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ORIGINAL ARTICLE

From the Lab to Patients: a Systematic Review and Meta-Analysis of Mesenchymal Stem Cell Therapy for Stroke

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Abstract

There may be the potential to improve stroke recovery with mesenchymal stem cells (MSCs); however, questions about the efficacy and safety of this treatment remain. To address these issues and inform future studies, we performed a preclinical and clinical systematic review of MSC therapy for subacute and chronic ischemic stroke. MEDLINE, Embase, the Cochrane Register of Controlled Trials, and PubMed were searched. For the clinical review, interventional and observational studies of MSC therapy in ischemic stroke patients were included. For the preclinical review, interventional studies of MSC therapy using *in vivo* animal models of subacute or chronic stroke were included. Measures of safety and efficacy were assessed. Eleven clinical and 76 preclinical studies were included. Preclinically, MSC therapy was associated with significant benefits for multiple measures of motor and neurological function. Clinically, MSC therapy appeared to be safe, with no increase in adverse events reported (with the exception of self-limited fever immediately following injection). However, the efficacy of treatment was less apparent, with significant heterogeneity in both study design and effect size being observed. Additionally, in the only randomized phase II study to date, efficacy of MSC therapy was not observed. Preclinically, MSC therapy demonstrated considerable efficacy. Although MSC therapy demonstrated safety in the clinical setting, efficacy has yet to be determined. Future studies will need to address the discordance in the continuity of evidence as MSC therapy has been translated from "bench-to-bedside".

Keywords Stem cells · Stroke · Ischemic stroke · Systematic review · Clinical · Preclinical

Introduction

Worldwide, stroke is the second leading cause of death and the third leading cause of disability with ischemic causes accounting for almost 85% of all stroke cases [1, 2]. Despite the massive societal and economic burden of stroke, treatments remain limited. Currently, tissue plasminogen activator (tPA) and mechanical thrombectomy are the only validated therapeutic interventional treatments for ischemic stroke patients [3].

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While long-term recovery from stroke is possible, accepted therapies to date are most optimal when initiated during a critical acute time window for recovery post-stroke [4]. By three months post-stroke, the rate of recovery rapidly decelerates and typically plateaus at approximately six months; this has often been referred to as a "closing of the window of recovery" [5, 6]. Recent advances in regenerative medicine suggest that there may be the possibility for enhanced recovery following stroke, and stem cell therapies may in fact reopen and/or expand the window of recovery following stroke [7].

Mesenchymal stem cells (MSCs) are one such promising cell therapy. MSCs can modulate tissue injury and inflammation [8, 9]. In preclinical studies using in vivo models of ischemic stroke, administration of MSCs has resulted in enhanced recovery of sensorimotor function [10], promotion of synaptogenesis, stimulation of nerve regeneration [11], decreased tissue plasminogen activator-induced brain damage [12], and favorable immunomodulatory effects [13]. Clinically, MSC administration has been shown to be safe in the stroke population in small, early phase trials [7, 14]. However, questions about the efficacy of MSC treatment for subacute and chronic stroke remain [15].

Before further resource-intensive clinical trials are undertaken, it is imperative that an up-to-date, methodologically rigorous review of the current literature is performed in order to analyze the safety and efficacy of MSC therapy in preclinical and clinical stroke. This may identify knowledge gaps along the bench-to-bedside continuum, which may inform and improve the design of future studies. Moreover, given that chronic stroke is one of the top five conditions that patients seek MSC treatment for in international, for-profit, cell therapy clinics [16, 17], it is imperative that an objective, current overview is available for patients and clinicians. Therefore, the purpose of this systematic review is to evaluate the safety and efficacy of MSC therapy for ischemic stroke, both in the preclinical and clinical settings.

Methods

We conducted two systematic reviews, one clinical and one preclinical. The clinical review was registered on PROSPERO (CRD42016033503). The preclinical review protocol was posted a priori on CAMARADES (http://www.camarades.info). This final manuscript was prepared in accordance with the PRISMA checklist (Supplemental Table 1) [18].

Eligibility Criteria

For clinical studies, we included both interventional and observational studies that compared MSC therapy to placebo, standard of care, or no treatment in chronic or acute ischemic stroke patients. We used the criteria defined by the International Society for Cellular Therapy as a general guide to define MSCs [19]. Studies using MSCs that were differentiated to other cell types, were administered as part of a co-treatment with other cell types or experimental therapies, or that had been genetically modified were excluded.

For preclinical studies, we included all controlled interventional studies that compared MSC therapy to placebo/vehicle or no treatment in preclinical in vivo models of ischemic stroke. In order to be included, animals in the control arm must have had a stroke induced (studies with only treatment and sham arms were not eligible for inclusion). We used the criteria defined by the International Society for Cellular Therapy as a general guide to define MSCs [19]. To match the non-acute stroke setting in humans, MSCs had to be administered ≥ 3 days after induction of stroke. If multiple doses were used, the first dose must have been administered no earlier than 3 days. Differentiated MSCs, MSCs administered as part of a co-treatment with other cell types or therapies, or MSCs which had been genetically modified were excluded.

Outcomes

Our primary outcome in the clinical review was safety, as characterized by adverse events. Adverse events were recorded and grouped by the organ system affected as well as classified as either immediate (< 24 h) or long term (> 24 h). Adverse events recorded included the following: fever, stroke, cardiovascular, neurological, hematological, gastrointestinal, renal, infectious, and local complications. Long-term adverse events (i.e., malignancy and death) were also recorded. Our secondary outcome of interest was efficacy, measured by assessments of neurological function (e.g., Barthel Index (BI) [20], National Institutes of Health Stroke Scale (NIHSS) [21], Modified Rankin Scale (mRS) [22]).

In the preclinical setting, our primary outcomes of interest were measures of skilled forelimb and hind limb function (walking tasks, skilled reaching tests, adhesive removal test), as these are considered sensitive tests of motor function [23, 24] and align with consensus guidelines on preclinical stroke recovery research [25]. Secondary outcomes of interest included other measures of neurological status (rotarod, modified neurological severity score) and safety (mortality and adverse events).

Literature Search Strategy

Two comprehensive literature search strategies (one for clinical studies and one for preclinical studies) were developed in collaboration with an information specialist (Risa Shorr, MLS, Ottawa Hospital Library Services) and a clinical expert in the field of stroke research (DD). A second information specialist reviewed the search strategies according to the PRESS framework [26]. The final search strategies were used to search MEDLINE, Embase, The Cochrane Central Register of

Controlled Trials (clinical review only), and PubMed. Clinicaltrials.gov and www.strokecenter.org/trials/ were also searched to identify ongoing clinical trials. We did not impose any language or date restriction on our searches. The search was last updated on April 20, 2018. Search strategies from both the clinical and preclinical searches of MEDLINE are provided in Supplemental Methods 1.

Study Selection Process

All citations identified by our literature searches were imported into DistillerSR® (Evidence Partners, Ottawa, Canada). Titles and abstracts were screened for inclusion by two independent reviewers. Those deemed potentially relevant were recorded, and the full-text articles obtained. Two independent reviewers then screened the full-text articles for final eligibility. Any disagreements were settled by consultation with a senior team member (MML). Although systematic reviews were not eligible for inclusion, their reference lists were reviewed to identify additional relevant articles.

Data Extraction

Two independent reviewers extracted relevant data from included studies using a standardized and pilot-tested data extraction form, created in DistillerSR®. Reviewers collected data relating to the study characteristics, study populations, intervention characteristics, outcomes, and risk of bias. Any disagreements were settled by consultation with a senior team member (MML).

