Meta-analysis of Preclinical Studies of Mesenchymal Stromal Cells to Treat Rheumatoid Arthritis

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SUPPLEMENTARY MATERIALS

MATERIALS AND METHODS

Literature search and inclusion criteria. This meta-analysis review was conducted according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidance(50). We conducted a comprehensive literature search for articles evaluating the therapeutic function of MSC in rheumatoid arthritis. The search terms used were sufficiently broad to capture the majority of the published data using MSC to treat animal models of arthritis. The search terms used were (mesenchymal OR mesenchymal stem cell OR mesenchymal stromal cell OR MSC) AND (rheumatoid arthritis OR rheumatoid OR arthritis OR RA). The electronic search strategy excluded non-English articles, and all studies included in this meta-analysis review were done in animal models of rheumatoid arthritis treated using non-genetic modified native MSC. Only the data documented therapeutic effects on arthritis, which means MSC were administrated at least one day after the initial RA induction, were included in our study. Studies with high risk of any bias were excluded from the meta-analysis if they scored "No" on any one question stated in the SYRCLE's risk of bias tool (below). In addition, studies that failed to present sample sizes, standard deviations, or missed numerical/graphical results required for evaluating the effect sizes objectively, were also excluded in the parametric meta-analysis.

Data extraction. Data were extracted from all available sources in each paper, including text and graphs. When only graphical presentation was available, values for mean and SD or SEM were obtained using GraphClick (Arizona Software, Phoenix, AZ) under high magnification by two independent investigators.

Evaluating the risk of bias. To evaluate the quality of the studies and risk of any bias, two independent investigators used the SYRCLE's risk of bias tool, which includes 10 defined criteria: (1) sequence generation, (2) baseline characteristics, (3) allocation concealment, (4) random housing, (5) blinding against performance bias, (6) random outcome assessment, (7) blinding against detection bias, (8) incomplete outcome data, (9) selective outcome reporting, and (10) other sources of biases including contamination and inappropriate influence of funders(49). In order to assign a judgment of low, high, or unclear risk of bias to each item mentioned in the tool, the following signaling questions were used: Q1: Was the allocation sequence adequately generated and applied? Q2: Were the groups similar at baseline or were they adjusted for confounders in the analysis? Q3: Was the allocation adequately concealed? Q4: Were animals randomly housed during the experiment? Q5: Were the caregivers and/or investigators blinded from knowledge of which intervention each animal received during the experiment? Q6: Were animals selected at random for outcome assessment? Q7: Was the outcome assessor blinded? Q8: Were incomplete outcome data adequately addressed? Q9: Are reports of the study free of selective outcome reporting? Q10: Was the study apparently free of other problems that could result in high risk of bias? A "yes" judgment indicates a low risk of bias, a "no" judgment indicates high risk of bias, and if insufficient details were reported to assess the risk of bias properly, the judgment of bias was recorded as "unclear"(49). As suggested in SYRCLE's risk of bias tool, we focused on evaluating the potential risk of bias instead of calculating the quality score for each article.

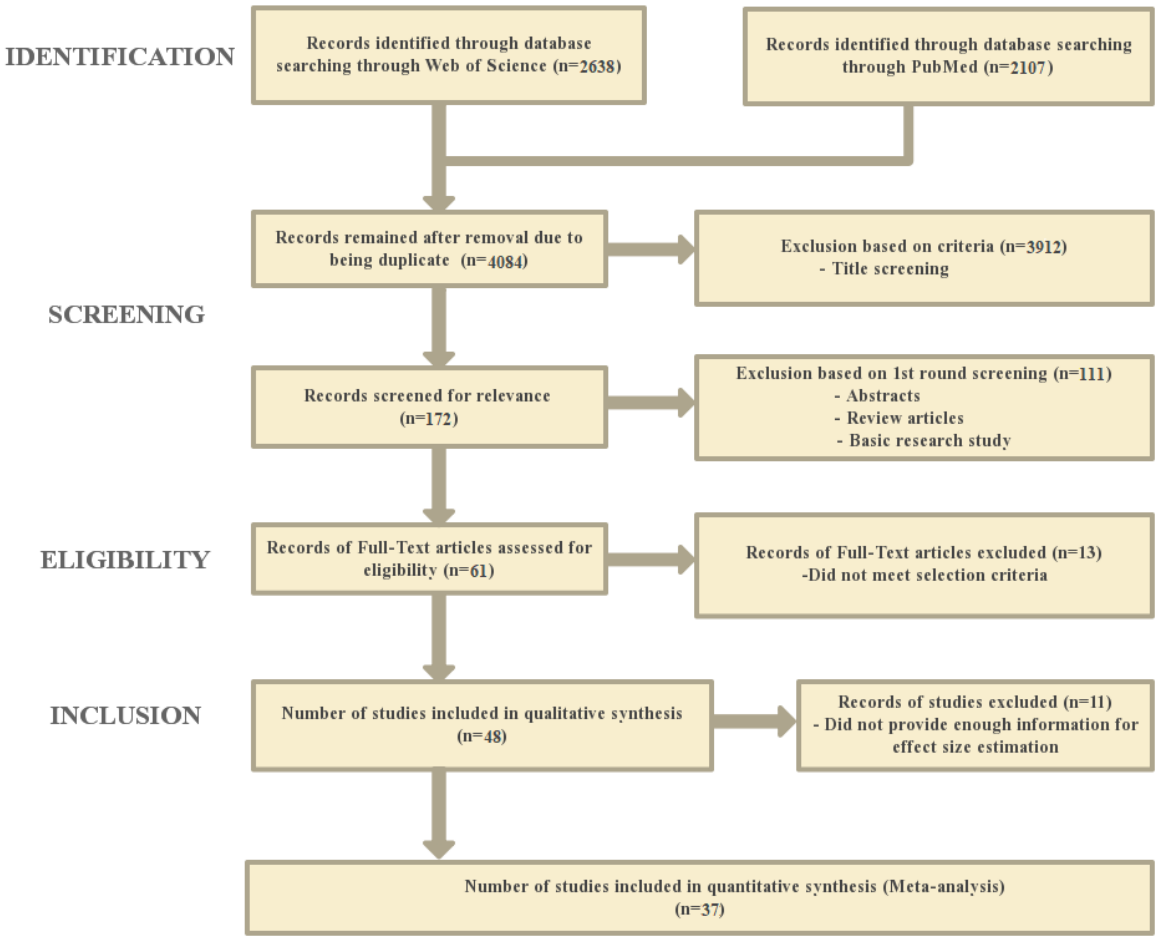


Figure S1

Flowchart of meta-analysis search and review process, conducted in accordance with PRISMA (preferred reporting items for systematic reviews and meta-analyses) statement criteria. Down arrows indicate the progression of studies that passed the previous criteria (number passed in parentheses). Side arrows indicate the number of studies excluded at each stage, and why they were excluded.

