Supplemental Material

Methods

Studies on cell therapy for ischemic stroke published before April 3rd 2018 were identified from PubMed, Web of Science and Scopus. The study protocol is available on PROSPERO (CRD42018093214 and CRD42018096257).

Inclusion and exclusion criteria

The full-text of relevant original English publications was retrieved after review of the titles and abstracts by two independent investigators (L.C. and E.H.). Preclinical studies were excluded if: 1) no focal cerebral ischemia model was used; 2) cell transplantation was not applied after ischemic stroke; 3) two or more types of cells were transplanted concomitantly; 4) neither infarct size nor behavioral outcome assessments including modified Neurological Severity Score (mNSS), rotarod test, and adhesive removal test (ART) were reported; 5) no full text was available. Both blinded and non-blinded, but only controlled clinical studies were included.

Data extraction

Three independent investigators (L.C., D.G., and M.M.) extracted the data. A fourth investigator (J.J.) was consulted in case of no consensus. The data were extracted from the text or by requesting the particular information from the authors; if neither option was possible, from figures in the publication. In the case that the figures were the only available source of information, values of mean and standard deviation (S.D.) or standard error (S.E.) were obtained from highly magnified images using Image J software. For studies with multiple intervention arms, we assessed each arm as an independent intervention.

A quality score was designed to estimate the methodological quality of preclinical studies. It included 10 criteria based on good laboratory practice guidelines for in vivo stroke modeling[1] as well as the CAMARADES quality checklist[2]: 1) publication in a peer-reviewed journal; 2) control of body temperature during surgery; 3) randomization when assigning experimental groups; 4) allocation concealment; 5) blinded outcome assessment; 6) statement describing use or preferably avoidance of anesthetics with intrinsic neuroprotective properties after ischemia induction; 7) use of animals with relevant comorbidities; 8) a priori sample-size calculation; 9) statement of compliance with animal welfare regulations; 10) statement declaring conflicts of interest. For each criterion, one point was given if reported in the publication.

The primary outcomes of interest were: 1) infarct size; 2) mNSS; 3) rotarod test performance; 4) ART performance, as these have been the most commonly used outcomes for evaluating the effect size of cell therapy in animals with ischemic stroke. The effect size values for the infarct size, mNSS and ART were multiplied by -1 because (in contrast to the rotarod test) larger values represent worse outcomes. When outcomes were reported at multiple time points, we only examined the final time point. In the clinical studies, the primary outcomes were National Institutes of Health Stroke Scale (NIHSS), modified Barthel index (mBI), modified Rankin scale (mRS); and Fugl-

Meyer scale (FMS) at the last reported time point after cell treatment. Similarly, the effect size values of NIHSS and mRS were multiplied by -1 as larger values represent worse outcomes.

Statistical analysis

Preclinical data analysis was based on the practical guide prepared by Vesterinen et al.[3]. The standardized mean differences (SMD), 95% confidence intervals (95% CI) and significances were examined using the inverse-variance method. For each outcome, the effect size was calculated using Hedges' g and heterogeneity was calculated as I^2. A random effects model was used to estimate the pooled effect size due to the substantial heterogeneity between the various studies. The potential of publication bias in preclinical studies was examined using funnel plots and Egger's regression. Adjusted effect sizes were estimated after using the Tweedie and Duval trim and fill approach.

The univariate meta-regression was conducted in preclinical studies to explore the sources of heterogeneity in different effect sizes, i.e. the variables that influence outcomes. The following variables were explored: 1) impact factor of published journal; 2) ischemia type (permanent/transient); 3) stroke model; 4) transplanted cell type (MSCs/NSCs/MNCs/others); 5) transplanted cell source (e.g. bone marrow, umbilical cord); 6) cell stemness (pluripotent/multipotent/unipotent/others); 7) type of manipulation (e.g. cell pretreatment, genetic engineering); 8) recipient species; 9) recipient age; 10) recipient sex; 11) degree of immunogenicity (autologous, syngeneic, allogeneic, or xenogeneic); 12) use of immunosuppressant; 13) passage of transplanted cells; 14) cell dose; 15) cell cryopreservation; 16) cell viability; 17) delivery route; 18) delivery time relative to stroke onset; 19) quality score of studies; 20) time of outcome assessment; 21) use of animals with common comorbidities. The significance level was set at $P<0.05$, and adjusted R^2 was calculated to measure the proportion of heterogeneity explained by the independent variables.