Risk of Bias Assessment and Construct Validity

For clinical studies, the risk of bias was assessed in duplicate using the appropriate Cochrane Risk of Bias tool: the RCT tool for randomized studies, and the ROBINS-I for non-randomized studies. For preclinical studies, risk of bias was assessed in duplicate using a modified version of the Cochrane Risk of Bias Tool that we have previously used for preclinical systematic reviews [27]. In addition to the risk of bias, we assessed construct validity for preclinical studies, to evaluate the clinical generalizability of the experimental conditions. This is of particular importance since a mismatch between the preclinical experimental conditions and the clinical manifestation of the disease may result in false estimates of effect [28]. Construct validity items evaluated in each study included (1) use of adult animals, (2) use of animals with relevant comorbidities, (3) evidence of persistent impairment, (4) rehabilitation of animals, (5) use of a battery of sensory-motor recovery tests (as opposed to a single test), and (6) size of infarct. Each item was assigned either a "yes," "no," or "unclear."

Data Analysis

Studies were pooled using Comprehensive Meta-Analysis (version 3; Biostat Inc., USA). Dichotomous safety outcomes were analyzed via fixed effects meta-analyses using Peto odds ratios and presented with accompanying 95% confidence intervals. Peto's method was used due to the rarity of events [29]. For continuous outcomes, a mean difference (MD) or standardized mean difference (SMD) was calculated, dependent on the outcome. MD and SMD were calculated using random effects inverse variance meta-analyses and presented with accompanying 95% confidence intervals. Statistical heterogeneity was assessed using the I^2 statistic, as well as the chi^2 test or the Cochrane Q test, depending on the analysis method. The presence of publication bias was assessed using funnel plots when sufficient data was available.

For preclinical studies, where sufficient data were available, we performed a priori defined subgroup analyses on the primary outcomes to determine whether the effect of MSCs on animal function varied by species, stroke induction model, MSC source, MSC compatibility, timing, or route of administration. Sensitivity analyses were performed to remove extreme values thought to be driving the overall effect, where applicable.

Assessment of the Continuity of Evidence

We first performed a cross-citation analysis to assess the evidence base used to justify clinical translation (and evaluate the continuity of evidence from the preclinical to clinical setting). Bibliographies of included clinical studies were examined to determine which included preclinical or clinical studies had been cited. Next, we used the Accumulating Evidence and Research Organization (AERO) model, to visually represent the totality of evidence and consistency of results along the preclinical to clinical translational pathway [30]. This model plots the accumulated evidence over time and stratifies it by study phase (i.e., preclinical, phase I, phase II, etc.). Each study is represented visually by a circle, with the color of the circle representing the success of the study (green, yellow, or red). Study success was determined by a combination of statistical significance of results and study author conclusions.

Deviations from Protocol

In the preclinical review, tertiary outcomes listed in the protocol are not reported in this review. These outcomes (e.g., vessel density, dendritic spine density) were not reported in the included studies. Planned exploratory subgroup analyses of biological sex, presence of comorbidities, duration of occlusion, and use of co-interventions were not performed due to either insufficient data or inconsistent reporting. In the clinical

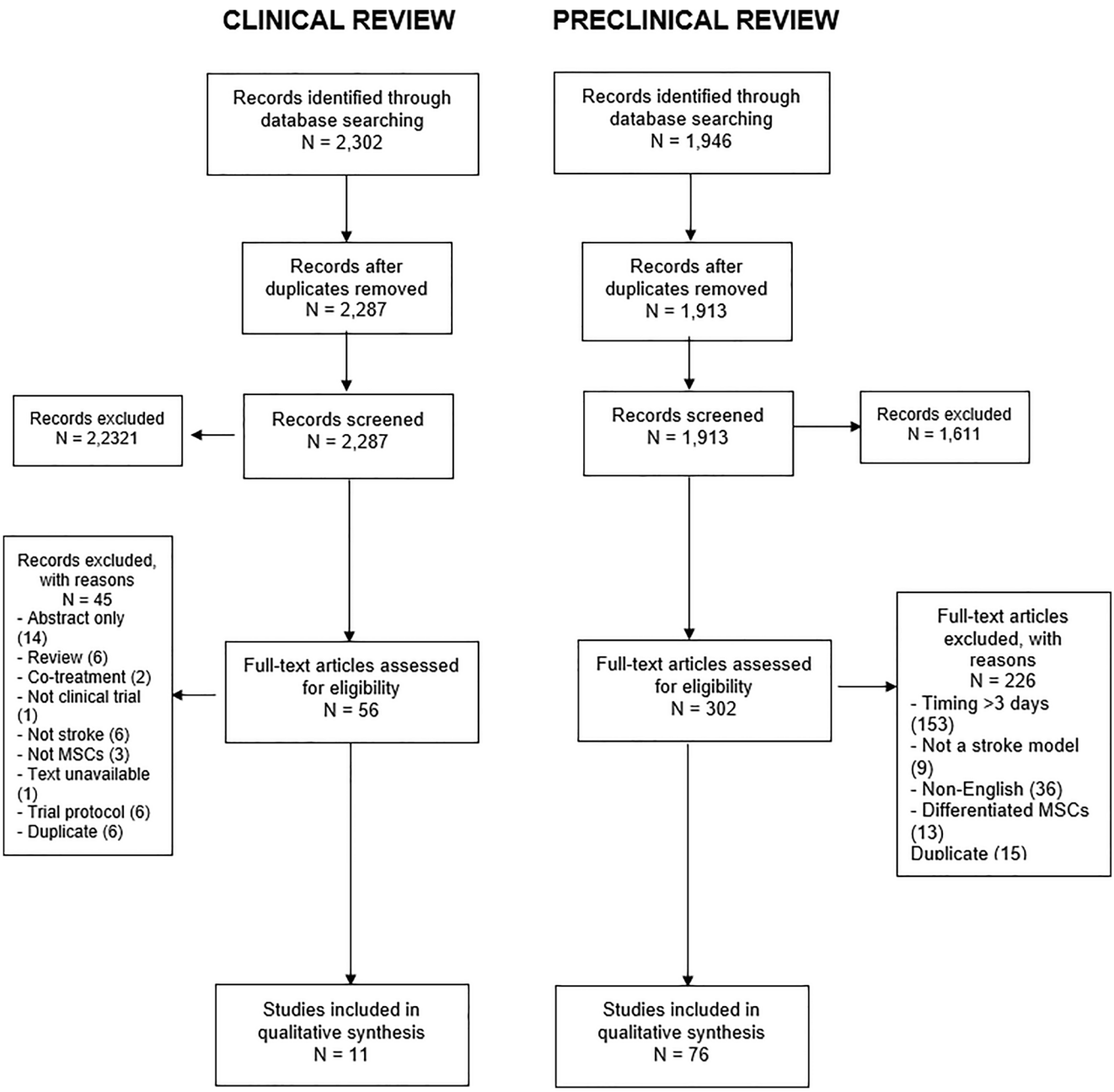


Fig. 1 Study selection flow diagram

review, the tertiary outcome of infarct volume was inconsistently reported in included studies, and therefore not analyzed.

Results

A total of 2287 and 1913 references were identified by the electronic searches for clinical and preclinical studies, respectively. Upon completion of the screening process, a total of 11 citations from 10 clinical studies [7, 14, 15] [S1–8], and 76 preclinical studies were included in our review (Fig. 1) [S9–84].

Characteristics of Included Clinical and Preclinical Studies

Clinical studies were published between 2009 and 2017 from six different countries and consisted of six interventional studies (3 RCTs and 3 non-RCTs) and 4 observational studies (3 case series and 1 retrospective cohort study) (Table 1). Study sample sizes ranged from 4 patients to 129 patients, and the duration of follow-up ranged from 24 to 208 weeks.