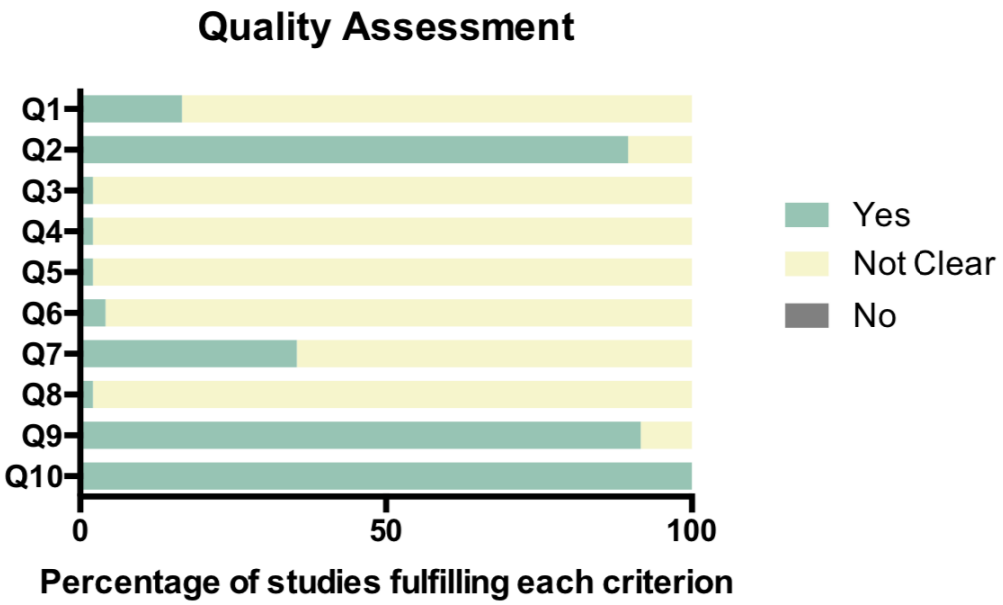
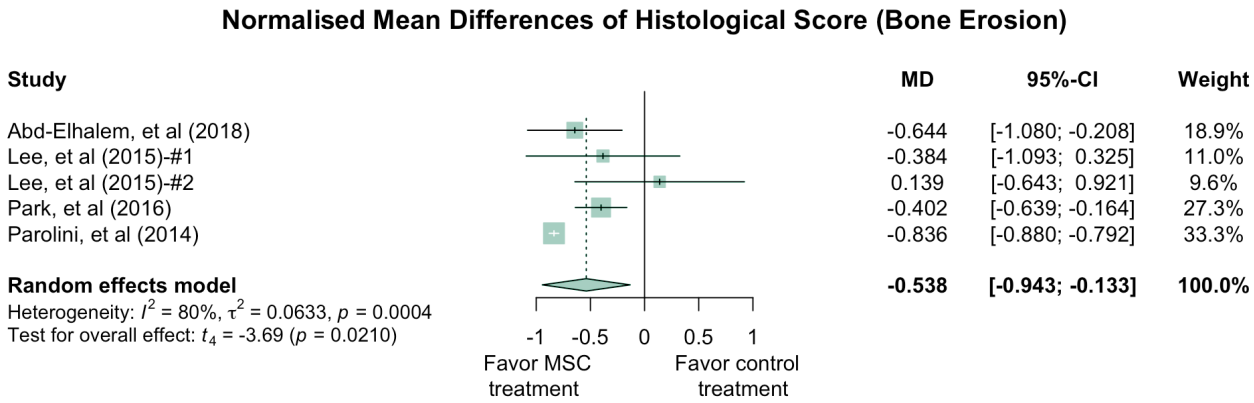


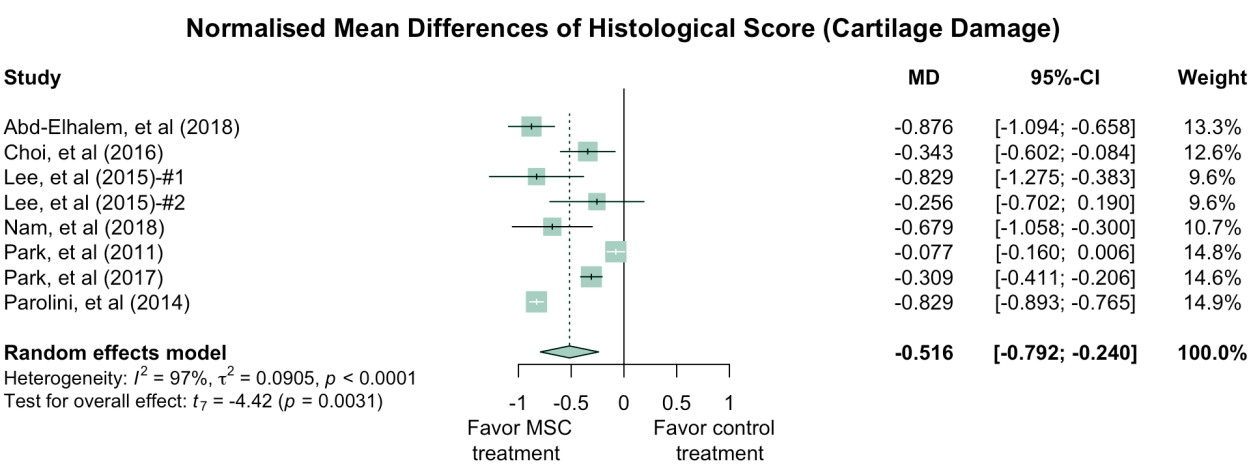
Figure S2

Quality assessment of literature. Horizontal axis indicates percentage of answers to the questions in SYRCLE's risk of bias tool. Green indicates "Yes"; dark gray indicates "No"; and yellow indicates "Not clear".

