96, 1.00]
Subtotal (95% CI)   1.81 [0.62, 3.01]
Heterogeneity: $Tau^2 = 1.28$; $Chi^2 = 21.78$, df = 4 (P = 0.0002); $I^2 = 82\%$
Test for overall effect: Z = 2.97 (P = 0.003)

1.3.9 Adhesive removal test
Chen J 2000 (89)   0.44 [−0.77, 1.66]
Chen J 2002 (91)   0.96 [−0.03, 1.96]
Chen J 2004 (92)   0.56 [−0.34, 1.45]
He XY 2011 BMSCs (96)   5.39 [3.33, 7.46]
He XY 2011 EPCs (96)   5.39 [3.33, 7.46]
Jang DK 2011 (99)   3.11 [0.97, 5.26]
Lim JY 2011 IT (51)   0.90 [−0.22, 2.01]
Lim JY 2011 IV (51)   1.10 [−0.05, 2.25]
Liu Z 2010 (107)   1.14 [0.06, 2.21]
Ma S 2013 (56)   4.91 [3.19, 6.63]
Mitkari B 2014 Early (62)   −0.03 [−0.87, 0.81]
Mitkari B 2014 Late (62)   −0.25 [−1.21, 0.70]
Shen LH 2007 (70)   1.88 [0.64, 3.11]
Stroemer P 2009 High (49)   0.98 [0.06, 1.90]
Stroemer P 2009 Low (49)   0.06 [−0.80, 0.91]
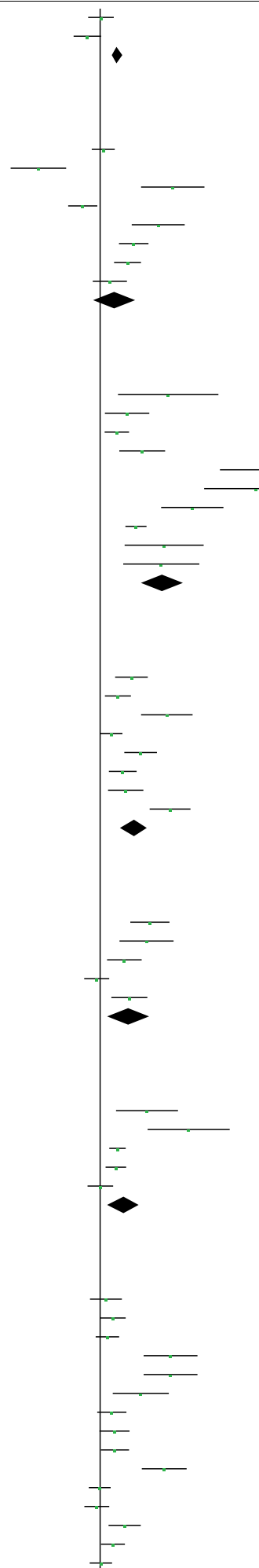
Stroemer P 2009 Medium (49)	0.55 [−0.33, 1.43]	
Veizovic T 2001 (77)	2.61 [1.38, 3.83]	
Yan T 2015 (38)	1.28 [0.18, 2.39]	
Yang B 2013 IV High (50)	6.88 [4.79, 8.98]	
Yang B 2013 IV Low (50)	1.11 [0.25, 1.97]	
Zhang L 2013 (41)	1.00 [−0.06, 2.06]	
Subtotal (95% CI)	**1.64 [1.04, 2.24]**	

Heterogeneity: Tau^2 = 1.55; Chi^2 = 117.26, df = 20 (P < 0.00001); I^2 = 83%
Test for overall effect: Z = 5.37 (P < 0.00001)

Total (95% CI)   **1.83 [1.58, 2.07]**
Heterogeneity: Tau^2 = 1.47; Chi^2 = 695.71, df = 127 (P < 0.00001); I^2 = 82%
Test for overall effect: Z = 14.70 (P < 0.00001)
Test for subgroup differences: Chi^2 = 26.08, df = 8 (P = 0.001), I^2 = 69.3%

Figure 3: Forest plot of functional outcome.

Study or Subgroup	Std. Mean Difference IV, Random, 95% CI
1.2.1 % contralateral	
Andrews EM 2008 (72)	
Chen J 2000 (89)	
Chen J 2002 (91)	
Chen J 2004 (92)	
Cui X 2012 (94)	
Gu N 2014 (95)	
Gutiérrez–Fernandéz M 2011 IA (118)	
Gutiérrez–Fernandéz M 2011 IV (118)	
Gutiérrez–Fernandéz M 2015 hADMSCs (119)	
Gutiérrez–Fernandéz M 2015 rADMSCs (119)	
Heo JS 2013 (97)	
Jiang C 2013 (100)	
Jiang W 2014 (101)	
Kaengkan P 2013 (102)	
Karlupia N 2014 cbMNCs High (53)	
Karlupia N 2014 cbMSCs Low (53)	
Karlupia N 2014 cmMSCs Low (53)	
Li D 2015 (64)	
Lim JY 2011 IT (51)	
Lim JY 2011 IV (51)	
Liu Z 2010 (107)	
Ma S 2013 (56)	
Mora–Lee S 2012 hMAPCs (109)	
Mora–Lee S 2012 hMSCs (109)	
Park HW 2015 (113)	
Shen LH 2007 (70)	
Toyoshima A 2015 (65)	
Yan T 2015 (38)	
Yang B 2013 IV High (50)	
Yang B 2013 IV Low (50)	
Yang Z 2015 (54)	
Zhang L 2013 (41)	
Zhang P, Zhao J 2009 (58)	
Zhao MZ 2006 Early (87)	
Zhao MZ 2006 Late (87)	
Subtotal (95% CI)	

Heterogeneity: Tau^2 = 0.67; Chi^2 = 103.72, df = 34 (P < 0.00001); I^2 = 67%
Test for overall effect: Z = 6.84 (P < 0.00001)

1.2.2 Absolut volume	
Brenneman M 2010 Young (39)	
Chu K 2004 (93)	
Hicks AU 2009 (75)	
Horita Y 2006 (85)	
Ikegame Y 2011 ADMSCs (42)	
Ikegame Y 2011 BMSCs (42)	
Jang DK 2011 (99)	
Kamiya N 2008 IA (66)	
Liu H 2006 (86)	
Mitkari B 2014 Early (62)	
Mitkari B 2014 Late (62)	
Moubarik C 2011 (110)	
Nomura T 2005 (111)	
Omori Y 2008 High (52)	
Omori Y 2008 Low (52)	
Onda T 2008 (112)	
Patkar S 2012 (47)	
Riegelsberger UM 2011 (37)	
Stroemer P 2009 High (49)	
Stroemer P 2009 Low (49)	

Stroemer P 2009 Medium (49)
Sugiyama M 2011 (114)
Ukai R 2007 BMSCs (115)
Ukai R 2007 PMSCs (115)
Yavagal DR 2014 (44)
Subtotal (95% CI)
Heterogeneity: $Tau^2 = 1.06$; $Chi^2 = 92.68$, df = 24 (P < 0.00001); $I^2 = 74\%$
Test for overall effect: Z = 4.32 (P < 0.0001)

1.2.3 % whole brain
Du G 2014 IA (67)
Du G 2014 IC (67)
Du G 2014 IV (67)
Hattori H 2012 (78)
kim DY 2007 (63)
Li D 2012 (104)
Liu YP 2009 (36)
Nam HS 2015 (61)
Veizovic T 2001 (77)
Subtotal (95% CI)
Heterogeneity: $Tau^2 = 1.53$; $Chi^2 = 39.12$, df = 8 (P < 0.00001); $I^2 = 80\%$
Test for overall effect: Z = 2.62 (P = 0.009)

Total (95% CI)
Heterogeneity: $Tau^2 = 0.88$; $Chi^2 = 244.25$, df = 68 (P < 0.00001); $I^2 = 72\%$
Test for overall effect: Z = 8.29 (P < 0.00001)
Test for subgroup differences: $Chi^2 = 0.17$, df = 2 (P = 0.92), $I^2 = 0\%$

Figure 4: Forest plot of structural outcome.