Preclinical studies were published between 2000 and 2018 from 12 different countries (Table 2). Study sample sizes ranged from 8 animals to 149 animals. Seventy-four studies

Table 1 Clinical study characteristics

Author (year)	Study type	Country	Patients included	Intervention (donor origin, tissue origin, fresh or frozen)	Administration route and timing	Dose (total cells)	Comparison	Follow-up duration (weeks)
Interventional								
Hess (2017) [15]	RCT	USA, UK	129	Allogeneic, BM, frozen	IV, 24–48 h	4×10^8-12×10^8	Placebo	52
Bhasin (2017 and 2011) [S4–5]	NRCT	USA, India	12	Autologous, BM, fresh	IV, 9.3 months (7–12 months)	5-6×10^7	Standard rehabilitation	208
Steinberg (2016) [7]	NRCT	USA	18	Allogeneic, BM, NR	IC, 22 months (7–36 months)	2.5×10^6, 5.0×10^6, or 10×10^6	N/A	52
Chen (2012) [S6]	NRCT	China	60	Autologous, BM, unclear	IV, 2 and 4 weeks	3-5×10^6	Standard rehabilitation	26
Lee (2010) [14]	RCT	South Korea	52	Autologous, BM, fresh	IV, 5 and 7 weeks	5×10^7	Standard rehabilitation	118
Meng (2009) [S7]	RCT	China	60	Autologous, BM, fresh	IV, NR	$16.2–51.3 \times 10^8$	Standard rehabilitation	24
Observational								
Wanamaker (2015) [S3]	Cohort	USA	10	NR, NR, NR	IC, 6–60 months	NR	Healthy Control, No treatment	52
Qiao (2014) [S2]	Case Series	China	6	Allogeneic, UB, NR	IV, 8 or 12 weeks	3.75×10^7	No comparison	104
Jiang (2013) [S1]	Case Series	China	4	Allogeneic, UB, fresh	IA, 1–7 weeks	2×10^7	No comparison	26
Honmou (2011) [S8]	Case Series	Japan	12	Autologous, BM, frozen	IV, 9 weeks	$0.6–1.8 \times 10^8$	No comparison	52

Table 2 Preclinical study characteristics

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
Chau, 2018 [S11]	USA	16	Mouse, C57BL/6	MCA ligation, permanent; CCA ligation, transient	Fresh, BM, xenogeneic	3	1e6	IN	14
Kho, 2018 [S12]	South Korea	78	Rat, Sprague-Dawley	Intraluminal suture, transient	Frozen, placental, xenogeneic	7	1e6	IV	28
Zhang, 2018 [S13]	China	36	Rat, Sprague-Dawley	MCAO, permanent	Fresh, BM, allogeneic*	7	0.5–3e6	IC, IV, IA	42
*Ding, 2017 [S14]	USA	16	Rat, Wistar	MCAO, transient	NR, BM, unclear	3	5e6	IV	35
He, 2017 [S15]	France	32	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, xenogeneic	8	3e6	IV	16
Hu, 2017 [S16]	China	24	Rat, Sprague-Dawley	MCAO, permanent	Fresh, BM, allogeneic	9	1e6	IV	14
Huang, 2017 [S17]	China	20	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, allogeneic	3	2e6	IV	14
Ding, 2016 [S18]	USA	16	Rat, Wistar	MCAO, transient	NR, BM, unclear	3	5e6	IV	35
He, 2016 [S19]	China	NR	Rat, Sprague-Dawley	MCAO, permanent	NR, BM, allogeneic	3	5e4, 5e5, 2e6	IV	7
Lee, 2016 [S20]	South Korea	28	Rat, Sprague-Dawley	MCAO, permanent	Fresh, adipose tissue, xenogeneic	1, 4, 7	3 × 10e6	IV	28
Moisan, 2016 [S21]	France	78	Rat, Sprague-Dawley	MCAO, transient	Frozen, BM, xenogeneic	8	3e6	IV	49
Nam, 2016 [S22]	South Korea	15	Rat, Sprague-Dawley	MCAO, permanent	Fresh, BM, xenogeneic	3	2e6	IV	7
Pourheydar, 2016 [S23]	Iran	40	Rat, Wistar	CCA suture, transient	Fresh, BM, allogeneic	7	1e6	IV	10
Zhang, 2016 [S24]	China	120	Rat, Sprague-Dawley	MCAO, permanent	Frozen, BM, allogeneic	3	5e5	IC	28
Acosta, 2015 [S25]	USA	30	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, xenogeneic	60	4 × 10e6	IV	71
Hosseini, 2015 [S26]	Iran	50	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, BM, allogeneic	1 h, 12 h, 1, 3, 5 or 7 days	2 × 10e5	IV	28
Lowrance, 2015 [S27]	USA	24	Rat, Sprague-Dawley	Endothelin 1 vasoconstriction, permanent	Fresh, BM, allogeneic	7	4.0x10e5	IC	53

Table 2 (continued)

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
Shichinohe, 2015 [S28]	Japan	20	Mouse, Balb/c	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, allogeneic	7	2x10e5	IC	35
Tan, 2015 [S29]	Japan	22	Rat, Wistar	Other, permanent	Fresh, BM, allogeneic	7	5x10e5	IC	300
Wu, 2015 [S30]	China	24	Mouse, C57Bl/6; rat, Sprague-Dawley	Tamura (permanent, transcranial MCAO coagulation model), permanent; MCAO, permanent	Fresh, unclear, xenogeneic, and allogeneic	3	1x10e6	IC	31
Yang, 2015 [S31]	USA	16	Rat, Sprague-Dawley	Intraluminal suture, transient	Frozen, BM, allogeneic	3	5e6	IV	25
Yang, 2015 [S32]	China	70	Rat, Sprague- Dawley	Intraluminal suture, transient	Fresh, BM, allogeneic	3	5 × 10e6	IV	17
Jeong, 2014 [S33]	South Korea	69	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, xenogeneic	3	5 × 10e5	IC	28
Jiang, 2014 [S34]	China	40	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, adipose tissue, autologous	3	2 × 10e6	IA	28
Mitkari, 2014 [S35]	Finland	60	Rat, Han:Wistar	Intraluminal suture, transient	Frozen, BM, xenogeneic	7	1 × 10e6	IA	42
Wang, 2014 [S36]	China	16	Rat, Sprague-Dawley	Tamura (permanent, transcranial MCAO coagulation model), Permanent	Fresh, BM, allogeneic	3 h, 24 h and 7 days	2 × 10e6 or 1 × 10e7	IV	21
Yamauchi, 2014 [S37]	Japan	27	Rat, Sprague-Dawley	MCAO, Permanent;	Fresh, BM, xenogeneic	7	5 × 10e5	IC	56

Table 2 (continued)