a



b



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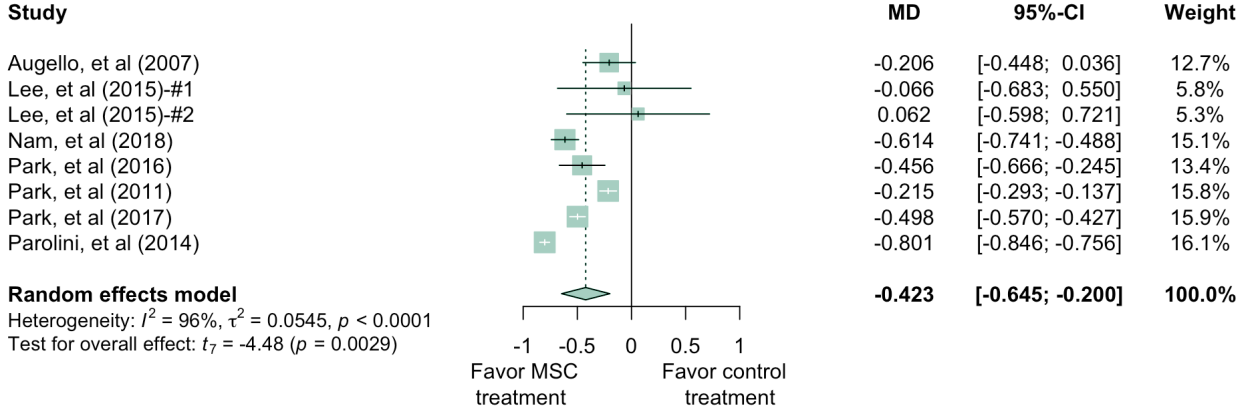
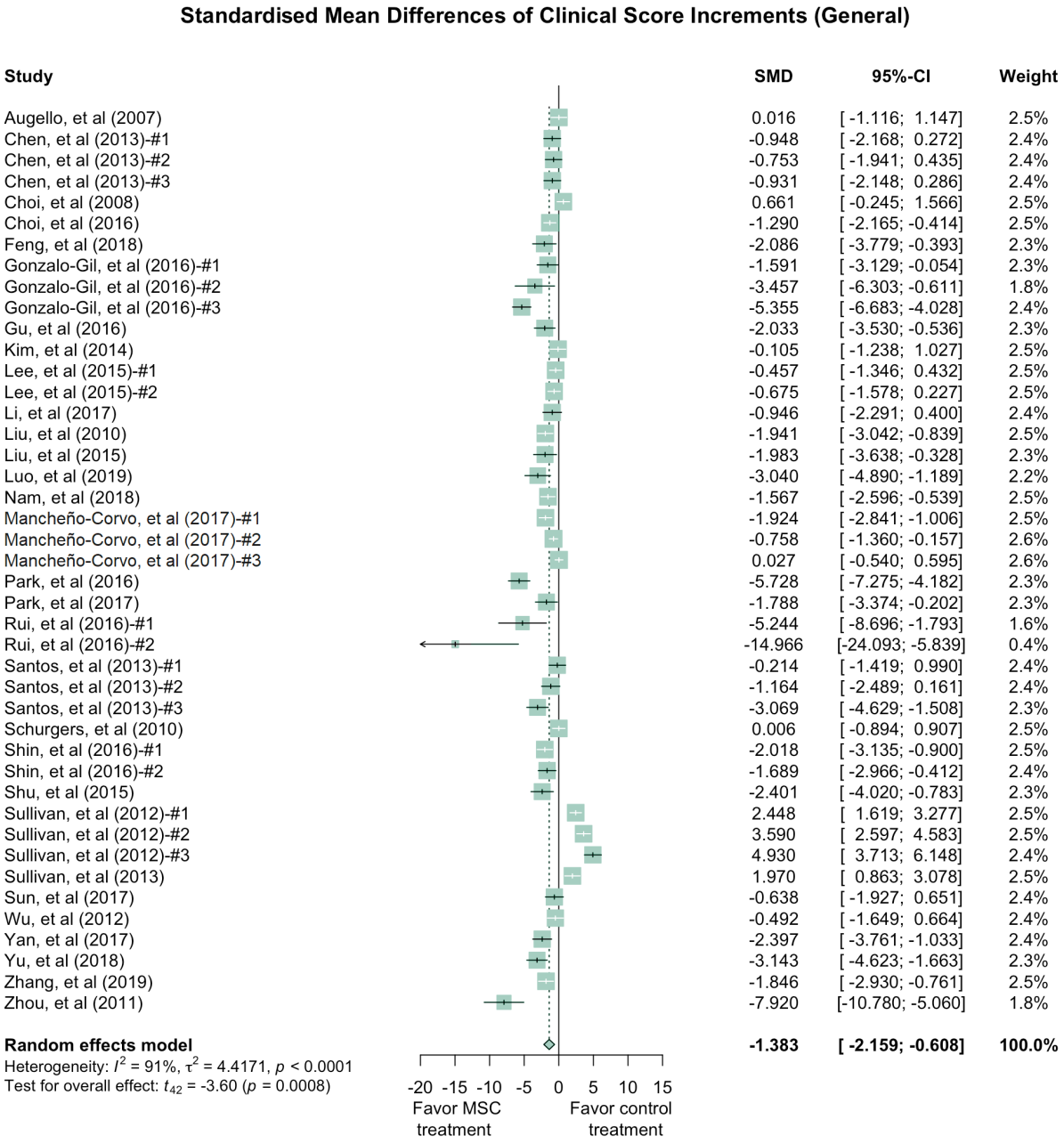


Figure S3

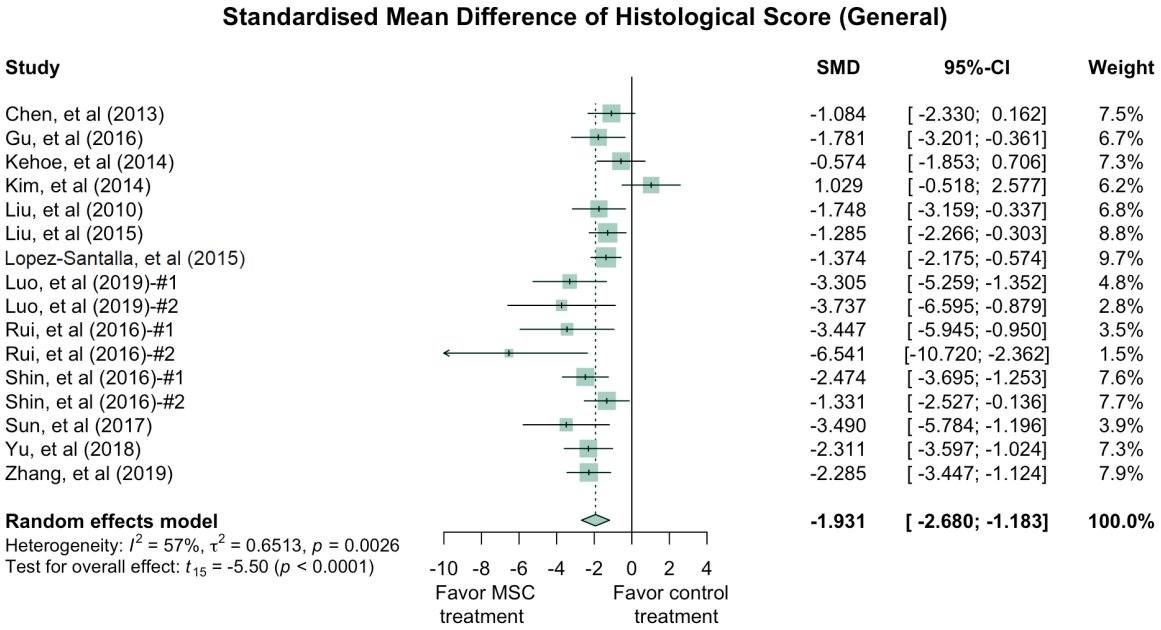
Forest plots showing the normalised mean difference (MD) and 95% CI for histological scores with a subgroup of (a) bone erosion; (b) cartilage damage; (c)inflammation. The graphs were

generated using the *meta* package in R. All results have been normalised with the sham control group as described in the methods. For all the plots, the vertical line indicates no effect, left hand side indicates favouring MSC treatment while right side indicates favouring PBS control treatment. The size of the box indicates the weighting of each study, and the thin horizontal whisker indicates the 95% CI. Random-effects model was used to summarise the effect sizes. Heterogeneity is denoted by the I^2 and τ^2.