Table 3: Methodological quality of included RCTs.

Estimation of risk of bias	Prasad K, et al. 2014 [18]	Chen DC, et al. 2014 [46]	Bang OY, et al. 2005 [59]	Lee JS, et al. 2010 [60]
Allocation concealment	Yes	No	Yes	Yes
Blinding of outcomes	Yes	Yes	Yes	Yes
Intention-to-treat analysis	Yes	Yes	No	No
Losses to follow-up (incomplete outcome data addressed)	Yes	Yes	No	No

| | **Mean Difference** | | **Mean Difference** |
Study or Subgroup	IV, Random, 95% CI		IV, Random, 95% CI
Bang OY 2005 (59)	−22.33 [−42.90, −1.76]		
Prasad K 2014 (18)	0.50 [−10.18, 11.18]		
Total (95% CI)	**−9.15 [−31.26, 12.95]**		

Heterogeneity: $Tau^2 = 190.66$; $Chi^2 = 3.73$, df = 1 (P = 0.05); $I^2 = 73\%$
Test for overall effect: Z = 0.81 (P = 0.42)

Figure 5: Forest plot of Barthel index.

| | **Mean Difference** | | **Mean Difference** |
Study or Subgroup	IV, Random, 95% CI		IV, Random, 95% CI
Bang OY 2005 (59)	−0.45 [−1.20, 0.30]		
Chen D 2014 (46)	−0.60 [−0.90, −0.30]		
Lee J 2010 (60)	−0.64 [−1.65, 0.37]		
Prasad K 2014 (18)	0.14 [−0.32, 0.60]		
Total (95% CI)	**−0.35 [−0.78, 0.08]**		

Heterogeneity: $Tau^2 = 0.11$; $Chi^2 = 7.28$, df = 3 (P = 0.06); $I^2 = 59\%$
Test for overall effect: Z = 1.61 (P = 0.11)

Figure 6: Forest plot of Modified ranking scale.

the Cochrane criteria [7]. In each study, allocation concealment, blinding of outcome evaluators, analysis by intention- to-treat and losses to follow-up were investigated. The heterogeneity of incorporated data was assessed using the I^2-test. A considerable degree of heterogeneity was seen in all the included studies (Figure 5, Figure 6 and Figure 7).

Effect size: The BI score, with 138 included patients, and the mRS, with 220 included patients, showed no

Study or Subgroup	Mean Difference IV, Random, 95% CI
Chen D 2014 (46)	−3.20 [−4.52, −1.88]
Prasad K 2014 (18)	−1.10 [−3.10, 0.90]
Total (95% CI)	−2.29 [−4.33, −0.25]

Heterogeneity: Tau^2 = 1.46; Chi^2 = 2.95, df = 1 (P = 0.09); I^2 = 66%
Test for overall effect: Z = 2.20 (P = 0.03)

Figure 7: Forest plot of National Institute of Health Stroke scale.
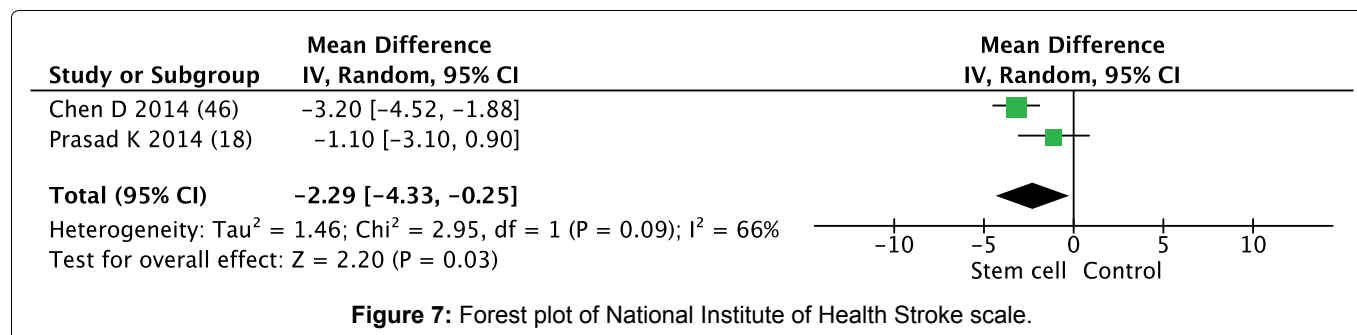
significant difference between the stem cell transplanted group and the control group. The NIHSS, with 109 included patients, did show a significant improvement in neurological function in the transplanted group (P = 0.03) (Figure 5, Figure 6 and Figure 7).

Discussion

Preclinical studies

Preclinical studies of stem cell therapy for ischemic stroke in rodent models displays an overall significant improvement in behavior and histology after stem cell transplantation. The preclinical pooled data analyses all established large beneficial effect sizes. In comparison with previously performed meta-analyses, the trend in our result was similar to the published data [13,14,19].

Clinical studies

Based on the evidence gathered from preclinical studies, several clinical trials were designed. To our knowledge, this is the first systematic review of randomized controlled trials on the use of stem cell therapy in clinical stroke models. Even though the fact that preclinical-pooled data analyses all established large beneficial effect sizes, stem cell therapy does not significantly improve the functional outcomes of patients with ischemic stroke. Neurological outcome did show a significant improvement, but only 2 studies calculated the NIHSS, which makes this the least prevailed factor. We can conclude that there is a trend for improvement by cell-based therapy, but currently this does not lead to a significant result.

We found 2 other non-randomized clinical trials with comparable baseline characteristics that reported efficacy of stem cell therapy in clinical setting [20,21]. The first study confirmed our results as it reported that no significant differences in neurological function were found [20]. The second study showed a statistically significant improvement in the modified BI in the transplanted group. However, due to a small sample size, to non-randomization and an unblinded outcome assessment in this study, make it less convincing [21].

Translation from preclinical models to clinic

Despite several attempts, the documented efficacy in preclinical rodent studies has not yet been translated to successive clinical trials. The study qualities or the therapeutic modalities could explain this failure.

Study quality:

Preclinical studies: The mean quality score for the included preclinical studies was only 4/10. Quality of preclinical studies was also reviewed in other meta-analyses and this shortcoming was highlighted [13,14,19]. This deficiency raises the consideration that authors and/or the reviewers did not adequately identify the potential limitations of the experimental design and/or data analysis. Lacks sample size and allocation concealment were two reported criteria of the poor-quality score. The absence of both sample size calculation and allocation concealment can cause an overestimation of the treatment effects. Low methodological quality reduces the benefit of the obtained results, since several studies have indicated that there is a relationship between increasing study quality and declining efficacy of stem cell therapy. Inadequate reporting thus correlates with overestimated efficacy [14,22,23]. However, some contrasting information is gained in a recent meta-analysis where higher study quality score was associated with larger behavioral gains related to mesenchymal stem cell administration [13].

The considerable degree of heterogeneity is an affirmation of the lack in standardization in the administration and reporting of experiments. The stroke research community recognized these inadequate study designs as one of the major reasons for the failed translation of positive results from animal studies to clinical trials [23]. The quality of preclinical studies has an important relevance in the translational potential of preclinical results. To overcome these barriers in translation, a conference of academicians and industry representatives was gathered to recommend guidelines for the preclinical improvement of quality of acute ischemic stroke therapies [24]. This meeting led to the first Stroke Therapy Academic Industry Roundtable (STAIR) publication in 1999, which was updated in 2009 [22]. The STAIR group reconciled in 2001 as well, but then to discuss various aspects of the design of clinical trials for stem cell therapy for stroke. This is equally important in the translational process, as the chances for future success trials in clinical setting [25].

Despite the initial guidelines, many questions and unresolved issues remained, so in 2007 another meeting with investigators from academia, industry leaders, and members of the National Institutes of Health was held.

This Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) meeting established on the roadmap of the STAIR meeting and explored the remaining concerns with the intention of better understanding of the status of the field. Their goal was to create a plan for future laboratory and clinical investigations in order to accelerate the process towards an effective cellular therapy for stroke [26].

After the publication of the original STEPS guidelines in 2009, an enormous growth in the number of cellular products and new laboratory experimentations occurred. The newly generated information had an impact on the safety and efficacy of cell-based therapies for stroke, so in 2010, a second meeting called STEPS 2 was convened. The purpose of this meeting was to identify critical gaps in knowledge and research areas, as well as update the prior recommendations and formulate new guidelines to direct future investigations [27]. Although meetings such as STAIR and STEPS integrate free debate between important investigators in the field and have the intention to improve (pre) clinical trials by this interchange, application of the STAIR model seemed too difficult to achieve and the first STEPS meetings did recognize these issues, but without offering specific solutions [28].

In 2012, a workshop on Improving the Quality of Preclinical and Clinical Research through Rigorous Study Design and Transparent Reporting (RIGOR) was held. The objective of the workshop was to draft guidelines to ensure worldwide consistency in stroke research practices. The RIGOR guidelines should be incorporated into translational grant applications and are recommended for all manuscripts submitted to Translational Stroke Research [29].

In December 2011, investigators from academia, industry leaders and members of the National Institutes of Health and FDA gathered again at the third STEPS meeting, STEPS 3. They discussed emerging data on the mechanisms of action of cell therapy, the barriers to successful clinical translation of preclinical evidence and the design of current clinical trials for acute and chronic stroke. Since the prior STEPS meetings, the field has continued to progress, and pilot clinical studies started to show safety for some cell types. It became clear that the formulation of a new set of guidelines, which focus on topics not covered in previous STEPS publications, was necessary. STEPS 3 displays a collection of recommendations that focus on more advanced stages of clinical testing [phase II/III], as well as testing of cell therapies in a broader stroke population like chronic stroke [30].

Many similarities exist in the guidelines advised by STAIR, STEPS, and RIGOR. They all strive for adherence to good laboratory (GLP) practices, including the need for several animal modeling studies with blinding to condition and outcome, randomization and complete power analysis and statistical analysis. They also request the clear statement of these GLP when submitting translational grant applications and manuscripts [31].

A positive note to this whole issue is that study quality has improved over time. There is a statistically significant correlation between quality score and year of publication [19]. This result may be suggestive of better incorporation of and adherence to the quality standards over time. Although there might be some improvement, there is still a great lack of consistency in the field which makes it difficult to compare studies and truly ascertain optimal conditions. Standardization of outcomes must be an important focus for experts in the field [19,32].

Clinical studies: The problem could also lie in the methodology of the clinical studies; however, we would expect that study bias would favor the transplanted group. The transplanted group did not do significantly better, so lack of methodological quality in clinical studies is not likely a reason for failed translation to clinic. Nevertheless, study quality is of major importance to make accurate conclusions. Several of the included RCTs reported lack of blinding of patients and due to ethical considerations, no sham or cell aspiration could be performed in the control groups. One RCT had no allocation concealment and 2 RCTs did not use an intention-to-treat analysis. Because of the small sample sizes, the clinical trials have limited power. Two of the studies did not report missing data. All these methodological inaccuracies are important and should be addressed in future clinical trials.

Besides study quality, equally essential in this quest for translation to clinic, is to find the optimal *in vivo* animal model and to determine the administration conditions with maximum efficacy.

Therapeutic modalities:

Animal model: Questions could be raised if the uses of rodent models for stroke for preclinical testing are the reason for the failed translation to the clinic. By other means, are rodents the correct animal model for preclinical testing trials? To translate stem cell therapy from the laboratory to the clinic in a successful manner, the resulting pathophysiological phenomenon of each stroke model should mimic the human disease condition as closely as possible. The anatomy of non-human primates (NHP) most closely resembles the human brain; besides their similar vascular anatomy there are numerous reasons to support the use of NPH models. NHP are gyrencephalic, researchers could target the basal ganglia through occlusion and their size makes it possible to test them by using human clinical techniques such as: CT, MRI, angiography, etc. NHP models are the best models to assess the cerebral ischemia pathology but, they require laborious post-operative care and are costly. This makes it difficult to accomplish in a large sample size, so safety rather than efficacy may be the

more appropriate outcome measure [19,31].

Despite the benefits of NHP models, rodents have been mainly used for testing therapeutic windows in acute, subacute and chronic stages of stroke and many mechanisms of cell therapy on stroke were clarified by the use of these rat models [13,31]. The use of rodents does also have many advantages like ease in post-operative care, feasibility, the possibility of large sample sizes and low costs. Like humans, rats have an internal carotid artery that branches off into anterior and middle cerebral arteries, but unlike human, they display variability in the vascular supply [19,31]. Research also concluded that mouse models are not sufficient to study human inflammatory diseases [33]. Nevertheless, the logistical and practical advantages of rodents' stroke models overcome these technical limitations; present animal models for stroke are assigned to rodents even though this preclinical model does not completely reflect the human stroke model [13].

Most focal ischemic stroke models in rodents are produced by ligation or occlusion of the proximal or distal middle cerebral artery (MCA) by the insertion of sutures, photothrombosis or infusion of blood clots using various methods. Ligation of the middle cerebral artery is the most popular, but it does have limitations, including the high rate of variability with the location and size of the lesion [31]. Although these models can mimic both complete and transient occlusion, none of them accurately mimic a clinical stroke. Because in humans who do suffer a stroke, complete occlusion rarely occurs and there is usually a spontaneous recanalization. Furthermore, the context in which the animal stroke model is assembled differs in the circumstances found in a clinical stroke. At first, stroke patients vary in the most relevant characteristics and represent a very heterogenic group [33,34]. Not only do patients differ in premorbid neurological function, age and site of ischemic lesion, a range of other clinical considerations, like multiple stroke risk factors and concomitant diseases are also often present. These factors do not only play a critical role in the resulting pathology of stroke, they also affect the response to therapeutics and the recovery after stroke [31,34].

The problem is that the present preclinical models mostly evaluate the effect of stem cell therapy in homogenous, adult, healthy male animals and co-morbidity factors and complications are not addressed. As a reaction on this, the STAIR publications emphasized that inclusion of comorbidities such as hypertension, diabetes and hypercholesterolemia in preclinical investigations is necessary for the successful translation of stem cell therapy for ischemic stroke. Female and aged animals should be included to generate a more heterogenic group of rodents so that animal models mimic human disease as closely as possible [19,22,29]. The previous recommendations were based on the

idea that studies in animal models with comorbidities would better replicate the pathophysiological state of human stroke patients. So, to effectively translate preclinical models to clinic, the use of appropriate animal models that mimic human diseases were seen as mandatory [29,31]. However, this hypothesis is challenged by the fact that human strokes occur in a mixed gender aged population in which most people have a history of hypertension or diabetes, but these are usually controlled by one or more drugs. On top of that some diabetic stroke patients do not respond to standard dose thrombolytic therapy and are extremely difficult to treat [29]. Considering the previous information and the refractory phenomenon seen in some diabetic patients, questions are raised if using a standard naive-hypertensive rodent will be adequate to anticipate drug efficacy in a heterogeneous population of stroke patients [29]. Some preclinical studies used spontaneous hypertensive or diabetic rats but no significant improvement in behavioral or histologic outcome was seen when the results of these studies were pooled [35-38]. This observation does support the use of more complex experimental models before translation to clinic.

3 studies conducted stem cell transplantation in aged rodents. We know that advanced age is associated with a decrease in brain plasticity and higher vulnerability to ischemic damage. Aged rats thus respond differently to experimentally induced stoke. They had larger infarct volume and higher overall mortality than younger animals and they also had a poorer functional recovery and significant decrease in angio- and neogenesis. Despite the differences in age, transplantation of stem cells resulted in significant improvement in both young and old animals, so the aging-related micro-environment does not impede an effective response to stem cell transplantation [39-41].