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
				CCA suture, transient					
Byun, 2013 [S38]	South Korea	40	Rat, Sprague-Dawley	Photothrombosis, permanent	Fresh, BM, xenogeneic	3	2.5 × 10e5	IV and IA	5
Ishizaka, 2013 [S39]	Japan	93	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, xenogeneic	1, 4 or 7	1 × 10e6	IA	21
Kawabori, 2013 [S40]	Japan	40	Rat, Sprague-Dawley	MCAO, permanent; CCA suture, transient	Fresh, BM, allogeneic	7 or 28	1 × 10e5 or 1 × 10e6	IC	56
Lu, 2013 [S41]	China	14	Dog, Beagle	MCA embolism, permanent	Fresh, BM, autologous	7	3 × 10e6	IA	35
Ruan, 2013 [S42]	China	45	Rat, Sprague-Dawley	Intraluminal suture, permanent	Fresh, BM, allogeneic	7	1 × 10e5	Other	28
Saito, 2013 [S43]	Japan	23	Rat, Sprague-Dawley	MCAO, permanent; CCA suture, transient	Fresh, BM, allogeneic	7	1 × 10e6	IC	42
Shichinohe, 2013 [S44]	Japan	15	Rat, Wistar	Ouabain injection, permanent	Fresh, BM, allogeneic	7	5 × 10e5	IC	49
Yoo, 2013 [S45]	South Korea	86	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, BM, xenogeneic	3	5 × 10e5	IC	7
Moisan, 2012 [S46]	France	20	Rat, Sprague-Dawley	Intraluminal suture, transient	Unclear, BM, xenogeneic	8	4 × 10e5	IC	21
Bao, 2011 [S47]	China	36	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, BM, xenogeneic	3	5 × 10e5	IC	84
Ding, 2011 [S48]	China	40	Rat, Sprague-Dawley	Intraluminal suture, permanent	Fresh, BM, allogeneic	5	5 × 10e6	IV	26
Ito, 2011 [S49]	Japan	25	Rat, Sprague-Dawley	MCAO, permanent; CCA suture, transient subgroup	Fresh, BM, xenogeneic	7	NR	IC	35

Table 2 (continued)

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
Lim, 2011 [S50]	South Korea	74	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, umbilical, xenogeneic	3	1 × 10e6	IT or IV	10
Sugiyama, 2011 [S51]	Japan	20	Rat, Sprague-Dawley	MCAO, Permanent; CCA suture, transient	Fresh, BM allogeneic	7	5 × 10e5	IC	63
Wang, 2011 [S52]	China	90	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, allogeneic	3	5 × 10e6	IV	28
Wang, 2011[S53]	China	21	Mouse, CD-1	Intraluminal suture, permanent	Fresh, BM, allogeneic	7	1 × 10e5	IC	30
Bao, 2010 [S54]	China	72	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, BM, xenogeneic	3	5 × 10e5	IC	28
Cho, 2010 [S55]	South Korea	100	Rat, Sprague-Dawley	MCAO, transient	Fresh, BM, xenogeneic	14	6 × 10e5	IC	35
Kawabori, 2010 [S56]	Japan	24	Rat, Sprague-Dawley	MCAO, permanent	Fresh, BM, allogeneic	7	1 × 10e6	IC or IV	35
Komatsu, 2010 [S57]	Japan	149	Rat, Sprague-Dawley	Intraluminal Suture, permanent	Fresh, BM, allogeneic	7, 14, 28	1 × 10e6	IV	84
Li, 2010 [S58]	China	8	Monkey, *Macaca fascicularis*	Photothro-mbosis, permanent	Fresh, BM, xenogenic	7	1 × 10e6 or 5 × 10e6	IC	21
Liu, 2010 [S59]	Taiwan	30	Rat, Sprague-Dawley	Intraluminal suture and three vessel (MCA surface, bilateral common carotid), transient	Fresh, umbilical, xenogeneic	7	1 × 10e6	IC	35
Osanai, 2010 [S60]	Japan	18	Mouse, Balb/c	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, syngeneic, and allogeneic	7	5 × 10e5	IC	63

Table 2 (continued)

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
Yang, 2010 [S61]	United States	NR	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, BM, allogeneic, and xenogeneic	7	5 × 10e5	IV	28
Zhang, 2010 [S62]	United States	88	Rat, Wistar	Intraluminal suture, transient	Fresh, umbilical, xenogeneic	7, 30, and 90	3 × 10e6	IV	60
Detante, 2009 [S63]	France	18	Rat, Sprague- Dawley	Intraluminal suture, transient	Fresh, BM, xenogeneic	7	3.4± 1.2 × 10e6	IV	8
Hokari, 2009 [S64]	Japan	16	Mouse, Balb/c	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, syngeneic	7	2 × 10e5	IC	35
Santos, 2009 [S65]	Brazil	14	Rat, Wistar	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, syngeneic	BMSCs 7, 14, or 30; MSCs 30	BMSCs 3 × 10e7, MSCs 3 × 10e6	IV	77
Yasuhara, 2009 [S66]	USA	54	Rat, Sprague-Dawley	Intraluminal suture, transient	Fresh, BM, xenogeneic	28–42	9.0 × 10e4 or 1.8 × 10e5	IC	112–140
Andrews, 2008 [S67]	USA	22	Rat, Long Evans black-hood	MCAO, permanent; CCA suture, transient	Fresh, BM, xenogeneic	7	1.5 × 10e6	IC	70
Kim, 2008 [S68]	South Korea	20	Rat, Fisher	Intraluminal suture, permanent	Fresh, BM, syngeneic	7	1 × 10e6	IC	28
Kim, 2008 [S69]	South Korea	60	Rat, Sprague-Dawley	Intraluminal suture, permanent	Fresh, BM, xenogeneic	3	1 × 10e6	IC	56
Kim, 2008 [S70]	South Korea	13	Rat, Sprague-Dawley	Intraluminal suture, permanent	Fresh, BM, xenogeneic	7	1.0 × 10e5	IC	77
Koh, 2008 [S71]	South Korea	36	Rat, Sprague- Dawley			14	6 × 10e5	IC	35

Table 2 (continued)

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
				Intraluminal suture, transient	Fresh, umbilical, xenogeneic				
Pavlichenko, 2008 [S72]	Russia	NR	Rat, Wistar – Kyoto	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, autologous	3	5 × 10e6	IV	42
Yoo, 2008 [S73]	South Korea	86	Rat, Sprague- Dawley	Intraluminal suture, transient	Fresh, BM, xenogeneic	3	5 × 10e5	IC	28
Cipriani, 2007 [S74]	Italy	28	Rat, Sprague- Dawley	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, amniotic fluid, xenogeneic	7	2.0 × 10e5	IC	97
Ding, 2007 [S75]	Taiwan	20	Rat, Sprague- Dawley	MCAO, transient; CCA suture, transient	Fresh, umbilical, xenogeneic	7	1 × 10e6	IC	35
Rehni, 2007 [S76]	India	36	Mouse, Swiss Albino	Intraluminal suture, transient	Fresh, umbilical, allogeneic	3	5 × 10e5 or 10 × 10e5	IC	7
Shen, 2007 [S10]	USA	25	Rat, Wistar	Intraluminal suture, transient	Fresh, BM, syngeneic	30	3 × 10e6	IV	120
Shyu, 2007 [S77]	Taiwan	35	Rat, Sprague-Dawley	Three vessel (MCA surface, bilateral common carotid), transient	NR, BM, xenogeneic	7	1 × 10e6	IC	35
Shichinohe, 2006 [S78]	Japan	15	Mouse, Balb/c	Tamura (permanent, transcranial MCAO coagulation	Fresh, BM, allogeneic	7	2 × 10e5	IC	35

Table 2 (continued)

Author	Country	Sample size	Species, strain	Stroke model	MSC source	Timing of administration (days post-stroke)	Dose	Route of administration	Follow-up (days)
				model), permanent					
Sokolova, 2006 [S79]	Russia	45	Rat, Wistar-Kyoto	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, syngeneic	3	5.0 × 10e6	IV	42
Gong-xiong, 2005 [S80]	China	48	Rat, Sprague-Dawley	Tamura (permanent, transcranial MCAO coagulation model), permanent	Fresh, BM, syngeneic	10	NR	IC	52
Mimura, 2005 [S81]	Japan	27	Rat, Wistar	MCA embolism, transient	Fresh, BM, syngeneic	7	2.4 × 10e4–4.8 × 10e4	IC	40 days
Li, 2005 [S82]	USA	21	Rat, Wistar	Intraluminal suture, permanent	Fresh, BM, syngeneic	7	3 × 10e6	IV	112
Gui, 2004 [S83]	China	12	Rat, Wistar	Intraluminal suture, permanent	NR, BM, syngeneic	7	1 × 10e4	IC	35
Chen, 2001 [S9]	USA	32	Rat, Wistar	Intraluminal suture, transient	Fresh, BM, NR	7	3 × 10e6	IV	35
Li, 2000 [S84]	USA	23	Mouse, C57BL/6J	MCA embolism, permanent	Fresh, umbilical, syngeneic	4	1 × 10e5	IC	28