a


Standardised Mean Differences of Clinical Score Increments (General)
Study
SMD
95%-CI
Weight
Augello, et al (2007) 0.016 [-1.116; 1.147] 2.5%
Chen, et al (2013)-#1 -0.948 [-2.168; 0.272] 2.4%
Chen, et al (2013)-#2 -0.753 [-1.941; 0.435] 2.4%
Chen, et al (2013)-#3 -0.931 [-2.148; 0.286] 2.4%
Choi, et al (2008) 0.661 [-0.245; 1.566] 2.5%
Choi, et al (2016) -1.290 [-2.165; -0.414] 2.5%
Feng, et al (2018) -2.086 [-3.779; -0.393] 2.3%
Gonzalo-Gil, et al (2016)-#1 -1.591 [-3.129; -0.054] 2.3%
Gonzalo-Gil, et al (2016)-#2 -3.457 [-6.303; -0.611] 1.8%
Gonzalo-Gil, et al (2016)-#3 -5.355 [-6.683; -4.028] 2.4%
Gu, et al (2016) -2.033 [-3.530; -0.536] 2.3%
Kim, et al (2014) -0.105 [-1.238; 1.027] 2.5%
Lee, et al (2015)-#1 -0.457 [-1.346; 0.432] 2.5%
Lee, et al (2015)-#2 -0.675 [-1.578; 0.227] 2.5%
Li, et al (2017) -0.946 [-2.291; 0.400] 2.4%
Liu, et al (2010) -1.941 [-3.042; -0.839] 2.5%
Liu, et al (2015) -1.983 [-3.638; -0.328] 2.3%
Luo, et al (2019) -3.040 [-4.890; -1.189] 2.2%
Nam, et al (2018) -1.567 [-2.596; -0.539] 2.5%
Mancheño-Corvo, et al (2017)-#1 -1.924 [-2.841; -1.006] 2.5%
Mancheño-Corvo, et al (2017)-#2 -0.758 [-1.360; -0.157] 2.6%
Mancheño-Corvo, et al (2017)-#3 0.027 [-0.540; 0.595] 2.6%
Park, et al (2016) -5.728 [-7.275; -4.182] 2.3%
Park, et al (2017) -1.788 [-3.374; -0.202] 2.3%
Rui, et al (2016)-#1 -5.244 [-8.696; -1.793] 1.6%
Rui, et al (2016)-#2 -14.966 [-24.093; -5.839] 0.4%
Santos, et al (2013)-#1 -0.214 [-1.419; 0.990] 2.4%
Santos, et al (2013)-#2 -1.164 [-2.489; 0.161] 2.4%
Santos, et al (2013)-#3 -3.069 [-4.629; -1.508] 2.3%
Schurgers, et al (2010) 0.006 [-0.894; 0.907] 2.5%
Shin, et al (2016)-#1 -2.018 [-3.135; -0.900] 2.5%
Shin, et al (2016)-#2 -1.689 [-2.966; -0.412] 2.4%
Shu, et al (2015) -2.401 [-4.020; -0.783] 2.3%
Sullivan, et al (2012)-#1 2.448 [1.619; 3.277] 2.5%
Sullivan, et al (2012)-#2 3.590 [2.597; 4.583] 2.5%
Sullivan, et al (2012)-#3 4.930 [3.713; 6.148] 2.4%
Sullivan, et al (2013) 1.970 [0.863; 3.078] 2.5%
Sun, et al (2017) -0.638 [-1.927; 0.651] 2.4%
Wu, et al (2012) -0.492 [-1.649; 0.664] 2.4%
Yan, et al (2017) -2.397 [-3.761; -1.033] 2.4%
Yu, et al (2018) -3.143 [-4.623; -1.663] 2.3%
Zhang, et al (2019) -1.846 [-2.930; -0.761] 2.5%
Zhou, et al (2011) -7.920 [-10.780; -5.060] 1.8%
Random effects model -1.383 [-2.159; -0.608] 100.0%
Heterogeneity: I^2 = 91%, τ^2 = 4.4171, p < 0.0001
Test for overall effect: t_{42} = -3.60 (p = 0.0008)
-20 -15 -10 -5 0 5 10 15
Favor MSC treatment
Favor control treatment

b



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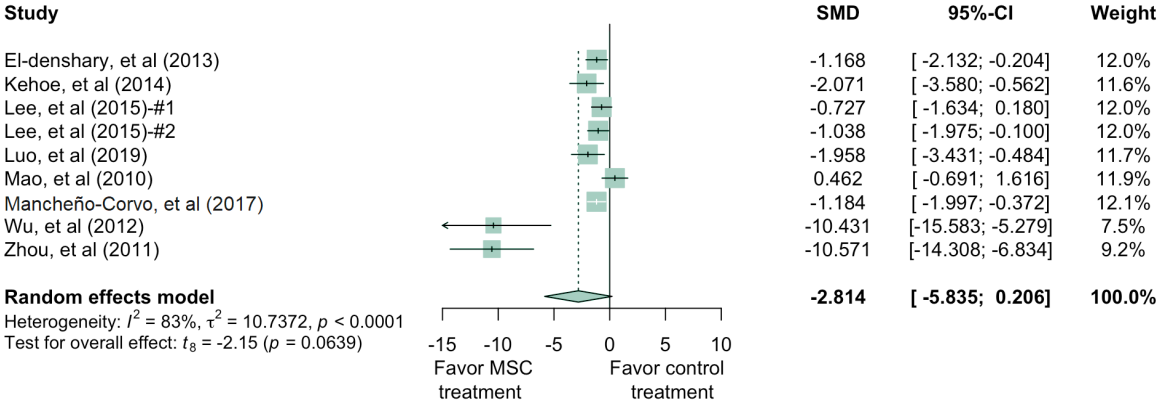


Figure S4

Forest plots showing the standardised mean difference (SMD) and 95% CI for (a) clinical score, (b) histological score, (c) paw thickness for each study included in the meta-analysis. The graphs were generated using the *meta* package in R. All results have been normalised with sham control group as described in the methods. For all the plots, the vertical line indicates no effect, left hand side indicates favouring MSC treatment while right side indicates favouring PBS control treatment. The size of the box indicates the weighting of each study, and the thin horizontal whisker indicates the 95% CI. Random-effects model was used to summarise the effect sizes. Heterogeneity is denoted by the I^2 and τ^2.

Normalised Mean Differences of Clinical Score Increments (MSC Donor Species + Tissue of Origin)