The guidelines set by STEPS, which also provide a basis for exploring stroke animal models for translation of cell-based therapy, emphasize the use of appropriate species, including larger animals. They also stated that clinically relevant stroke animal models, preferably in an NHP, are desirable for testing the potential of anti-stroke therapeutic strategies. There is still a requirement to improve the many existing animal models, so they can correctly reflect our ability to test possible clinical treatments [31].

Cell type: When choosing an adequate cell type, two important considerations need to be considered. First, stem cells can be divided into autologous or allogeneic stem cells. Allogeneic transplantation implies the chance for host immunity and graft-versus-host disease, while the risk for immune reactions is avoided when using autologous stem cells. The need for immunosuppressant when using allogeneic stem cells might be an important hurdle in the clinical practice of

De Meyer et al. Int J Crit Care Emerg Med 2018, 4:058

• Page 12 of 19 •

stem cell transplantation. Secondly, all cell types have the need for *in vitro*, or if possible *in vivo*, expansion to meet he adequate injection dose and the time and requirements needed to expand each cell type differ between cell types.

One of the most frequently used cell types in preclinical studies are mesenchymal stem cells (MSC). These cells are multipotent and are most commonly isolated from bone marrow and adipose tissue, but can be derived from amniotic fluid, the placenta and the umbilical cord as well. Both adipose- and bone marrow-derived MSCs have shown to be effective in preclinical trials. MSCs have the advantage of not carrying a risk for tumorigenesis, like pluripotent cells, and being immunoprivileged, with low major histocompatibility complex (MHC) expression. MSCs even have an immunosuppressive nature, which obviates the need for immunosuppressant in allotransplantation. Adipose tissue contains more than a thousand-fold more MSC compared with bone marrow, so preparing enough cells is much easier for adipose-derived stem cells. Harvesting of bone marrow-derived MSCs also involves a highly invasive procedure, while adipose-derived stem cells use a less invasive method. Additionally, the age, sex and drug usage of cell donors have a great influence on the quality of bone-marrow derived MSCs. Adipose tissue-derived MSCs are thus considered to be a more convenient option than bone marrow-derived MSCs [42-44].

Since MSCs require complicated culturing conditions for *in vitro* expansion, some studies used peripheral blood hematopoietic stem cells (PBSC). These are autologous stem cell without the need for *in vitro* expansion. After several days of granulocyte colony stimulating factor (G-CSF) injections, PBSC can be easily harvested from the bloodstream, so no invasive extraction procedure is needed [45,46].

Another type of frequently used stem cell is human umbilical cord blood cells (HUBCB). HUBCB have the advantage of easy availability as it is discarded post birth and lack of ethical conflicts. Since HUBCBs are allogeneic, they require HLA-matching, which limits their clinical application. However, one study declared that there is a tolerance to HLA-mismatch with non-to low severity from HLA mismatch [38].

In total, more than 20 different cell types have been used in our included preclinical studies, all with their advantages and disadvantages. The difference between autologous and allogeneic cell transplants seemed of major importance for clinical translation, but since evidence suggested that several allogeneic stem cells can be administered without immunosuppression. Still, the included clinical trials all used autologous stem cells. At present, there is no study comparing all these different cell types in a direct manner, so it is difficult to advise one specific cell type for clinical application. Embryonic

cells have ethical concerns, limited availability and the potential of tumor formation. Induced pluripotent stem cells also have the possibility of late oncogenesis and long-term safety is still a serious problem. So, although it is too early to withdraw conclusions, these cell types are unlikely to be proposed in the future [47,48].

Cell dose: To determine the correct cell dose, preclinical studies that investigate cell dose-response curves are necessary. Researchers observed dose-dependent immune-suppressive effect *in vitro*, so cell dose might play a crucial role in the effective translation of preclinical evidence [35].

When we evaluate studies, using different cell doses, most often a higher dose was more effective than a lower dose [49-51]. A single high dose of 3×10^6 cells is considered better than multiple low dose injections at different times [52]. One study declared that 2 different administrations were better than one single administration [35]. But here, the benefit is possible explained by the absolute higher dose caused by the double injection, rather than the double injection itself. The dose-response curve probably follows a u-shape rather than a linear progression, with higher number of cells leading to a ceiling effect. After all, too large doses of stem cell are observed to be destructive and therefore have diminished effects. One study described a severe ipsilateral eye inflammation followed by acute mortality of study animals after administration of 10^7 cells. Mesenchymal stem cells have the tendency to aggregate in multicellular globules, thus at higher concentrations could result in vascular embolization. This study indicated 5×10^6 cells as the maximum number of cells that could be safely transplanted [53]. This result was confirmed be the study of Yang, et al. were the injection of 5×10^7 cells caused high mortality rates due to embolisms, so 5×10^6 cells were seen as ideal cell dose therapy [54]. Another study showed that animals died when they received greater than 30×10^6 cells, stating $10\text{-}20 \times 10^6$ cells might be the optimal range [36].

Lower doses of cells did not manage to get enough cells entering the brain for effective treatment, so too low cell doses are also thought to be ineffective [13,50,51]. However, there are several studies with low cell doses ($\leq 10^5$) that showed a significant improved function [42,44,55-58].

One study did not find any dose-response relationship suggesting that inadequate dose is not a likely explanation for failure of translation to clinic [18].

Different cell doses have been used in the included RCTs. Based on mean body mass, Bang, et al. computed that 1×10^8 cells/patient is the human dose equivalent to the dose used in rodent stroke models. They considered 1×10^5 - 3×10^6 cells/rat as the effective dose used in rodents [59]. This dose was injected by Lee, et al. as well

[60]. The other two clinical trials used different doses so the optimal dose of stem cells in human stroke model still needs to be determined and high-quality dose-response studies are urgently needed. Another important point of investigation is whether the number of cell passages might downgrade the efficacy of stem cells. When extrapolating rodents' doses to humans, large numbers of cells are needed. This means that at least 5 or 6 or more passages are needed to achieve a sufficient dose [51,61]. If the efficacy of stem cells diminishes by more cell passages, this could partly explain the lack of efficacy of stem cell therapy in humans.

Besides the possible importance of the number of cells, the proposal has been made that cell size and velocity of injection are of equal value [62]. Slower injection might diminish sludging and thereby allow higher cell doses [44].

Administration route: The most appropriate route for administration still needs to be determined. Preclinical studies use mainly three different injection routes: Intracerebral (IC), intra-arterial (IA) and intravenous [IV]. In the included RCTs stereotactic and intravenous administration were used.

IC transplantation results in the highest number of cell deposits into the ischemic brain and is shown to be an effective administration route. However, this is also the most invasive route and studies have demonstrated that the stereotactic inoculation of cells can cause damage. IC injection can thus be potentially complicated by hemorrhage or injury, probably by needle insertion, as well as fluid loading, neuronal cell deaths, reactive gliosis and micro-calcification [36,63-65].

Both intravascular routes are less invasive and can avoid unnecessary brain damage. IA transplantation has been correlated with a decrease in infarct volumes, functional recovery and a high number of surviving stem cells. IA injection however, holds the risk of cerebral embolisms and reduction of blood flow with the occurrence of micro-occlusions when high doses of stem cells are infused [62,65]. IV injection is the less-invasive, simplest and practical route of administration, but has the disadvantage that there is a dispersal of cells throughout the body. Fewer cells reach the brain parenchyma because they get entrapped in the lungs and other peripheral organs [54,62,65].

IC administration is unambiguously the least applicable route in clinical setting, since it involves an invasive procedure with high risks of additional damage and possible neurological worsening of the patient's state. If IC injection is chosen, safety trials have opted for delivery of cells many months or years after stroke at the earliest. Patients need to be stable to undergo general anesthesia for this invasive procedure and no spontaneous recovery may be expected [28]. However, one of the included RCT used stereotactic

injection of stem cells and this appeared to be safe [46]. In contrast to IC admission, the recommendations regarding intravascular administration routes are less straightforward and differ between several preclinical studies.