BMC bone marrow-derived MSCs, *IA* intra-arterial, *IC* intracranial, *IV* intravenous, *IN* intranasal, *NR* not reported, *NRCT* nonrandomized controlled trial, *RCT* randomized controlled trial, *SD* standard deviation, *UBC* umbilical cord-derived MSCs

(97%) were performed in rodents, with most ($n = 65$, 86%) being performed in rats. There was one study performed in monkeys (1%) and one in dogs (1%). Stroke was induced via a number of methods, most commonly intraluminal suture ($n = 30$, 39%) and middle cerebral artery occlusion ($n = 24$, 32%). Follow-up ranged from 5 to 300 days.

Intervention Characteristics

Of the 10 clinical studies, five assessed the effects of autologous MSCs (50%), four studied allogeneic MSCs (40%), and one study did not report the type of MSC used (10%) (Table 1). The MSCs were bone marrow-derived in seven studies (70%), umbilical cord-derived in two studies (2%), and one study did not report this information (10%). The MSCs were given intravenously in seven studies (70%), intra-cerebrally in two (20%), and intra-arterially in another (10%). The dose of MSCs administered ranged from 2.5×10^6 to 51.3×10^8 total cells, and the timing of administration varied from 24 h to 60 months post-stroke.

Of the 76 included preclinical studies, a fresh product was used in 65 studies (86%). Tissue sources of MSCs included bone marrow ($n = 64$, 84%), adipose tissue ($n = 2$, 3%), umbilical cord ($n = 7$, 9%), placenta ($n = 1$, 1%), and amniotic fluid ($n = 1$, 1%). The tissue source of cells was unclear in one study (1%). Immunological compatibility between donor MSCs and recipients varied between xenogeneic (i.e., human cells to animal model, $n = 32$, 42%), syngeneic ($n = 10$, 13%), allogeneic ($n = 25$, 33%), autologous ($n = 3$, 4%), multiple compatibilities ($n = 3$, 4%), and unclear ($n = 3$, 4%). Routes of administration included intracerebral ($n = 38$, 50%), intravenous ($n = 28$, 37%), intraarterial ($n = 4$, 5%), intranasal ($n = 1$, 1%), and multiple methods ($n = 4$, 5%). Dosage administered ranged from 1×10^4 cells to 1×10^7 cells, while the timing of administration ranged from 3 to 60 days post-stroke (Table 2).

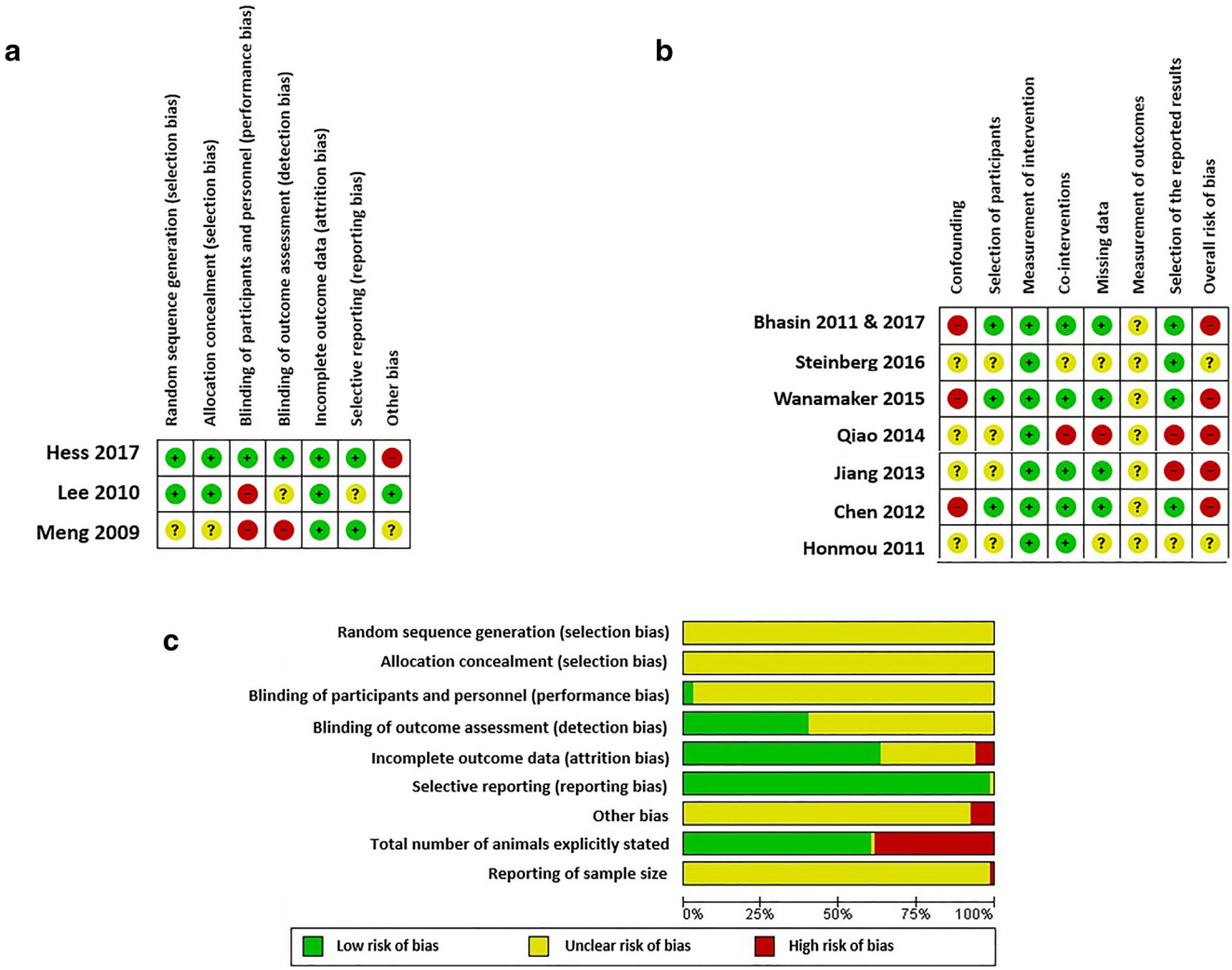


Fig. 2 Peto odds ratios (95% CI) and pooled estimate for the risk of death in clinical studies

Risk of Bias

For clinical studies, the overall study quality was poor with 9 of the 10 studies being classified at a high risk of bias in at least one domain (Fig. 2).

For preclinical studies, the quality of studies was also found to be poor, with the majority of studies at unclear risk of bias due to poor methodological reporting (Fig. 2).

Construct Validity

In terms of construct validity, 56 studies (74%) stated that an adult animal model was used, and three studies used animals with comorbidities (4%). Fifty-seven studies (75%) demonstrated persistent impairment in a control group, no studies subjected animals to rehabilitation, and 28 studies (37%) used a battery of sensory-motor recovery tests (as opposed to a single test). In 21 studies (28%), the infarct size was proportional to that seen in human stroke patients, and in 16 studies (21%), the duration of occlusion created a clinically relevant infarct size. A breakdown of construct validity by study, as well as a more detailed outline of our criteria, is available in Supplemental Table 2.