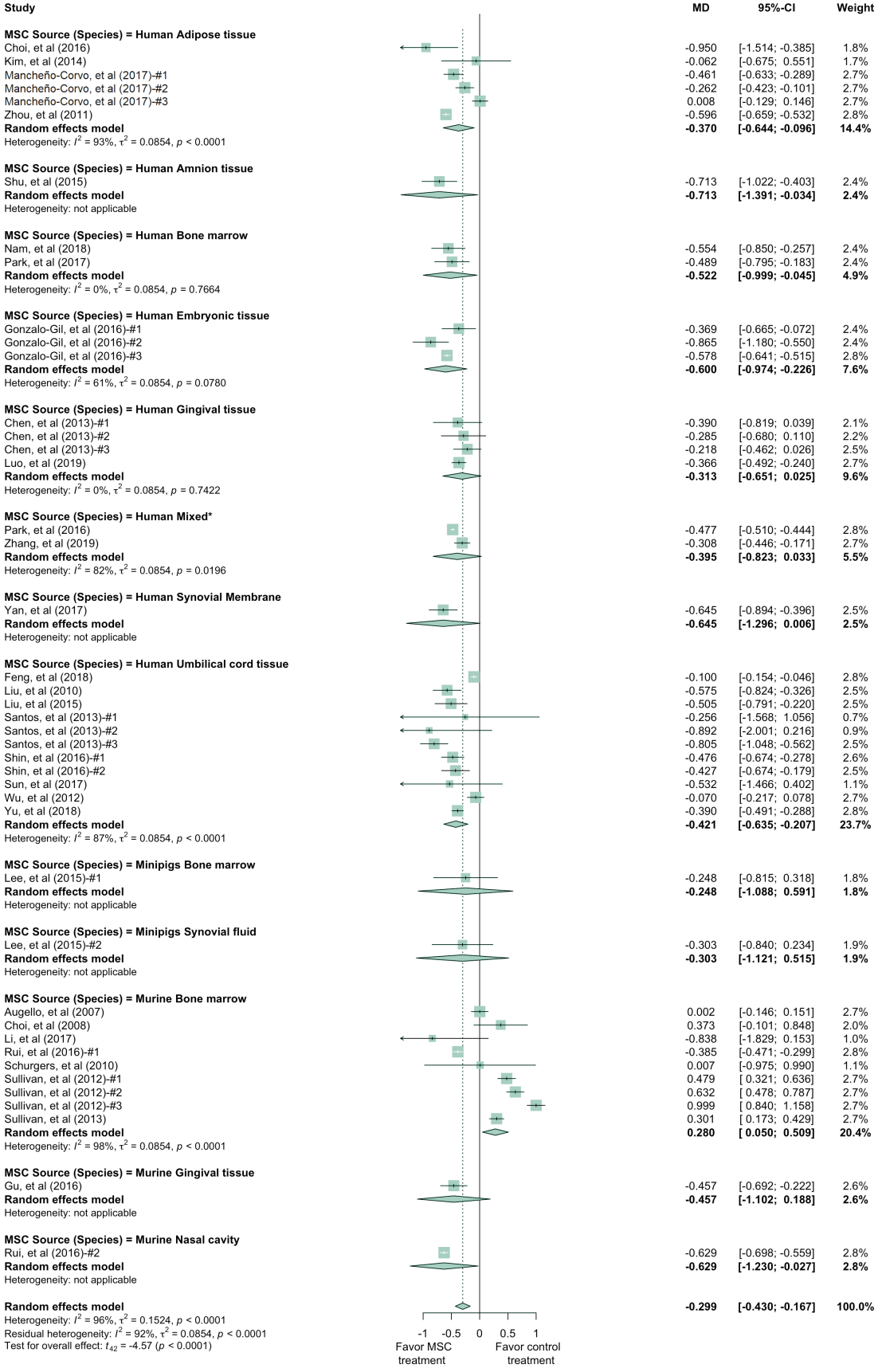


Figure S5

Forest plots showing the normalised mean difference (MD) and 95% CI for two important MSC donor species, human and mouse, and tissue of origin. The graphs were generated using the *meta* package in R. All results have been normalised with the sham control group as described in the methods. For all the plots, the vertical line indicates no effect, left hand side indicates favouring MSC treatment while right side indicates favouring PBS control treatment. The size of the box indicates the weighting of each study, and the thin horizontal whisker indicates the 95% CI. Random-effects model was used to summarise the effect sizes. Heterogeneity is denoted by the I^2 and τ^2.

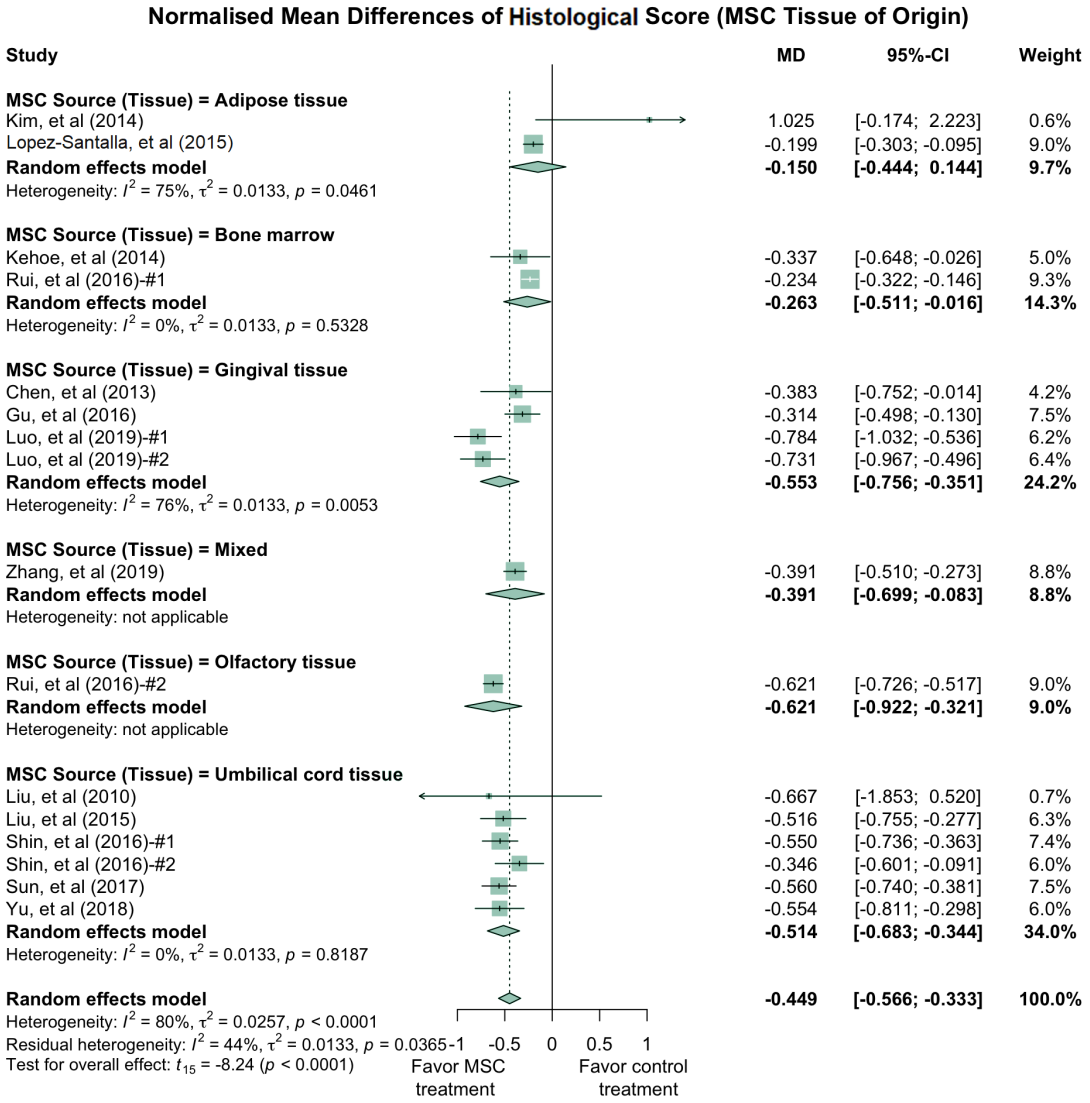
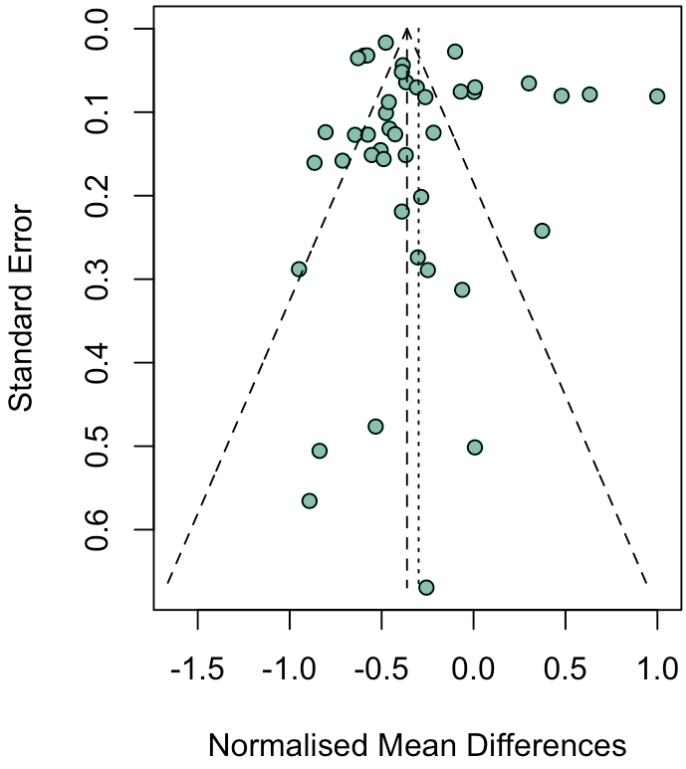