Some studies, comparing different administration routes, stated that IA and IC delivery resulted in a higher number of surviving stem cells and a higher improvement in histologic and behavioral outcome [13,44,66]. Other studies did not find a significant influence of route of administration on the efficacy of cell transplants. IA and IV infusion both resulted in improved functional status and/or histologic outcome with no added benefit of IA administration [12,14,50,51].

One study suggested that the number of cells delivered to the brain may be not critical to influence functional outcomes or that only a minimum threshold of cells delivered to the brain is needed and that higher cell presence does not lead to a greater effect on recovery in rodent stroke models. This would explain how IV delivery, with lesser cells achieving the brain, can be as effective as IA delivery [50]. This hypothesis is enhanced by the fact that paracrine effects of stem cell-associated growth factors are considered to be the main effective pathway [36].

Based on preclinical evidence, the three administration routes can be effective, so the route of delivery might not be the major hurdle in translating preclinical evidence to clinical practice. IV injection is sometimes seen as the least effective route, but clearly has some great safety and practical advantages. We presume that IV injection should be the route of predilection to consider in clinical practice, like 3 out of 4 RCTs [18,59,60] already did.

Timing: Several studies have compared different administration times. For example, the study of Komatsu, et al. delivered stem cell in 3 time periods from 7 days after middle cerebral artery occlusion (MCAo) until 28 days after MCAo. The stem cells injected at 7 days post-MCAo did significantly better than the cells transplanted at 28 days. Administration at 4 weeks post-MCAo might thus be too late to be effective [49]. The study of Nam, et al. on the other hand, compared 1 h, 1 day or 3 days post-MCAo injection. The 1 h post-MCAo transplanted group showed maximum neurological recovery, in both functional and structural way [61]. Other studies stated that 24 h is the most optimal administration time, because this might go along with the time of opening of the blood-brain barrier [44,65,67].

In general, preclinical evidence mostly supports early administration [52,56,68,69]. This early transplantation, however, has practical complications in clinic setting, especially when autologous stem cells are used which need time for expansion. Delayed inoculation of cells is a better representation of the planned clinical

scenario, where disability following stroke will need to be stabilized before treatment [49]. It is therefore beneficial that several preclinical studies show that late administration of stem cells still can be effective. 14 of the included studies delivered stem cells at 1 week or later after MCAo. 13 of these studies, including some in which stem cells were only administered 4 weeks after MCAo, reported an improvement in behavioral and/or histologic outcome [45,49,55,63,68,70-78]. The other study did not report significant improvement in histologic outcome and even worsening of behavior was noted [62].

When defining an optimal delivery time for clinical practice, several factors, besides the preclinical evidence, must be considered. First, pilot studies have shown that many patients undergo marked improvement or worsening in the first week after stroke. When deterioration is severe, they might even need hemicraniectomy, which would mask the possible effect of cell transplantation. Secondly, cytokines released from the intervention might destabilize the patients in the first week and cause serious adverse effects, which would be unethical to perform [18,60]. Lastly, after a sudden stroke, there is time needed to prepare autologous stem cells, which can only be delivered several weeks later [64]. So even though preclinical evidence suggests early stem cell transplantation, this is impossible in clinical practice. The present RCTs offered cell therapy to stroke patients after weeks and months of stroke onset. This late administration might be a possible explanation for lack of benefit, but the current available knowledge there is no possible manner to transplant cells at earlier time points. The exact optimal time is still unknown, so additional studies concerning the time of transplantation seem necessary [28,59]. However, since histologic and functional improvement have been described for cell transplantation time points from 1 h to 1 month upon MCAo, the time window for effective stem cell transplantation might eventually be less relevant [14,35].

Preconditioning and improving the post engravement cell survival: Despite immense advancements, a myriad of factors is hampering the acceptance and the survival of stem cell. Within hours, a very high number of donor cells die after transplantation [79]. This high mortality is incriminated to several pathological processes that encompasses local immunological and inflammatory responses, loss of trophic factors as well as local primary factors responsible for the initial ischemic insult (e.g. reduced perfusion and nutrients, hypoxia and others) [80,81].

Improvement of stem cell transplantation is related to the number of cells retained and remained active at the site of the cell graft. Increasing the number of cells engraftment may overcome the massive cell death and washout, but number of complications remain such

cell aggregation, vascular embolism, inflammation generated by cell death of the graft [36,44,53,54].

Hence, it is imperative to reinforce the donor cell to withstands the rigors of the microenvironment of the infarcted site by optimizing cell engraftment and this without increasing complications [82]. Authors had shown in stroke [83] and in myocardial infarction [80], that priming cells prior transplantation with cytokines to a state of "readiness" by stimulating their survival pathways will fortify cell engraftment leading to enhance survival and to overcome numbers of problems occurring during the post engraftment period.

Conclusions and Limitations

Stem cell therapy seems promising in animal models, but before extrapolation to humans large and well-designed preclinical trials are needed. We consider preclinical study quality as one of the main factors for failed translation to clinic. Designing high-quality studies should focus first on the optimal cell type, dose, route of administration and timing of delivery. These issues were noticed by researchers in the field as well and gave rise to the several guidelines, but currently their effect on study quality is still lacking. Some researchers state that several issues concerning the translating of stem cell therapy to human, might be better directly addressed by careful studies in human stroke models, rather than supplementary preclinical studies [13]. This possible approach is supported by the excellent safety record of our included clinical trials.

If stem cell therapy is going to be used in clinic, research needs to be done to determine which stroke patients need to be considered. Because, it is possible that the efficacy of stem cell transplantation will be affected by the location, severity and chronicity of the stroke and the competence of blood supply [59]. Another strategy for the future is the investigation of modified stem cells. Several preclinical studies already showed that modified stem cell might have better functional outcomes than unmodified cells. Gene transduction into stem cells is a possible manner to enhance their current therapeutic potential [14,84-86].

Our study has some limitation since only 4 RCTs using human stroke models could be included in the systematic review. Although this is much more than the Cochrane meta-analysis in 2010, the sample sizes of the included studies are small, so our results are not conclusive. Our research should be repeated when more high-quality studies are available.

Declarations of Interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter of materials discusses in the manuscript. This includes employment, consultancies, honoraria, stock

De Meyer et al. Int J Crit Care Emerg Med 2018, 4:058

• Page 15 of 19 •

ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

1. Feigin VL, Krishnamurthi RV, Parmar P, Norrving B, Mensah GA, et al. (2015) Update on the global burden of ischemic and hemorrhagic stroke in 1990-2013: The GBD 2013 study. Neuroepidemiology 45: 161-176.

2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, et al. (2015) Heart disease and stroke statistics-2015 update: A report from the American Heart Association. Circulation 131: e29-e322.

3. Wahlgren N, Ahmed N, Davalos A, Ford GA, Grond M, et al. (2007) Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study [SITS-MOST]: An observational study. Lancet 369: 275-282.

4. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, et al. (2008) Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 359: 1317-1329.

5. Chopp M, Li Y (2002) Treatment of neural injury with marrow stromal cells. Lancet Neurol 1: 92-100.

6. Pereira AC, Martin PJ, Warburton EA (2001) Thrombolysis in acute ischaemic stroke. Postgrad Med J 77: 166-171.

7. Boncoraglio GB, Bersano A, Candelise L, Reynolds BA, Parati EA (2010) Stem cell transplantation for ischemic stroke. Cochrane Database Syst Rev CD007231.

8. Liu X, Ye R, Yan T, Yu SP, Wei L, et al. (2014) Cell based therapies for ischemic stroke: From basic science to bedside. Prog Neurobiol 115: 92-115.

9. Savitz SI (2015) Developing cellular therapies for stroke. Stroke 46: 2026-2031.

10. Janowski M, Wagner DC, Boltze J (2015) Stem cell-based tissue replacement after stroke: Factual necessity or notorious fiction? Stroke 46: 2354-2363.