Safety of MSCs in the Clinical Setting

Nine studies ($n = 339$ patients) reported data on mortality, three of which ($n = 19$ patients) were case series and were therefore not included in the meta-analysis. Patients who had been administered MSC therapy had a significantly reduced risk of death compared to control (Peto OR 0.43; 95% CI 0.20–0.90) (Supplementary Fig. 1). The decrease in pooled mortality was largely influenced by one study. In a sensitivity analysis removing this study, there were an equal number of deaths in each group (Peto OR 0.58; 95% CI 0.22–1.53) (Supplemental Fig. 2). MSC treatment was associated with a significantly increased risk of developing a fever immediately following study injection (Peto OR 6.88; 95% CI 2.48–19.08). There were no differences between groups in all other adverse events reported (Supplemental Table 3). Of note, in the largest RCT to date, similar rates of serious adverse events were seen in the MSC group and control group (34% and 39%, respectively) [15].

Safety of MSCs in the Preclinical Setting

Nine preclinical clinical studies (12%, $n = 249$ animals) reported data on mortality. There was no statistically significant difference in the risk of mortality between MSC and control groups (Peto OR 0.96; 95% CI 0.49–1.86) (Supplemental Fig. 3).

Efficacy of MSCs in the Clinical Setting

Clinical measures of efficacy reported by studies in our review included the NIHSS ($n = 5$, 50%), mRS ($n = 6$, 60%), BI ($n = 3$, 30%), Fugl-Meyer (FM) scale ($n = 3$, 30%), and the European Stroke Scale (ESS) ($n = 1$, 10%). Due to the heterogeneity of study designs, no formal pooling of data was performed, and data is presented descriptively. Of the five studies which reported data on the NIHSS, there was one RCT [15], one single-arm trial [7], one non-randomized interventional study [S6], and two case series [S2, S8]. In the single RCT, there was no significant difference in the NIHSS improvement between placebo and cell therapy groups at 1-year post-treatment. In the single arm study, the NIHSS decreased from baseline by 2.00 (95% CI, −2.7 to −1.3; $P < 0.001$) at 12 months. In the non-randomized study, both treatment and control groups saw a significant decrease in the NIHSS scores at 6 months post-treatment; however, the improvement seen in the MSC-treated group was significantly larger. In both case series, the NIHSS scores decreased post-treatment.

Of the six studies which reported data on mRS, there were two RCTs [14, 15], one single-arm trial [7], and three case series [S1–2, S8]. The first RCT reported that the proportion of patients with an mRS score 0–3 increased in the MSC group, but not in the control group. In the other RCT, the proportion of patients with an mRS score ≤ 1 was significantly larger in the MSC group at 1-year post-treatment ($p = 0.041$). However, the authors stated that the distribution of mRS scores was not significantly different between groups. In the single-arm study, there was no change in mRS score from baseline at 12 months post-treatment. In all three case series, the mRS score decreased post-treatment.

Of the three studies which reported data on BI, one was an RCT [15], one was a non-randomized study [S5], and one was a case series [S2]. The RCT found that a BI of 95 or more was equal between MSC and placebo-treated patients. In the non-randomized study, the modified BI increased significantly more in the treatment group compared to the control group at 208 and 156 weeks. In the case series, the BI increased post-treatment.

Of the three studies which reported data on FM score, there was one RCT [S7], one single-arm study [7], and one non-randomized study [S5]. In the RCT, the FM score improvement was significantly greater in the treatment group compared to the control group 6 months post-treatment. In the single-arm study, the FM score increased significantly from baseline at 12 months post-treatment. In the non-randomized study, there was no significant difference in FM score between treatment and control groups at 4-year follow-up.

One single-arm study reported data on European Stroke Scale scores [7] and saw a significant increase from baseline at 12 months post-treatment.

Efficacy of MSCs in the Preclinical Setting

Preclinical measures of efficacy reported by studies in our review included the rotarod test ($n = 19$, 25%), the adhesive removal test ($n = 15$, 20%), the modified neurological severity score ($n = 18$, 24%), the skilled reaching test ($n = 3$, 4%), and walking tasks ($n = 4$, 5%).

MSC therapy resulted in a significantly superior performance on walking tasks (SMD 1.37; 95% CI 0.63 to 3.49) but not in skilled reaching tests (SMD 1.11; 95% CI − 1.28 to 3.49) when compared to placebo-treated animals (Fig. 3a and b). Limited data prohibited subgroup analyses for these outcomes.

Animals that had been treated with MSCs post stroke performed significantly better on the adhesive removal test (MD − 25.54; 95% CI − 30.39 to − 20.69, $I^2 = 88.1\%$) (Fig. 3c) when compared to placebo-treated animals. Visual inspection of the funnel plot and Eggers regression test indicate a small degree of publication bias ($p = 0.011$) (Supplemental Fig. 4). In our a priori defined subgroup analyses, there was no difference in the measure of effect between species, or route of administration (Supplemental Figs. 5–6). Animals that received umbilical cord-derived MSCs performed better than those that received either bone marrow-derived or adipose tissue-derived MSCs ($p < 0.001$) (Supplemental Fig. 7). Animals that received autologous MSCs (compared to allogeneic, syngeneic, or xenogeneic), received MSC therapy in 3–30 days after stroke induction (compared to > 30 days), or had stroke induced with the MCAO model (compared to intraluminal suture or the Tamura model) performed worse than their counterparts (Supplemental Figs. 8–10), although limited evidence was available from those subgroups.

Animals who had been treated with MSCs performed significantly better on the rotarod test (MD 25.12; 95% CI 17.34 to 32.90, $I^2 = 91.1\%$) (Supplemental Fig. 11). No evidence of publication bias was detected via visual inspection of a funnel plot or via Egger's regression test (Supplemental Fig. 12). In post hoc exploratory subgroup analyses, there was no difference in the measure of effect between species, route of administration or stroke model (Supplemental Figs. 13–15). Animals that had been administered adipose-derived MSCs (compared to bone marrow or umbilical cord derived), or autologous MSCs (compared to allogeneic, syngeneic or xenogeneic) performed worse than their counterparts (Supplemental Figs. 16–17), although limited evidence was available from those subgroups. No evidence was available from studies which administered MSCs > 30 days after stroke induction; therefore, that subgroup analysis was not performed.

MSC-treated animals also had significantly lower modified neurological severity scores (MD − 1.64; 95% CI − 2.18 to − 1.09) compared to placebo treated animals (Supplemental Fig. 18). No evidence of publication bias was detected via visual inspection of a funnel plot or via Egger's regression test (Supplemental Fig. 19). In post hoc exploratory subgroup analyses, there was no difference in the measure of effect between species, MSC source, route of administration, MSC compatibility, or stroke model (Supplemental Figs. 20–24). Animals that received MSC therapy 3–30 days after stroke induction period performed better than those that received MSC therapy > 30 days, although limited evidence was available for the chronic period (Supplemental Fig. 25).

Preclinical to Clinical Continuity of Evidence

In our citation analysis, the median number of preclinical studies cited by each clinical study was 1 (range 0–4) (Supplemental Table 4). Only two preclinical studies (3%) were cited more than once. The consistency of evidence is noted in our AERO model (Fig. 4). Evidence is overwhelmingly positive (green) in the preclinical and early clinical stages of research, with very few studies demonstrating any unfavorable results. However, in the only randomized phase II study to date (i.e., the most methodologically rigorous study) [15], efficacy of MSC therapy was not observed.