Figure S6

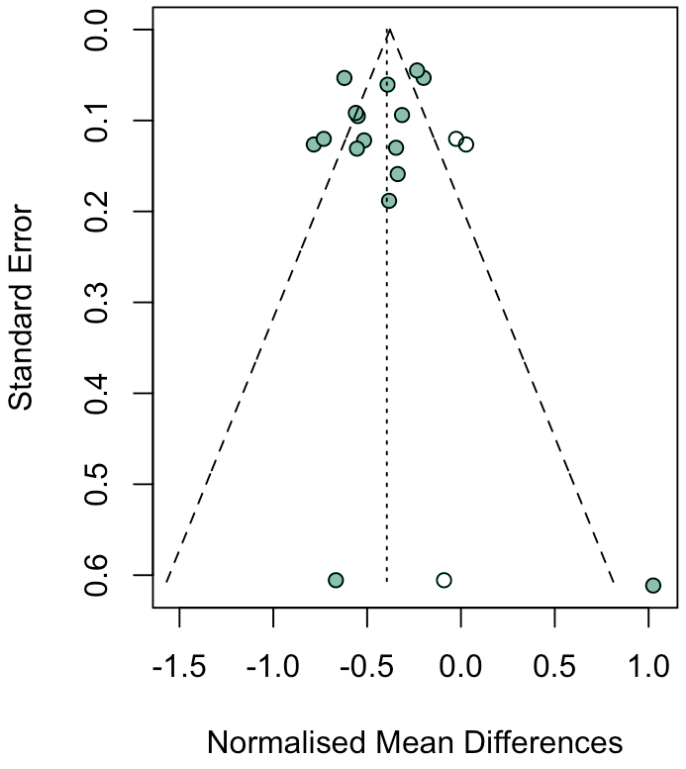
A forest plot showing the normalised mean difference (MD) and 95% CI of histological score of MSC tissue of origin. The graph was generated using the *meta* package in R. All the results have

been normalised with the sham control group as described in the methods. The vertical line indicates no effect, left hand side indicates favouring MSC treatment while right side indicates favouring control treatment. The size of the box indicates the weighting of each study, and the thin horizontal whisker indicates the 95% CI. Random-effects model was used to summarise the effect sizes. Heterogeneity is denoted by the I^2 and τ^2. Mixed indicates the treatment arm contains more than one type of MSC tissue of origin.

a


Standard Error
0.0
0.1
0.2
0.3
0.4
0.5
0.6
-1.5
-1.0
-0.5
0.0
0.5
1.0
Normalised Mean Differences

b


Standard Error
0.0
0.1
0.2
0.3
0.4
0.5
0.6
-1.5
-1.0
-0.5
0.0
0.5
1.0
Normalised Mean Differences

c

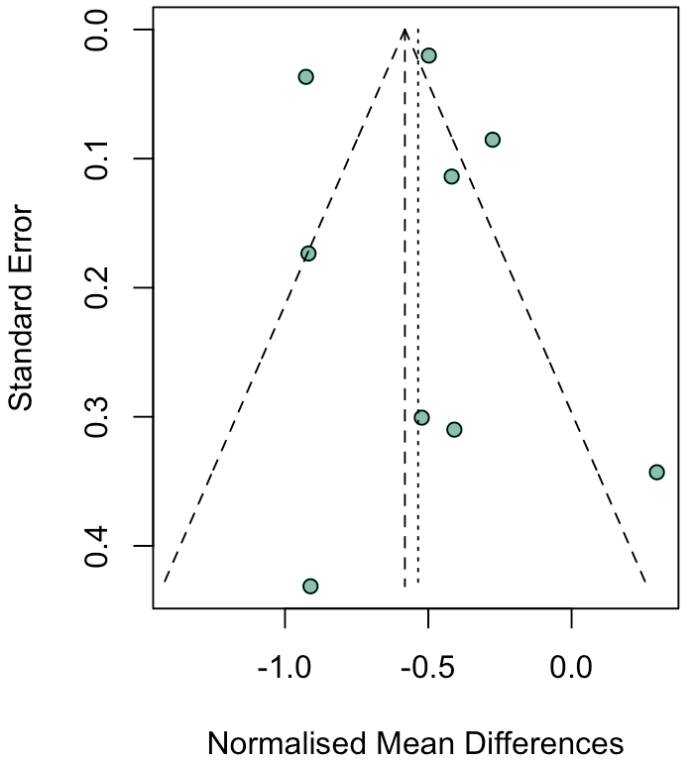


Figure S7

Funnel plot for (a) clinical score, (b) histological score and (c) paw thickness, after trim-and-fill correction. Each dot represents a study with the y-axis representing study quality and the x-axis representing the study results. The original studies are denoted by the green dots, while white dots represent the hypothetical studies added into the analysis.