11. Dulamea AO (2015) The potential use of mesenchymal stem cells in stroke therapy-From bench to bedside. J Neurol Sci 352: 1-11.

12. Du G, Liu Y, Dang M, Zhu G, Su R, et al. (2014) Comparison of administration routes for adipose-derived stem cells in the treatment of middle cerebral artery occlusion in rats. Acta Histochem 116: 1075-1084.

13. Vu Q, Xie K, Eckert M, Zhao W, Cramer SC (2014) Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. Neurology 82: 1277-1286.

14. Lees JS, Sena ES, Egan KJ, Antonic A, Koblar SA, et al. (2012) Stem cell-based therapy for experimental stroke: A systematic review and meta-analysis. Int J Stroke 7: 582-588.

15. (2014) Review Manager 5.3. The Nordic Cochrane Centre TCC, Copenhagen, Denmark.

16. Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, et al. (1990) A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab 10: 290-293.

17. Lin TN, He YY, Wu G, Khan M, Hsu CY (1993) Effect of brain edema on infarct volume in a focal cerebral ischemia model in rats. Stroke 24: 117-121.

18. Prasad K, Sharma A, Garg A, Mohanty S, Bhatnagar S, et al. (2014) Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: A multicentric, randomized trial. Stroke 45: 3618-3624.

19. Vahidy FS, Rahbar MH, Zhu H, Rowan PJ, Bambhroliya AB, et al. (2016) Systematic review and meta-analysis of bone marrow-derived mononuclear cells in animal models of ischemic stroke. Stroke 47: 1632-1639.

20. Moniche F, Gonzalez A, Gonzalez-Marcos JR, Carmona M, Pinero P, et al. (2012) Intra-arterial bone marrow mononuclear cells in ischemic stroke: A pilot clinical trial. Stroke 43: 2242-2244.

21. Bhasin A, Srivastava MV, Mohanty S, Bhatia R, Kumaran SS, et al. (2013) Stem cell therapy: A clinical trial of stroke. Clin Neurol Neurosurg 115: 1003-1008.

22. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, et al. (2009) Update of the stroke therapy academic industry roundtable preclinical recommendations. Stroke 40: 2244-2250.

23. Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, et al. (2012) A call for transparent reporting to optimize the predictive value of preclinical research. Nature 490: 187-191.

24. Stroke Therapy Academic Industry Roundtable (1999) Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 30: 2752-2758.

25. Stroke Therapy Academic Industry Roundtable II (2001) Recommendations for clinical trial evaluation of acute stroke therapies. Stroke 32: 1598-1606.

26. Stem cell therapies as an emerging paradigm in stroke participants (2009) Stem cell therapies as an emerging paradigm in stroke [STEPS]: Bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. Stroke 40: 510-515.

27. Savitz SI, Chopp M, Deans R, Carmichael T, Phinney D, et al. (2011) Stem cell therapy as an emerging paradigm for stroke [STEPS] II. Stroke 42: 825-829.

28. Kalladka D, Muir KW (2011) Stem cell therapy in stroke: Designing clinical trials. Neurochem Int 59: 367-370.

29. Lapchak PA, Zhang JH, Noble-Haeusslein LJ (2013) RIGOR guidelines: Escalating STAIR and STEPS for effective translational research. Transl Stroke Res 4: 279-285.

30. Savitz SI, Cramer SC, Wechsler L (2014) Stem cells as an emerging paradigm in stroke 3: Enhancing the development of clinical trials. Stroke 45: 634-639.

31. Tajiri N, Dailey T, Metcalf C, Mosley YI, Lau T, et al. (2013) In vivo animal stroke models: A rationale for rodent and non-human primate models. Transl Stroke Res 4: 308-321.

32. Borlongan CV, Chopp M, Steinberg GK, Bliss TM, Li Y, et al. (2008) Potential of stem/progenitor cells in treating stroke: The missing steps in translating cell therapy from laboratory to clinic. Regen Med 3: 249-250.

33. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, et al. (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 110: 3507-3512.

34. Cramer SC (2003) Clinical issues in animal models of stroke and rehabilitation. ILAR J 44: 83-84.

35. Kranz A, Wagner DC, Kamprad M, Scholz M, Schmidt UR, et al. (2010) Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in

rats. Brain Res 1315: 128-136.

36. Liu YP, Seçkin H, Izci Y, Du ZW, Yan YP, et al. (2009) Neuroprotective effects of mesenchymal stem cells derived from human embryonic stem cells in transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 29: 780-791.

37. Riegelsberger UM, Deten A, Pösel C, Zille M, Kranz A, et al. (2011) Intravenous human umbilical cord blood transplantation for stroke: Impact on infarct volume and caspase-3-dependent cell death in spontaneously hypertensive rats. Exp Neurol 227: 218-223.

38. Yan T, Venkat P, Chopp M, Zacharek A, Ning R, et al. (2015) Neurorestorative therapy of stroke in type 2 diabetes mellitus rats treated with human umbilical cord blood cells. Stroke 46: 2599-2606.

39. Brenneman M, Sharma S, Harting M, Strong R, Cox CS, et al. (2010) Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow Metab 30: 140-149.

40. Tang Y, Wang J, Lin X, Wang L, Shao B, et al. (2014) Neural stem cell protects aged rat brain from ischemia-reperfusion injury through neurogenesis and angiogenesis. J Cereb Blood Flow Metab 34: 1138-1147.

41. Zhang L, Yi L, Chopp M, Kramer BC, Romanko M, et al. (2013) Intravenous administration of human umbilical tissue-derived cells improves neurological function in aged rats after embolic stroke. Cell Transplant 22: 1569-1576.

42. Ikegame Y, Yamashita K, Hayashi S, Mizuno H, Tawada M, et al. (2011) Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy. Cytotherapy 13: 675-685.

43. Liu XL, Zhang W, Tang SJ (2014) Intracranial transplantation of human adipose-derived stem cells promotes the expression of neurotrophic factors and nerve repair in rats of cerebral ischemia-reperfusion injury. Int J Clin Exp Pathol 7: 174-183.

44. Yavagal DR, Lin B, Raval AP, Garza PS, Dong C, et al. (2014) Efficacy and dose-dependent safety of intra-arterial delivery of mesenchymal stem cells in a rodent stroke model. PLoS One 9: e93735.

45. Shyu WC, Lin SZ, Chiang MF, Su CY, Li H (2006) Intracerebral peripheral blood stem cell [CD34+] implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. J Neurosci 26: 3444-3453.

46. Chen DC, Lin SZ, Fan JR, Lin CH, Lee W, et al. (2014) Intracerebral implantation of autologous peripheral blood stem cells in stroke patients: A randomized phase II study. Cell Transplant 23: 1599-1612.

47. Patkar S, Tate R, Modo M, Plevin R, Carswell HV (2012) Conditionally immortalised neural stem cells promote functional recovery and brain plasticity after transient focal cerebral ischaemia in mice. Stem Cell Res 8: 14-25.

48. Yuan T, Liao W, Feng NH, Lou YL, Niu X, et al. (2013) Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. Stem Cell Res Ther 4: 73.

49. Stroemer P, Patel S, Hope A, Oliveira C, Pollock K, et al. (2009) The neural stem cell line CTX0E03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. Neurorehabil Neural Repair 23: 895-909.

50. Yang B, Migliati E, Parsha K, Schaar K, Xi X, et al. (2013) Intra-arterial delivery is not superior to intravenous delivery of autologous bone marrow mononuclear cells in acute ischemic stroke. Stroke 44: 3463-3472.

51. Lim JY, Jeong CH, Jun JA, Kim SM, Ryu CH, et al. (2011) Therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells after intrathecal administration by lumbar puncture in a rat model of cerebral ischemia. Stem Cell Res Ther 2: 38.