Discussion

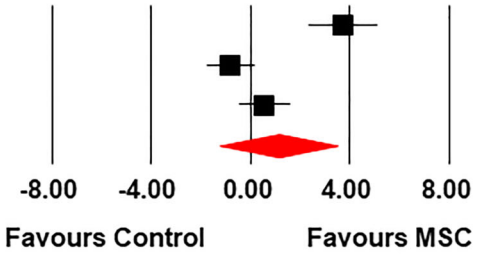
Our systematic review and meta-analysis evaluated and described the safety and efficacy of MSC therapy for ischemic stroke in both the clinical and preclinical settings. Our review included 10 clinical and 76 preclinical studies, all of which administered MSCs in either the subacute or chronic phase following stroke. Available evidence indicates that MSC therapy appears to be safe in both the preclinical and clinical settings. In the preclinical setting, MSC therapy had beneficial effects in multiple neurological and motor skills tests. In the clinical setting, MSC therapy appeared to be promising in early phase studies, but the most rigorous study performed to date failed to demonstrate efficacy. We noted a clear discontinuity of effect as MSC therapy was translated from the bench-to-bedside.

A complete and current synthesis of this area is needed at this time for several reasons. From a patient perspective, patients seeking cell therapy from "stem cell clinics" (most commonly autologous bone marrow derived MSC therapy) should be aware of the very limited current evidence to support efficacy this therapy. For both preclinical and clinical researchers, quantitative results from our study could be used to perform power calculations for these studies as they are prepared. Reassuringly for future trials, the therapy does appear to be safe overall with no increase risk in mortality noted in clinical studies. In addition, recognizing knowledge gaps and the discontinuity from preclinical to clinical development may inform better design of future preclinical and clinical studies.

Many potential reasons for the discordance between preclinical and clinical findings can be found in our synthesized

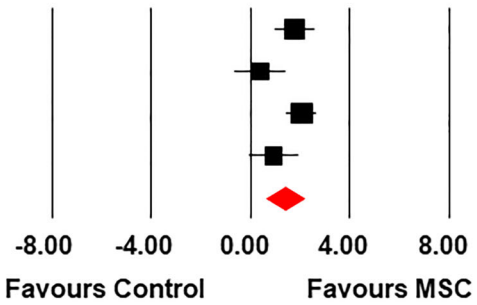
a

Study name	Year	Std diff in means	Lower limit	Upper limit
Andrews	2008	3.70	2.32	5.08
Mitkari	2014	-0.81	-1.79	0.18
Lowrance	2015	0.56	-0.48	1.59
OVERALL		1.11	-1.28	3.49



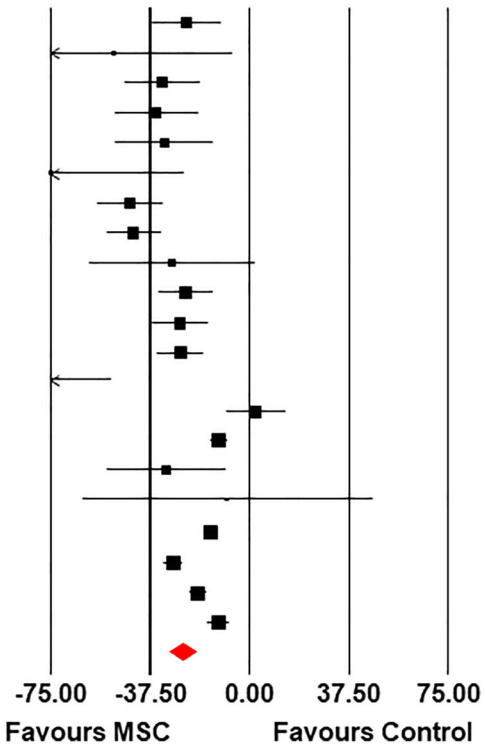
b

Study name	Year	Std diff in means	Lower limit	Upper limit
Gong-xiong	2005	1.77	0.95	2.58
Santos	2009	0.38	-0.68	1.44
Wang	2011	2.05	1.43	2.68
Yan	2015	0.92	-0.11	1.95
OVERALL		**1.37**	**0.63**	**2.11**



c

Study name	Year	Difference in means	Lower limit	Upper limit
Chen	2001	-23.64	-36.61	-10.67
Li	2005	-50.98	-95.39	-6.57
Shen	2007	-32.78	-47.16	-18.40
Rehni (b)	2007	-34.94	-50.77	-19.11
Rehni (a)	2007	-32.12	-50.63	-13.61
Kim	2008	-74.82	-125.23	-24.41
Zhang (a)	2010	-45.22	-57.89	-32.55
Zhang (b)	2010	-43.83	-54.21	-33.45
Zhang (c)	2010	-29.22	-60.59	2.15
Bao	2011	-24.20	-34.59	-13.81
Lim (a)	2011	-26.17	-37.00	-15.34
Lim (b)	2011	-26.07	-34.85	-17.29
Yoo	2013	-85.90	-119.59	-52.21
Mitkari	2014	2.46	-8.87	13.79
Jeong	2014	-11.63	-14.87	-8.39
Yang	2015	-31.47	-53.96	-8.98
Moisan	2016	-8.36	-63.33	46.62
Zhang (a)	2018	-14.40	-16.38	-12.42
Zhang (b)	2018	-28.90	-32.54	-25.26
Zhang (c)	2018	-19.60	-22.95	-16.25
Chau	2018	-11.77	-16.07	-7.47
OVERALL		**-25.54**	**-30.39**	**-20.69**



◀ **Fig. 3** Standardized mean differences (95% CI) and pooled estimate for **a** skilled reaching test, **b** walking tasks, and difference in means (95% CI) and pooled estimate for **c** adhesive removal tests

dataset. First, although MSC therapy for ischemic stroke has been well studied in the preclinical setting, investigators have focused on outcomes that may not be clinically relevant. For example, in a 2014 meta-analysis of 46 studies of MSC therapy for ischemic stroke in animal models, 44 studies reported that MSC therapy significantly improved outcomes [31]. However, this study did not capture the most sensitive tests of impairment that may be the most clinically relevant (e.g., reaching tasks) [23]. In our review, evidence was limited for both walking ($n = 4$) and reaching ($n = 3$) tasks, and results were marked with significant heterogeneity. The diminished efficacy in the potentially most clinically relevant of tests may provide insight into the lack of clinical efficacy in larger, methodologically rigorous trials of MSC therapy. Future preclinical studies should consider focusing on sensitive tests.

The timing of MSC therapy must be carefully considered for future studies. All patients in clinical studies have been administered MSC therapy in the subacute or chronic phase following stroke; however, preclinical work cited by those studies had focused on the acute administration of the cells. In addition, a previous preclinical systematic review of MSC therapy for stroke focused on acute administration of these cells [31]. Our review now provides a synthesis of preclinical studies that match the timing of clinical studies that have been performed to date. It is clear, however, from our review that a paucity of preclinical evidence exists for cell therapy in the chronic phase of stroke (i.e., administration after 30 days in stroke models), which is the timing of intervention for most clinical trials performed to date. Future studies will need to address this knowledge gap and use available clinical and preclinical evidence to rationally choose the timing of cell therapy. It may be possible that clinical studies will need to focus on more acute administration of MSCs following stroke in order to replicate the preclinical promise of this therapy.