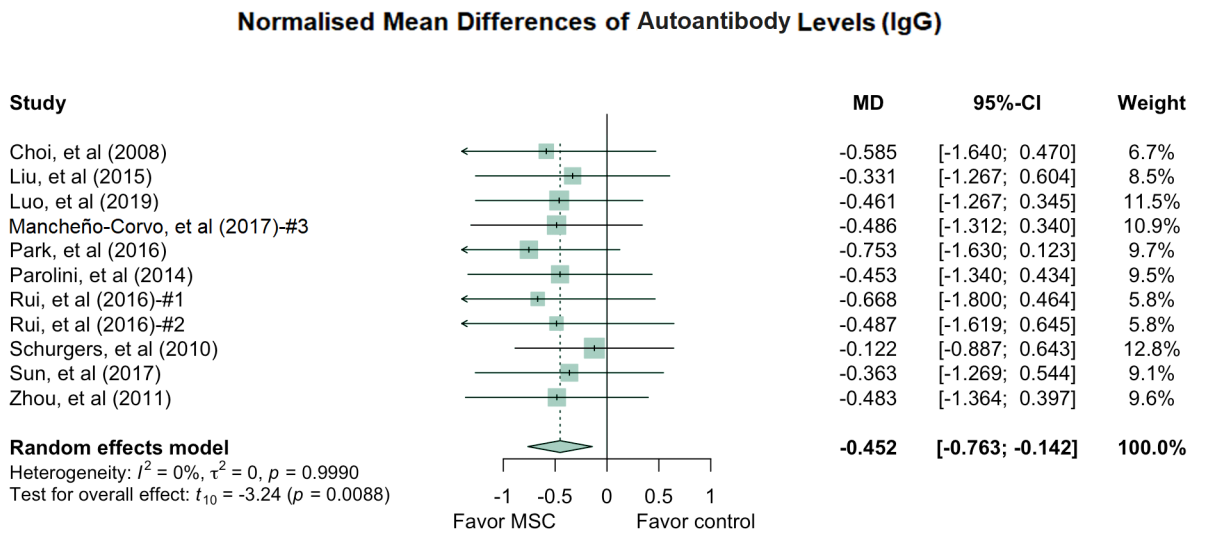


Figure S8

Forest plots showing the normalised mean difference (MD) and 95% CI for clinical scores with a subgroup of autoantibody levels (IgG). The graphs were generated using the *meta* package in R. All results have been normalised with the sham control group as described in the methods. For all the plots, the vertical line indicates no effect, left hand side indicates favouring MSC treatment while right side indicates favouring PBS control treatment. The size of the box indicates the weighting of each study, and the thin horizontal whisker indicates the 95% CI. Random-effects model was used to summarise the effect sizes. Heterogeneity is denoted by the I^2 and τ^2.

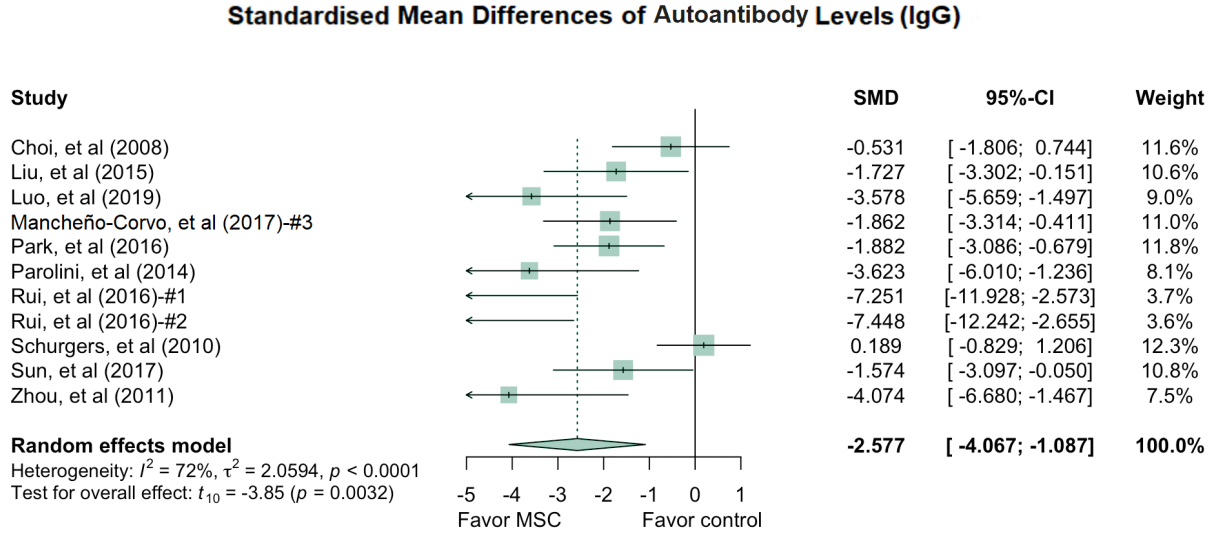


Figure S9

Forest plots showing the standardised mean difference (SMD) and 95% CI for clinical score, for each study included with subgroup of autoantibody levels (IgG) in the meta-analysis. The graphs were generated using the *meta* package in R. All results have been normalised with sham control group as described in the methods. For all the plots, the vertical line indicates no effect, left hand

side indicates favouring MSC treatment while right side indicates favouring PBS control treatment. The size of the box indicates the weighting of each study, and the thin horizontal whisker indicates the 95% CI. Random-effects model was used to summarise the effect sizes. Heterogeneity is denoted by the I^2 and τ^2.

Tables

Table S1: Autoantibodies summary of the pre-clinical studies using MSC to treat RA in this study

Author (year)	Arm	$P <$ 0.05	MSC favor?	Origin	Donor	Control	Transplant type	Treatment protocol	Rcpt	Target
Zhou, et al (2011)[S1]	1	Y	Y	AD	Human	PBS/ Other	Xenogenic	CIA, no booster, IV, Multiple	Mouse	IgG, IgG2a
#Garimella, et al (2015)[S2]	1	Y	Y	AD	Murine	PBS	Autologous	CIA, with booster, IP, Single	Mouse	IgG
#Chen, et al (2009)[S6]	1	Y	N	BM	Murine	Nil	Autologous	CIA, with booster, IV, Single	Mouse	IgG
Rui, et al (2016)[S13]	1	Y	Y	BM	Murine	PBS	Allogeneic	CIA, with booster, IV, Multiple	Mouse	IgG
	2	Y	Y	Other (OE)	Murine	PBS	Allogeneic	CIA, with booster, IV, Multiple	Mouse	IgG
Park, et al (2016)[S20]	1	Y	Y	BM	Human	Other	Xenogenic	CIA, with booster, IV, Multiple	Mouse	IgG
	2	Y	Y	BM	Human	Other	Xenogenic	CIA, with booster, IV, Multiple	Mouse	IgG
	3	Y	Y	BM	Human	Other	Xenogenic	CIA, with booster, IV, Multiple	Mouse	IgG
#Gonzalez, et al (2009)[S21]	1	Y	Y	AD	Human	PBS/ Other	Xenogenic	CIA, with booster, IP, Multiple	Mouse	IgG, IgG1, IgG2a
#Bouffi, et al (2010)[S23]	1	Y	Y	BM	Murine	N/A	Allogeneic	CIA, with booster, IV, Multiple	Mouse	9-10w
Schurgers, et al (2010)[S25]	1	N	N	BM	Murine	PBS	Autologous	CIA, no booster, IV, Single	Mouse	IgG
Liu, et al (2015)[S26]	1	Y	Y	UC	Human	PBS	Xenogenic	CIA, with booster, IV, Single	Mouse	IgG, Ig M
Choi, et al (2008)[S27]	1	N	N	BM	Murine	PBS	Autologous	CIA, with booster, IV, Multiple	Mouse	IgG
Parolini, et al (2014)[S29]	1	Y	Y	Other (AM)	Human	PBS	Xenogenic	CIA, with booster, IP, Multiple	Mouse	IgG, IgG1, IgG2a
Choi, et al (2016)[S32]	1	Y	Y	AD	Human	PBS	Xenogenic	CIA, with booster, IV, Multiple	Mouse	N/A
#Luz-Crawford, et al (2015)[S33]	1	Y	Y	BM	Murine	PBS/ Other	Allogeneic	CIA, with booster, IV, Multiple	Mouse	IgG2a, IgG1
Luo, et al (2019)[S36]	1	Y	Y	Other (GI)	Human	Nil	Xenogenic	CIA, IV, Single	Mouse	IgG, IgG1, IgG2a, IgG2b
	2	Y	Y	Other (GI)	Human	Nil	Xenogenic	CIA, IV, Single	Mouse	IgG, IgG1, IgG2a, IgG2b