52. Omori Y, Honmou O, Harada K, Suzuki J, Houkin K, et al. (2008) Optimization of a therapeutic protocol for intravenous injection of human mesenchymal stem cells after cerebral ischemia in adult rats. Brain Res 1236: 30-38.

53. Karlupia N, Manley NC, Prasad K, Schäfer R, Steinberg GK (2014) Intraarterial transplantation of human umbilical cord blood mononuclear cells is more efficacious and safer compared with umbilical cord mesenchymal stromal cells in a rodent stroke model. Stem Cell Res Ther 5: 45.

54. Yang Z, Cai X, Xu A, Xu F, Liang Q (2015) Bone marrow stromal cell transplantation through tail vein injection promotes angiogenesis and vascular endothelial growth factor expression in cerebral infarct area in rats. Cytotherapy 17: 1200-1212.

55. Daadi MM, Lee SH, Arac A, Grueter BA, Bhatnagar R, et al. (2009) Functional engraftment of the medial ganglionic eminence cells in experimental stroke model. Cell Transplant 18: 815-826.

56. Ma S, Zhong D, Chen H, Zheng Y, Sun Y, et al. (2013) The immunomodulatory effect of bone marrow stromal cells (BMSCs) on interleukin (IL)-23/IL-17-mediated ischemic stroke in mice. J Neuroimmunol 257: 28-35.

57. Yu H, Cao B, Feng M, Zhou Q, Sun X, et al. (2010) Combinated transplantation of neural stem cells and collagen type I promote functional recovery after cerebral ischemia in rats. Anat Rec 293: 911-917.

58. Zhang P, Li J, Liu Y, Chen X, Kang Q, et al. (2009) Human neural stem cell transplantation attenuates apoptosis and improves neurological functions after cerebral ischemia in rats. Acta Anaesthesiol Scand 53: 1184-1191.

59. Bang OY, Lee JS, Lee PH, Lee G (2005) Autologous mesenchymal stem cell transplantation in stroke patients. Ann Neurol 57: 874-882.

60. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, et al. (2010) A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells 28: 1099-1106.

61. Nam HS, Kwon I, Lee BH, Kim H, Kim J, et al. (2015) Effects of mesenchymal stem cell treatment on the expression of matrix metalloproteinases and angiogenesis during ischemic stroke recovery. PLoS One 10: e0144218.

62. Mitkari B, Nitzsche F, Kerkelä E, Kuptsova K, Huttunen J, et al. (2014) Human bone marrow mesenchymal stem/stromal cells produce efficient localization in the brain and enhanced angiogenesis after intra-arterial delivery in rats with cerebral ischemia, but this is not translated to behavioral recovery. Behav Brain Res 259: 50-59.

63. Kim DY, Park SH, Lee SU, Choi DH, Park HW, et al. (2007) Effect of human embryonic stem cell-derived neuronal precursor cell transplantation into the cerebral infarct model of rat with exercise. Neurosci Res 58: 164-175.

64. Li D, Zhang M, Zhang Q, Wang Y, Song X, et al. (2015) Functional recovery after acute intravenous administration of human umbilical cord mesenchymal stem cells in rats

with cerebral ischemia-reperfusion injury. Intractable Rare Dis Res 4: 98-104.

65. Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, et al. (2015) Intra-arterial transplantation of allogeneic mesenchymal stem cells mounts neuroprotective effects in a transient ischemic stroke model in rats: Analyses of therapeutic time window and its mechanisms. PLoS One 10: e0127302.

66. Kamiya N, Ueda M, Igarashi H, Nishiyama Y, Suda S, et al. (2008) Intra-arterial transplantation of bone marrow mononuclear cells immediately after reperfusion decreases brain injury after focal ischemia in rats. Life Sci 83: 433-437.

67. Komatsu K, Honmou O, Suzuki J, Houkin K, Hamada H, et al. (2010) Therapeutic time window of mesenchymal stem cells derived from bone marrow after cerebral ischemia. Brain Res 1334: 84-92.

68. Dai J, Li SQ, Qiu YM, Xiong WH, Yin YH, et al. (2013) Migration of neural stem cells to ischemic brain regions in ischemic stroke in rats. Neurosci Lett 552: 124-128.

69. Shen LH, Li Y, Chen J, Zacharek A, Gao Q, et al. (2007) Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab 27: 6-13.

70. Prasad K, Mohanty S, Bhatia R, Srivastava MV, Garg A, et al. (2012) Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischaemic stroke: A pilot study. Indian J Med Res 136: 221-228.

71. Andrews EM, Tsai SY, Johnson SC, Farrer JR, Wagner JP, et al. (2008) Human adult bone marrow-derived somatic cell therapy results in functional recovery and axonal plasticity following stroke in the rat. Exp Neurol 211: 588-592.

72. Braun RG, Andrews EM, Kartje GL (2012) Kinematic analysis of motor recovery with human adult bone marrow-derived somatic cell therapy in a rat model of stroke. Neurorehabil Neural Repair 26: 898-906.

73. Ding DC, Shyu WC, Chiang MF, Lin SZ, Chang YC, et al. (2007) Enhancement of neuroplasticity through upregulation of beta1-integrin in human umbilical cord-derived stromal cell implanted stroke model. Neurobiol Dis 27: 339-353.

74. Hicks AU, Lappalainen RS, Narkilahti S, Suuronen R, Corbett D, et al. (2009) Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: Cell survival and functional recovery. Eur J Neurosci 29: 562-574.

75. Li F, Miao ZN, Xu YY, Zheng SY, Qin MD, et al. (2012) Transplantation of human amniotic mesenchymal stem cells in the treatment of focal cerebral ischemia. Mol Med Rep 6: 625-630.

76. Veizovic T, Beech JS, Stroemer RP, Watson WP, Hodges H (2001) Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells. Stroke 32: 1012-1019.

77. Hattori H, Suzuki S, Okazaki Y, Suzuki N, Kuwana M (2012) Intracranial transplantation of monocyte-derived multipotential cells enhances recovery after ischemic stroke in rats. J Neurosci Res 90: 479-488.

78. Zhang ZH, Wang RZ, Wang RZ, Li GL, Wei JJ, et al. (2008) Transplantation of neural stem cells modified by human neurotrophin-3 promotes functional recovery after transient focal cerebral ischemia in rats. Neurosci Lett 444: 227-230.

79. Qu Z, Balkir L, van Deutekom JC, Robbins PD, Pruchnic R, et al. (1998) Development of approaches to improve cell survival in myoblast transfer therapy. J Cell Biol 142: 1257-1267.

80. Haider HK, Muhammad Ashraf M (2008) Strategies to promote donor cell survival: Combining preconditioning approach with stem cell transplantation. J Mol Cell Cardiol 45: 554-566.

81. Haider HK (2018) Bone marrow cell therapy and cardiac reparability: Better cell characterization will enhance clinical success. Regen Med 13: 457-475.

82. Haider HK, Ashraf M (2010) Preconditioning and stem cell survival. J Cardiovasc Trans Res 3: 89-102.

83. Tabeshmehr P, Haider HK, Salmannejad M, Sani M, Hosseini SM, et al. (2017) Nicorandil potentiates sodium butyrate induced preconditioning of neurons and enhances their survival upon subsequent treatment with H_2O_2. Transl Neurodeger 6: 29.

84. Horita Y, Honmou O, Harada K, Houkin K, Hamada H, et al. (2006) Intravenous administration of glial cell line-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in the adult rat. J Neurosci Res 84: 1495-1504.

85. Liu H, Honmou O, Harada K, Nakamura K, Houkin K, et al. (2006) Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. Brain 129: 2734-2745.

86. Zhao MZ, Nonoguchi N, Ikeda N, Watanabe T, Furutama D, et al. (2006) Novel therapeutic strategy for stroke in rats by bone marrow stromal cells and ex vivo HGF gene transfer with HSV-1 vector. Journal of Cerebral Blood Flow and Metabolism 26: 1176-1188.