The poor methodological rigor of both preclinical and early clinical studies may have contributed to a high risk of bias in their findings. For instance, only a small minority of preclinical studies employed randomization and blinding, which are well recognized to increase validity of findings (i.e., absence of these methodologies spuriously increases the magnitude of outcomes in preclinical studies) [32, 33]. Moreover, despite the large number of studies published, it appears none of them were specifically designed to be confirmatory studies, which are increasingly considered to be a necessary preamble to clinical trials [34, 35]. Similar issues with design were also noted for clinical studies as the majority with positive results had significant issues with design (e.g., lack of blinding for outcome assessment). Four of the ten clinical studies also did not include a control group. The interpretation of data from these studies is difficult, as the natural history for ischemic stroke is

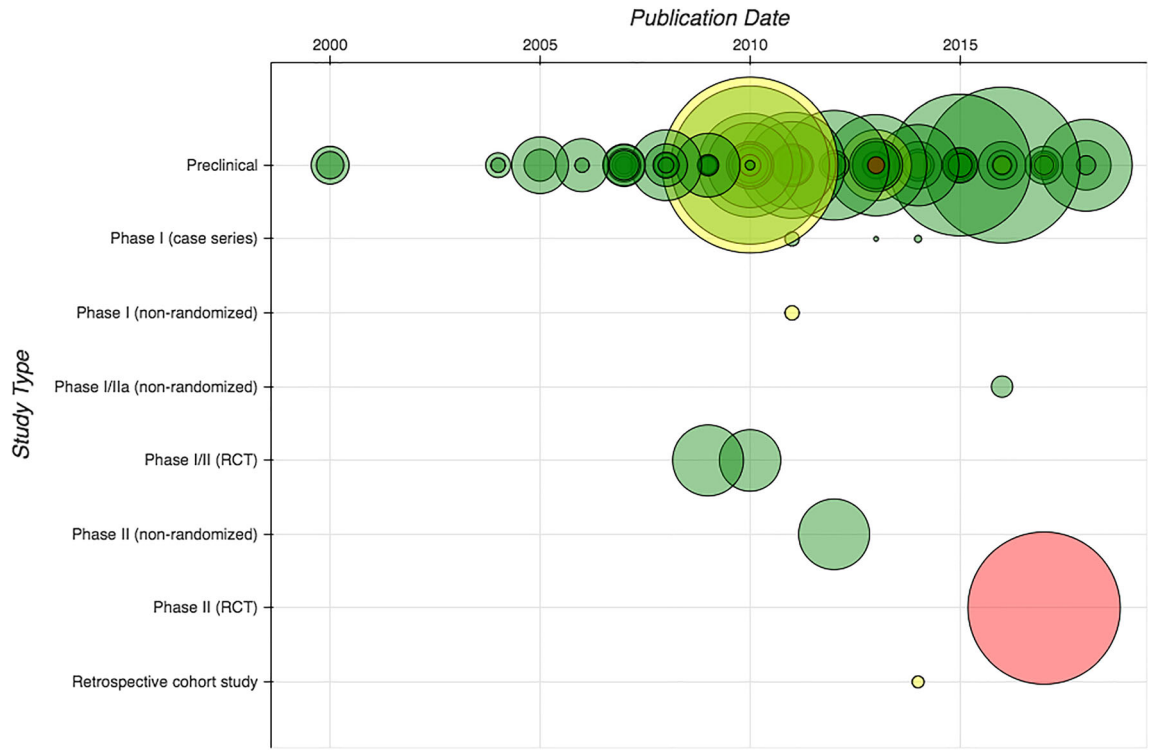


Fig. 4 AERO model demonstrating the consistency of results across the translational pathway. The size of each circle is proportional to the sample size of the study. A green circle represents a study with positive findings, yellow represents mixed findings, and red represents negative findings

improvement with standard rehabilitation. It is perhaps not surprising then that preclinical and clinical evidence is overwhelmingly positive up to the point where MSC therapy was tested in a methodologically rigorous manner (demonstrated through our AERO diagram, Fig. 4). Increased methodological rigor will be needed for future laboratory and clinical studies.

The clinical relevance of the stroke models used by many of the included preclinical studies needs to be considered in more detail. Studies have demonstrated that poor construct validity affects reproducibility and downstream clinical translation [36, 37]. In our assessment of construct validity of included preclinical studies, key characteristics seen in ischemic stroke patients were missing or under-reported. For example, up to 75% and 68% of ischemic stroke patients have hypertension and hyperglycemia respectively, [38, 39] yet only three included preclinical studies (4%) used animals with any comorbidities. Additionally, only 16 (21%) of studies created a clinically relevant infarct size by selecting an appropriate duration of occlusion (i.e., < 90 min) and only 21 studies (28%) infarcted less than 40% of the brain. The majority of human strokes, especially the treatable ones, are significantly smaller in size than what has been seen in preclinical models [40, 41]. Severe differences between the preclinical experimental conditions and the clinical manifestation of the disease have been demonstrated to provide false estimates of effect [28]. This may contribute to the discordance between the preclinical and clinical evidence presented by this review. Future preclinical studies should carefully consider parameters regarding their choice of animal model, in an effort to increase construct validity.

Differences in donor cell characteristics may have also contributed to differences in preclinical and clinical results. The vast majority of preclinical studies (> 90%), used a fresh (i.e., not cryopreserved) MSC product from healthy, very young donors. However, in the clinical setting, half of the included studies used an autologous MSC product. This may be significant as cell therapy products derived from non-healthy individuals have been demonstrated to be less efficacious than those taken from healthy donors [42, 43]. The one large clinical RCT included did use allogeneic cells from a healthy donor; however, it did use a cryopreserved product and the viability of cells post-thawing was not described. Issues with poor viability of cryopreserved MSCs have been associated with poor clinical efficacy [44]. In order to better match preclinical conditions demonstrated to be efficacious, future clinical trials should consider using allogeneic cells from healthy donors (e.g., umbilical cord) and carefully assess (and report) the viability of cells used.

Conclusions from our systematic review are limited by several factors. First, our conclusions are tempered by the quality of the studies included in our analysis. All included clinical studies were deemed to be at a high risk of bias across at least one domain. In addition, the majority of preclinical studies were deemed to be at an unclear risk of bias due to poor methodological reporting. Second, there was considerable heterogeneity seen across studies. Due to significant methodological heterogeneity seen in clinical studies, no meta-analysis was performed. This considerable heterogeneity precludes any definitive conclusions regarding efficacy; however, it does clearly demonstrate the scarcity of data available. With regard to preclinical studies, significant statistical heterogeneity was observed across all performed analyses. Subgroup analyses did not resolve any of the statistical heterogeneity observed. Despite the observed limitations, this review provides a comprehensive overview of all available data concerning MSC therapy for ischemic stroke in both the clinical and preclinical settings. Although some promising results exist, it is evident from our review that high-quality RCT evidence is required before conclusions can be made regarding the efficacy of MSC therapy in the chronic phase of ischemic stroke.

Summary

Our systematic review and meta-analysis provides a comprehensive, up-to-date evaluation of the safety and efficacy of MSC therapy for chronic stroke. With the exception of an increased risk in fever immediately following injection, MSC therapy was not associated with an increase in adverse events. Preclinically, MSC therapy demonstrated considerable efficacy with regard to several neurological and motor function tests; however, in the most sensitive tests, signals of efficacy were diminished. In the clinical setting, significant methodologic heterogeneity and a scarcity of data precludes definitive conclusions regarding efficacy of treatment, although the largest and most methodologically sound trial to date demonstrated a lack of efficacy. Future preclinical and clinical studies should consider issues we have highlighted including study design, methodological rigor, interventions, and outcomes. Ultimately, well-designed RCTs examining MSC therapy are needed to determine the effect of MSC therapy in the treatment of chronic stroke.

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Compliance with Ethical Standards

Conflict of Interest DS is an unpaid consultant and has an equity interest in Northern Therapeutics (Montréal, QC, Canada).

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors

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