Mancheño-Corvo, et al, (2017)[S44]	1	Y	Y	AD	Human	Ringer	Xenogenic	CIA, with booster, IL, Multiple	Mouse	IgG
	2	Y	Y	AD	Human	Ringer	Xenogenic	CIA, with booster, IL, Multiple	Mouse	IgG
	3	N	N	AD	Human	Ringer	Xenogenic	CIA, with booster, IV, Multiple	Mouse	IgG
Sun, et al, (2017)[S50]	1	N	Y	UC	Human	PBS	Xenogenic	CIA, with booster, IP, Single	Mouse	IgG

Table S2: Summary of meta-regression statistics

Covariate(s) included	τ^2	R^2	Test for moderator (*p*-value)	Interaction test (*p*-value)
No regression	0.1542	N/A	N/A	N/A
Treatment dosage (A)	0.1505	1.25%	0.2078	N/A
Number of injections (B)	0.1294	15.08%	0.0147	N/A
MSC tissue of origin (C)	0.1104	27.58%	0.0315	N/A
Donor species (D)	0.1030	32.43%	0.0006	N/A
Transplant types (E)	0.1035	32.11%	0.0006	N/A
Routes of administration (F)	0.1393	8.58%	0.1383	N/A
A and B	0.1307	14.26%	0.0332	N/A
A and B with interaction	0.1221	19.91%	0.0205	0.0722
C and D	0.0966	36.60%	0.0132	N/A
C and D with interaction	0.0854	43.94%	0.0052	0.0413
A and B with interaction, C, D and E (Final model)	0.0639	58.04%	0.0017	N/A

A simple mixed-effects model linear regression was used. Normalised clinical score was the dependent variable. Moderators were added to the model through addition, while potential interaction terms were also added if necessary. τ^2 is the estimation between study variance, and R^2 is the percentage of variance that has been accounted for by the regression model.

Table S3: Meta-regression of the effect of treatment dosage with subgroup of number of injections

Subgrouping strategy	τ^2	R^2	Test for moderator (*p*-value)	Significance of adding quadratic term test (*p*-value)
Single injection				
Linear regression	0.1645	14.78%	0.1043	
Quadratic regression	0.1643	14.85%	0.2786	0.9370
Multiple injection				
Linear regression	0.0520	2.34%	0.7041	
Quadratic regression	0.0346	35.03%	0.1339	0.0391

Testing the non-linear effect of treatment dosage on normalised clinical score differences with mixed-effects model regression. τ^2 is the estimation between study variance, and R^2 is the percentage of variance that has been accounted for by the regression model.

Table S4: Results of clinical studies using MSC to treat RA

Study	Wang, et.al. (2013)	Wang, et.al. (2016)	Liang, et.al. (2012)	Álvaro-Gracia, et.al. (2017)	Ghoryani, et.al. (2019)	Park, et.al. (2018)	Shadmanfar, et.al. (2018)
Clinical Phase	1/2	Pilot	Pilot	1/2	N/A	1	1/2
Study Population	Subjects with Active RA	Subjects with JIA	Subjects with Refractory RA	Subjects with Refractory RA	Subjects with Refractory RA	Subjects with Active RA	Subjects with knee involved RA
Primary Objective	Safety and Efficacy	Safety and Efficacy	Safety and Efficacy	Safety, Tolerability and Efficacy	Safety and Efficacy	Safety and Tolerability	Safety and Efficacy
Primary Endpoint	Primary: Safety (Prevalence of AEs) Secondary: ACR20, ACR50, ACR 70, DAS28, HAQ-DI at month 3, 6 and 8	Primary: Safety (Prevalence of AEs) Secondary: DAS28. ESR, CRP at month 3 and 6	VAS pain score, DAS28, EULAR, CRP, ESR at month 1, 3, 6, 12, and then every half year	Primary: Safety (Prevalence of AEs) Secondary: ACR20, ACR50, ACR 70, DAS28-ESR, CRP, SF-36 every month until Month 3	DAS28-ESR, VAS, ESR, CRP, RF, anti-CCP, measure immunological factors at month 1, 6, 12	Primary: Safety (Prevalence of AEs) Secondary: DAS28, WOMAC score, VAS at month 3, 6, 12	Primary: Safety (Prevalence of AEs) Secondary: DAS28, HAQ, VAS, ESR at Week 4
Trial Design	Open label	Open label	N/A	Randomised, Multicentre, Double blind, Placebo-controlled	N/A	Open label	Randomised, Triple-blind, Single-centre, Placebo-controlled
Control Arm	Placebo	None	None	Placebo	None	None	Placebo
Random Scheme	None	None	N/A	Randomised, 3:1 test article to placebo	N/A	None	Block (Size 4) randomisation
Blinding	None	None	N/A	Single blinded for safety; Double blinded for efficacy	N/A	None	Triple blinded
Patients Enrolled	172	10	4	67	9	9	30
Patients Treated with Active Drug	136	10	4	42	9	9	15
Number of sites	1	1	1	18	1	1	1
Route of Delivery	IV	IV	IV	IV	IV	IV	Intra-articular
Dose(s)	4.0×10^7 cells / infusion, single or two IV infusion(s)	4.0×10^7 cells / infusion, two IV infusions	1.0×10^6/kg, IV infusions	1.0×10^6/kg/infusion (Corhort A), 2.0×10^6/kg/infusion (Cohort B), 4.0×10^6/kg/infusion (Corhort C), three IV infusions	1.0×10^6/kg/ infusion, single IV infusion	2.5×10^7cells /infusion, 5.0×10^7cells /infusion or 1.0×10^8cells /infusion, single IV infusion	$42 \pm 4\times10^6$ cells /injection, single injection to knee joint
Outcomes / Major Findings	Six cases of 136 patients (4%) showing mild flu-like symptoms during the infusion. No other	No AEs were observed after MSC infusion. 7 patients (70%, 7/10) achieved	3 of 4 patients achieved a reduction in ESR, DAS28, and pain VAS score at 1 and 6	Only 1 of the 8 AE grade 3 from 53 patients is serious. Significant increased ACR 20 response rate	Significant reduction in DAS28-ESR. VAS score showed significant	DAS-28 reduced 1.60 ± 1.57. Reduced levels of IL-1β, IL-6, IL-8, and TNF-α at 24	No adverse effects reported. Achieved superior findings according to WOMAC, VAS, time to jelling and pain-

	AEs reported. Significant increased ACR 20 response rate and reduction in DAS28, HAQ-DI	DAS28<2.6. Reduction in ESR, CRP	months after administration. 2 of 3 had a EULAR moderate response at 6 months but experienced a relapse at 7 and 23 months, respectively. No one achieved DAS28 remission in the follow-up period. No SAEs were reported.		decreasing trend. No significant difference for serum CRP and anti-CCP levels after intervention	hours were observed. The HAQ score and pain VAS changes at week 4 were (-0.15 ± 0.48)	free walking distance. The improvement cannot be significantly sustained over 12 months

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