In the clinical studies, the standardized mean differences (SMD), 95% confidence intervals (95% CI) and significances were also examined using the inverse-variance method. The Cochrane risk of bias tool was used to evaluate potential publication bias[4]. $P<0.05$ was considered as statistically significant; $0.05<=P<0.10$ was considered as a trend towards statistical significance. Data were analyzed using Stata version 14.0 (Stata-Corp).

Supplemental References

1. Macleod MR, Fisher M, O'Collins V, et al. Good laboratory practice: preventing introduction of bias at the bench. Stroke 2009; 40: e50–e52.
2. Macleod MR, O'Collins T, Howells DW, et al. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke 2004; 35: 1203–1208.
3. Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: a practical guide. J Neurosci Methods 2014; 221:92-102.
4. Higgins JP, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomized trials. BMJ 2011; 343: d5928.

Supplemental Table 1. Preclinical study characteristics.

Characteristics	All studies	MSC	NSC	MNC	Others
No. of publications	355	194	72	47	42
No. of treatment arms	704	376	137	112	79
No. of animals	10830	5575	2355	1610	1290
Impact factor, mean (SD)	3.54 (2.34)	3.38 (1.72)	3.96 (3.70)	3.36 (2.01)	3.75 (2.06)
Stroke model, n (%)					
Intraluminal	8720 (80.52)	4850 (87.00)	1927 (81.83)	1022 (63.48)	921 (71.40)
Distal MCAO	1426 (13.17)	598 (10.73)	147 (6.24)	503 (31.24)	178 (13.80)
Photothrombotic	289 (2.67)	20 (0.18)	101 (4.29)	16 (1.00)	152 (11.78)
ET-1	181 (1.67)	20 (0.18)	77 (3.27)	45 (2.80)	39 (3.02)
Embolic	214 (1.96)	87 (0.80)	103 (4.37)	24 (1.49)	0 (0.00)
Ischemia type, n (%)					
Transient	8028 (74.13)	4161 (38.42)	1975 (83.86)	1019 (63.29)	873 (67.67)
Permanent	2802 (25.87)	1414 (13.06)	380 (16.14)	591 (36.71)	417 (32.33)
Cell donors, n (%)					
Rat	4752 (43.88)	2808 (25.93)	778 (33.04)	792 (49.19)	374 (28.99)
Human	4334 (40.11)	2411 (43.25)	626 (26.58)	674 (41.86)	623 (48.29)
Mouse	1616 (14.92)	247 (4.43)	951 (40.38)	144 (8.94)	274 (21.24)
Others	54 (0.50)	35 (0.63)	0 (0.00)	0 (0.00)	19 (1.47)
Unspecified	74 (0.68)	74 (1.33)	0 (0.00)	0 (0.00)	0 (0.00)
Recipients, n (%)					
Rat	8916 (82.33)	4965 (89.06)	1606 (68.20)	1429 (88.76)	916 (71.01)
Mouse	1841 (17.00)	569 (10.21)	734 (31.17)	181 (11.24)	357 (27.67)
Primates	21 (1.94)	0 (0.00)	15 (0.64)	0 (0.00)	6 (0.47)
Others	52 (4.80)	41 (0.74)	0 (0.00)	0 (0.00)	11 (0.85)
Donor gender, n (%)					
Male	2862 (26.43)	1617 (29.00)	297 (12.61)	538 (33.42)	410 (31.78)
Female	2205 (20.36)	1293 (23.19)	67 (2.85)	576 (35.78)	269 (20.85)
Unspecified	5594 (51.65)	2622 (47.03)	1879 (79.79)	496 (30.81)	597 (46.28)
Both	169 (1.56)	43 (0.77)	112 (4.76)	0 (0.00)	14 (1.09)
Recipient gender, n (%)					
Male	9214 (85.08)	4592 (82.37)	2144 (91.04)	1464 (90.93)	1014 (78.60)
Female	656 (6.06)	476 (8.54)	31 (1.32)	38 (2.36)	111 (8.60)
Unspecified	918 (8.48)	479 (8.60)	172 (7.30)	108 (6.71)	159 (12.33)
Both	36 (0.33)	28 (0.50)	8 (0.34)	0 (0.00)	0 (0.00)
Age, n (%)					
Young	10152 (93.74)	5209 (93.43)	2244 (95.29)	1471 (91.37)	1228 (95.19)
Middle-aged	195 (1.80)	156 (2.80)	0 (0.00)	39 (2.42)	0 (0.00)
Aged	104 (0.96)	56 (1.00)	14 (0.59)	22 (1.37)	12 (0.93)
Unspecified	279 (2.58)	54 (0.97)	97 (4.12)	78 (4.84)	50 (3.88)
Cell doses, n (%)					
0-5 million/kg	5572 (51.45)	2901 (52.04)	1514 (64.29)	491 (30.50)	666 (51.63)