87. Chen B, Gao XQ, Yang CX, Tan SK, Sun ZL, et al. (2009) Neuroprotective effect of grafting GDNF gene-modified neural stem cells on cerebral ischemia in rats. Brain Res 1284: 1-11.

88. Chen J, Li Y, Chopp M (2000) Intracerebral transplantation of bone marrow with BDNF after MCAo in rat. Neuropharmacology 39: 711-716.

89. Chen J, Sanberg PR, Li Y, Wang L, Lu M, et al. (2001) Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke 32: 2682-2688.

90. Chen J, Li Y, Wang L, Lu M, Chopp M (2002) Caspase inhibition by Z-VAD increases the survival of grafted bone marrow cells and improves functional outcome after MCAo in rats. J Neurol Sci 199: 17-24.

91. Chen J, Li Y, Zhang R, Katakowski M, Gautam SC, et al. (2004) Combination therapy of stroke in rats with a nitric oxide donor and human bone marrow stromal cells enhances angiogenesis and neurogenesis. Brain Res 1005: 21-28.

92. Chu K, Kim M, Park KI, Jeong SW, Park HK, et al. (2004) Human neural stem cells improve sensorimotor deficits in the adult rat brain with experimental focal ischemia. Brain Res 1016: 145-153.

93. Cui X, Chopp M, Shehadah A, Zacharek A, Kuzmin-Nichols N, et al. (2012) Therapeutic benefit of treatment of stroke with simvastatin and human umbilical cord blood cells: Neurogenesis, synaptic plasticity, and axon growth. Cell Transplant 21: 845-856.

94. Gu N, Rao C, Tian Y, Di Z, Liu Z, et al. (2014) Anti-inflammatory and antiapoptotic effects of mesenchymal stem cells transplantation in rat brain with cerebral ischemia. J Stroke Cerebrovasc Dis 23: 2598-2606.

De Meyer et al. Int J Crit Care Emerg Med 2018, 4:058

• Page 18 of 19 •

95. He XY, Chen ZZ, Cai YQ, Xu G, Shang JH, et al. (2011) Expression of cytokines in rat brain with focal cerebral ischemia after grafting with bone marrow stromal cells and endothelial progenitor cells. Cytotherapy 13: 46-53.

96. Heo JS, Choi SM, Kim HO, Kim EH, You J, et al. (2013) Neural transdifferentiation of human bone marrow mesenchymal stem cells on hydrophobic polymer-modified surface and therapeutic effects in an animal model of ischemic stroke. Neuroscience 238: 305-318.

97. Ishizaka S, Horie N, Fukuda Y, Satoh K, Nishida N, et al. (2013) Intra-arterial cell transplantation provides timing-dependent cell distribution and functional recovery after stroke. Stroke 44: 720-726.

98. Jang DK, Park SI, Han YM, Jang KS, Park MS, et al. (2011) Motor-evoked potential confirmation of functional improvement by transplanted bone marrow mesenchymal stem cell in the ischemic rat brain. J Biomed Biotechnol 2011: 238409.

99. Jiang C, Wang J, Yu L, Ou C, Liu X, et al. (2013) Comparison of the therapeutic effects of bone marrow mononuclear cells and microglia for permanent cerebral ischemia. Behav Brain Res 250: 222-229.

100. Jiang W, Liang G, Li X, Li Z, Gao X, et al. (2014) Intracarotid transplantation of autologous adipose-derived mesenchymal stem cells significantly improves neurological deficits in rats after MCAo. J Mater Sci Mater Med 25: 1357-1366.

101. Kaengkan P, Baek SE, Kim JY, Kam KY, Do BR, et al. (2013) Administration of mesenchymal stem cells and ziprasidone enhanced amelioration of ischemic brain damage in rats. Mol Cells 36: 534-541.

102. Leu S, Lin YC, Yuen CM, Yen CH, Kao YH, et al. (2010) Adipose-derived mesenchymal stem cells markedly attenuate brain infarct size and improve neurological function in rats. J Transl Med 8: 63.

103. Li D, Fang Y, Wang P, Shan W, Zuo Z, et al. (2012) Autologous transplantation of adipose-derived mesenchymal stem cells attenuates cerebral ischemia and reperfusion injury through suppressing apoptosis and inducible nitric oxide synthase. Int J Mol Med 29: 848-854.

104. Li N, Wang P, Ma XL, Wang J, Zhao LJ, et al. (2014) Effect of bone marrow stromal cell transplantation on neurologic function and expression of VEGF in rats with focal cerebral ischemia. Mol Med Rep 10: 2299-2305.

105. Lin YC, Ko TL, Shih YH, Lin MYA, Fu TW, et al. (2011) Human umbilical mesenchymal stem cells promote recovery after ischemic stroke. Stroke 42: 2045-2053.

106. Liu Z, Li Y, Zhang ZG, Cui X, Cui Y, et al. (2010) Bone marrow stromal cells enhance inter-and intracortical axonal connections after ischemic stroke in adult rats. J Cereb Blood Flow Metab 30: 1288-1295.

107. Mäkinen S, Kekarainen T, Nystedt J, Liimatainen T, Huhtala T, et al. (2006) Human umbilical cord blood cells do not improve sensorimotor or cognitive outcome following transient middle cerebral artery occlusion in rats. Brain Res 1123: 207-215.

108. Mora-Lee S, Sirerol-Piquer MS, Gutiérrez-Pérez M, Gomez-Pinedo U, Roobrouck VD, et al. (2012) Therapeutic effects of hMAPC and hMSC transplantation after stroke in Mice. PLoS ONE 7: e43683.

109. Moubarik C, Guillet B, Youssef B, Codaccioni JL, Piercecchi MD, et al. (2011) Transplanted late outgrowth endothelial progenitor cells as cell therapy product for stroke. Stem Cell Rev 7: 208-220.

110. Nomura T, Honmou O, Harada K, Houkin K, Hamada H, et al. (2005) I.V. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Neuroscience 136: 161-169.

111. Onda T, Honmou O, Harada K, Houkin K, Hamada H, et al. (2008) Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. J Cereb Blood Flow Metab 28: 329-340.

112. Park HW, Moon HE, Kim HSR, Paek SL, Kim Y, et al. (2015) Human umbilical cord blood-derived mesenchymal stem cells improve functional recovery through thrombospondin1, pantraxin3, and vascular endothelial growth factor in the ischemic rat brain. J Neurosci Res 93: 1814-1825.

113. Sugiyama M, Iohara K, Wakita H, Hattori H, Ueda M, et al. (2011) Dental pulp-derived CD31/CD146 side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A 17: 1303-1311.

114. Ukai R, Honmou O, Harada K, Houkin K, Hamada H, et al. (2007) Mesenchymal stem cells derived from peripheral blood protects against ischemia. J Neurotrauma 24: 508-520.

115. Wang J, Chao F, Han F, Zhang G, Xi Q, et al. (2013) PET demonstrates functional recovery after transplantation of induced pluripotent stem cells in a rat model of cerebral ischemic injury. J Nucl Med 54: 785-792.

116. Wu Y, Wu J, Ju R, Chen Z, Xu Q (2015) Comparison of intracerebral transplantation effects of different stem cells on rodent stroke models. Cell Biochem Funct 33: 174-182.

117. Gutiérrez-Fernández M, Rodríguez-Frutos B, Alvarez-Grech J, Vallejo-Cremades MT, Expósito-Alcaide M, et al. (2011) Functional recovery after hematic administration of allogenic mesenchymal stem cells in acute ischemic stroke in rats. Neuroscience 175: 394-405.

118. Gutierrez-Fernandez M, Rodriguez-Frutos B, Ramos-Cejudo J, Otero-Ortega L, Fuentes B, et al. (2015) Comparison between xenogeneic and allogeneic adipose mesenchymal stem cells in the treatment of acute cerebral infarct: Proof of concept in rats. J Transl Med 13: 46.

De Meyer et al. Int J Crit Care Emerg Med 2018, 4:058

• Page 19 of 19 •