5-10 million/kg	1112 (10.27)	941 (16.88)	15 (0.64)	70 (4.35)	86 (6.67)
10-20 million/kg	1799 (16.61)	1138 (20.41)	280 (11.89)	207 (12.86)	174 (13.49)
20-30 million/kg	514 (4.75)	242 (4.34)	211 (8.96)	37 (2.30)	24 (1.86)
30-50 million/kg	1199 (11.07)	295 (5.29)	275 (11.68)	351 (21.80)	278 (21.55)
50-100 million/kg	213 (1.97)	58 (1.04)	0 (0.00)	133 (8.26)	22 (1.71)
⩾100 million/kg	236 (2.18)	0 (0.00)	52 (2.21)	184 (11.43)	0 (0.00)
Unspecified	185 (1.71)	0 (0.00)	8 (0.34)	137 (8.51)	40 (3.10)
Delivery route, n (%)					
Intravenous	5585 (51.57)	3285 (58.92)	512 (21.74)	1336 (82.98)	452 (35.04)
Intra-arterial	957 (8.84)	535 (9.60)	54 (2.29)	172 (10.68)	196 (15.19)
Intracortical	3509 (32.40)	1427 (25.60)	1462 (62.08)	56 (3.49)	564 (43.72)
Intraventricular	562 (5.19)	209 (3.75)	299 (12.70)	0 (0.00)	54 (4.19)
Intrathecal	67 (0.62)	51 (0.91)	16 (0.68)	0 (0.00)	0 (0.00)
Others	150 (1.39)	68 (1.22)	12 (0.51)	46 (2.86)	24 (1.86)
Transplantation time, n (%)					
0-8h	2506 (23.14)	1283 (23.01)	423 (17.96)	440 (27.33)	360 (27.91)
>8-24h	4808 (44.40)	2896 (51.95)	643 (27.30)	887 (55.09)	382 (29.61)
>24h-1w	3051 (28.17)	1155 (20.72)	1187 (50.40)	216 (13.42)	493 (38.22)
>1w-1m	293 (2.71)	187 (3.35)	66 (2.80)	21 (1.30)	19 (1.47)
>1m	70 (0.65)	24 (0.43)	0 (0.00)	46 (2.86)	0 (0.00)
Unspecified	102 (0.94)	30 (0.54)	36 (1.53)	0 (0.00)	36 (2.79)
Cell immunogenicity, n (%)					
Autologous	380 (3.51)	61 (1.09)	16 (0.68)	258 (16.02)	45 (3.49)
Allogeneic	5190 (47.92)	2811 (50.42)	1396 (59.28)	559 (34.72)	424 (32.87)
Xenogeneic	4999 (46.16)	2577 (46.22)	943 (40.04)	674 (41.86)	805 (62.40)
Syngeneic	131 (1.21)	12 (0.22)	0 (0.00)	119 (7.39)	0 (0.00)
Unspecified	130 (1.20)	114 (2.04)	0 (0.00)	0 (0.00)	16 (1.24)
Cell immunophenotyping, n (%)					
No	4631 (42.76)	2025 (36.32)	1220 (51.80)	911 (56.58)	475 (36.82)
Yes	5751 (53.10)	3295 (59.10)	1102 (46.79)	607 (37.70)	747 (57.91)
Unspecified	448 (4.14)	255 (4.58)	33 (1.41)	92 (5.72)	68 (5.27)
Cell cryopreservation, n (%)					
No	3587 (33.12)	1445 (25.92)	966 (41.02)	846 (52.55)	330 (25.58)
Yes	1116 (10.30)	605 (10.85)	0 (0.00)	417 (25.90)	94 (7.29)
Unspecified	6127 (56.58)	3525 (63.23)	1389 (58.98)	347 (21.55)	866 (67.13)
Comorbidities, n (%)					
No	10134 (93.57)	5381 (96.52)	2355 (100.00)	1196 (74.29)	1202 (93.18)
Hypertension	449 (4.15)	74 (1.33)	0	365 (22.67)	10 (0.78)
T1DM	87 (0.80)	72 (1.29)	0	15 (0.93)	0 (0.00)
T2DM	160 (1.48)	48 (0.86)	0	34 (2.11)	78 (6.05)

MSCs: mesenchymal stem cells, MNCs: mononuclear cells, NSCs: neural stem cells, MCAO: middle cerebral artery occlusion, ET-1: endothelin-1.

Supplemental Table 2. Percentage of studies meeting each quality score criteria.

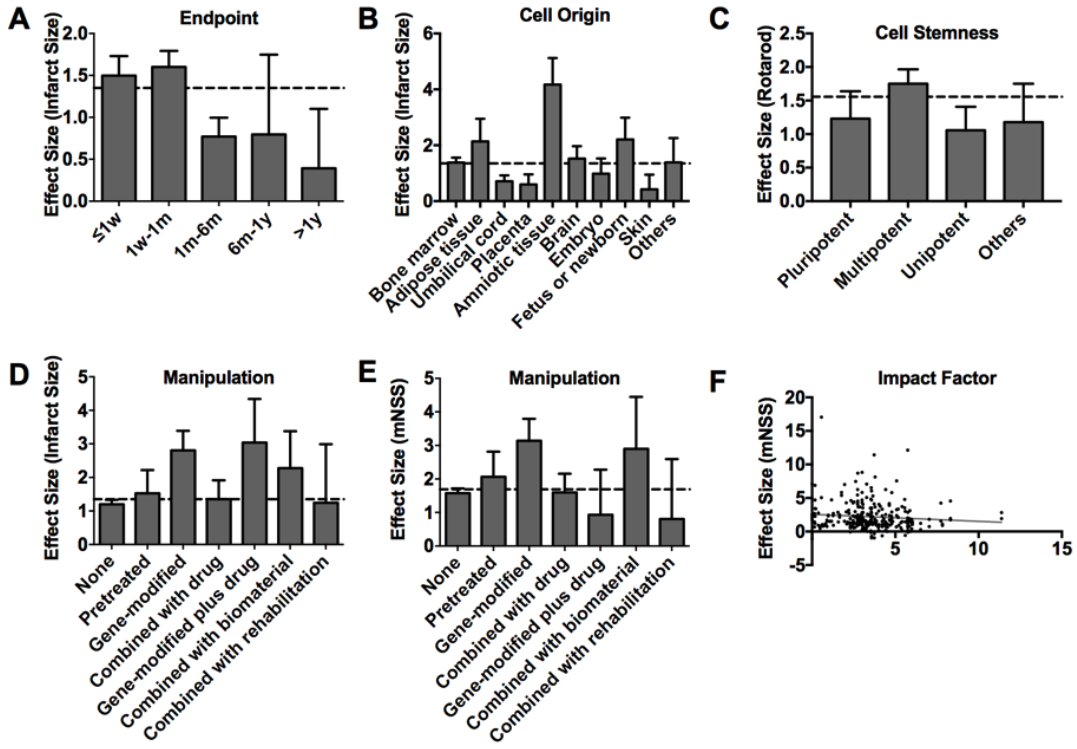
Quality score criteria	Studies meeting criteria (%)				
	All studies	MSCs	NSCs	MNCs	Others
Total quality score, median (IQR)	5 (4-6)	5 (4-6)	5 (4-7)	5 (4-7)	5 (4-6)
Publication in peer-reviewed journal	355 (100.00%)	194 (100.00%)	72 (100.00%)	47 (100.00%)	42 (100.00%)
Statement of compliance with animal welfare regulations	343 (96.62%)	185 (95.36%)	70 (97.22%)	47 (100.00%)	41 (97.62%)
Statement of anesthetics	301 (84.79%)	168 (86.60%)	58 (80.56%)	42 (89.36%)	33 (78.57%)
Control of body temperature	212 (59.72%)	111 (57.22%)	49 (68.06%)	29 (61.70%)	23 (54.76%)
Randomization	222 (62.54%)	136 (70.10%)	31 (43.06%)	32 (68.09%)	23 (54.76%)
Blinding	203 (57.18%)	106 (54.64%)	49 (68.06%)	34 (72.34%)	14 (33.33%)
Statement of conflict of interest	193 (54.37%)	105 (54.12%)	38 (52.78%)	24 (51.06%)	26 (61.90%)
Allocation concealment	23 (6.48%)	7 (3.61%)	10 (13.89%)	6 (12.77%)	0 (0.00%)
Animals with comorbidities	24 (6.76%)	9 (4.64%)	0 (0.00%)	12 (25.53%)	3 (7.14%)
Sample size calculation	10 (2.82%)	2 (1.03%)	2 (2.78%)	4 (8.51%)	2 (4.76%)

MSCs: mesenchymal stem cells, MNCs: mononuclear cells, NSCs: neural stem cells, IQR: interquartile range

Supplemental Table 3. Characteristics of included clinical studies.

Characteristics	All studies	MSCs	MNCs	Others
No. of publications	10	3	6	1
No. of patients	460	187	243	30
(treated/control)	(223/237)	(85/102)	(123/120)	(15/15)
RCT/NRCT	6/4	2/1	3/3	1
Sex, n (%)				
Male	291 (63.26)	109 (58.29)	162 (66.67)	20 (66.67)
Female	169 (36.74)	78 (41.71)	81 (33.33)	10 (33.33)
Age (mean)				
40~60	252 (54.78)	9 (4.81)	213 (87.65)	30 (100.00)
>60	208 (45.22)	178 (95.19)	30 (12.35)	0 (0.00)
Comorbidity, n (%)				
None	25 (5.43)	19 (10.16)	6 (2.47)	0 (0.00)
Hypertension	176 (38.26)	38 (20.32)	119 (48.97)	19 (63.33)
Diabetes	116 (25.22)	26 (13.90)	82 (33.74)	8 (26.67)
Dyslipidemia	76 (16.52)	13 (6.95)	55 (22.63)	8 (26.67)
Heart disease	141 (30.65)	86 (45.99)	49 (20.16)	6 (20.00)
Stroke history	20 (4.35)	7 (3.74)	13 (5.35)	0 (0.00)
Others	277 (60.22)	110 (58.82)	160 (65.84)	7 (23.33)
Cell source, n (%)				
Bone marrow	208 (93.27)	85（100）	123 (100)	0
Peripheral blood	15 (6.73)	0	0	15 (100)
Immunogenicity, n (%)				
Autologous	158 (70.85)	20 (23.53)	123 (100)	15 (100)
Allogeneic	65 (29.15)	65 (76.47)	0	0
Cell dose, n (%)				
0-5 million/kg	88 (39.46)	20 (23.53)	53 (43.09)	15 (100)
5-10 million/kg	135 (60.54)	65 (76.47)	70 (56.91)	0
Cryopreservation, n (%)				
No	158 (70.85)	20 (23.53)	123 (100)	15 (100)
Yes	65 (29.15)	65 (76.47)	0	0
Delivery time, n (%)				
24h-1w	73 (32.74)	65 (76.47)	8 (6.50)	0
1w-1m	94 (42.15)	4 (4.71)	90 (73.17)	0
>1m	56 (25.11)	16 (18.82)	25 (20.33)	15 (100)
Delivery route, n (%)				
Intravenous	167 (74.89)	85 (100）	82 (66.67)	0
Intra-arterial	41 (18.39)	0	41 (33.33)	0
Intracortical	15 (6.72)	0	0	15 (100)

MSCs: mesenchymal stem cells, MNCs: mononuclear cells, RCT: randomized controlled trial, NRCT: non-randomized controlled trial



Supplemental Figure 1. Study characteristics that significantly accounted for effect size heterogeneity in different outcome measures. (A) effect of assessment endpoint on infarct size reduction; (B) effect of cell origin on infarct size reduction; (C) effect of cell stemness on rotarod test performance; (D) effect of cell manipulation on infarct size reduction; (E) effect of cell manipulation on mNSS performance; (F) effect of impact factor on mNSS performance.