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Mesenchymal Stromal Cell Therapy in Bronchopulmonary Dysplasia: Systematic Review and Meta-Analysis of Preclinical Studies

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Key Words. Stem cells • Lung injury • Preterm birth • Animal model • Meta-analysis

ABSTRACT

Extreme prematurity is the leading cause of death among children under 5 years of age. Currently, there is no treatment for bronchopulmonary dysplasia (BPD), the most common complication of extreme prematurity. Experimental studies in animal models of BPD suggest that mesenchymal stromal cells (MSCs) are lung protective. To date, no systematic review and meta-analysis has evaluated the preclinical evidence of this promising therapy. Our protocol was registered with Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies prior to searching MEDLINE (1946 to June 1, 2015), Embase (1947 to 2015 Week 22), Pubmed, Web of Science, and conference proceedings (1990 to present) for controlled comparative studies of neonatal animal models that received MSCs or cell free MSC-derived conditioned media (MSC-CM). Lung alveolarization was the primary outcome. We used random effects models for data analysis and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses reporting guidelines. We screened 990 citations; 25 met inclusion criteria. All used hyperoxia-exposed neonatal rodents to model BPD. MSCs significantly improved alveolarization (Standardized mean difference of −1.330, 95% confidence interval [CI −1.724, −0.94, I^2 69%]), irrespective of timing of treatment, source, dose, or route of administration. MSCs also significantly ameliorated pulmonary hypertension, lung inflammation, fibrosis, angiogenesis, and apoptosis. Similarly, MSC-CM significantly improved alveolarization, angiogenesis, and pulmonary artery remodeling. MSCs, tested exclusively in hyperoxic rodent models of BPD, show significant therapeutic benefit. Unclear risk of bias and incomplete reporting in the primary studies highlights nonadherence to reporting standards. Overall, safety and efficacy in other species/large animal models may provide useful information for guiding the design of clinical trials. STEM CELLS TRANSLATIONAL MEDICINE *2017;6:2079–2093*

SIGNIFICANCE STATEMENT

Bronchopulmonary dysplasia (BPD) is the most common complication of extreme prematurity and lacks effective treatment. Mesenchymal stromal cells (MSCs) are lung protective, and first clinical trials are under way in preterm infants. This first systematic review and meta-analysis assessing all preclinical studies of MSCs for BPD shows significant therapeutic benefit of MSC therapy on several outcome measures. More importantly, the study highlights methodological short-comings and the need to implement reporting standards such as Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines to ensure safe, evidence-based, and timely clinical translation of this promising therapy.

INTRODUCTION

Every year, an estimated 15 million babies are born preterm (before 37 completed weeks of gestation). Preterm birth has surpassed infectious diseases as the number one cause of under-5 mortality in children [1]. The most common complication of extreme preterm birth is bronchopulmonary dysplasia (BPD), a chronic lung disease [2] that complicates the course of approximately 40% of infants born <28 weeks gestation [3, 4]. BPD is strongly predictive of late death or disability [5, 6] and has a high economic burden [7]. Despite improvements in perinatal care, the incidence of BPD has increased over the last decade. BPD is a multifactorial disease in which extreme preterm birth, perinatal inflammation, mechanical ventilation, and oxidative stress contribute to impaired lung growth [8].

Survival of more immature infants born during the canalicular and saccular stages of lung development disrupt the normal program of alveolar and vascular development, resulting in

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alveolar simplification, dysmorphic capillaries, and remodeling of the vascular and airway smooth muscle layer [9]. Consequently, prevention of lung injury in ever more prematurely born infants has become increasingly challenging.

Although, many pharmacological and nonpharmacological approaches have been tested for the prevention and treatment of BPD, only few have contributed modestly in decreasing the incidence/severity of BPD [10]. Postnatal systemic corticosteroids remain controversial because of their association with adverse neurodevelopmental outcomes [11].

Recent insights into stem cell biology have unraveled the therapeutic potential of stem cells. Stem cells can self-renew and differentiate into specialized cell types thereby promoting organogenesis, tissue regeneration, maintenance, and repair [12]. Mesenchymal stromal cells (MSCs) attracted particular interest because of their ease of isolation, expansion, apparent multipotency, and pleiotropic effects in various injury models [13]. In experimental neonatal lung injury, MSCs are lung protective and exert their therapeutic benefit mainly through a paracrine activity [14]. These data suggest MSCs as a promising therapy to reduce the incidence/severity of BPD in extreme premature infants.

To date, there has been no systematic review and/or meta-analysis on the therapeutic potential of MSC in experimental BPD. Translation of potentially life-saving therapies is unacceptably slow. Even more concerning is overall failure, with less than 5% of high impact preclinical reports being clinically translated and only 11% of clinically tested agents ultimately receiving licensing [15]. In a first step to ensure the evidence-based translation of this promising MSC therapy into the clinic for patients at risk of developing BPD, we have performed this systematic review to assess the current evidence to help guide the design of clinical trials.

Materials and Methods

Protocol

The protocol, developed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-P checklist [16] was prospectively registered and is available on the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies website (CAMARADES) [17]. We followed the PRISMA [18] guidelines for this manuscript.

Inclusion and Exclusion Criteria

We included preclinical, controlled comparative studies of neonatal animal models mimicking features observed in human BPD and evaluated the therapeutic potential and safety of MSCs or cell free MSC-derived conditioned media. MSCs were defined using the minimal criteria set out in the International Society for Cellular Therapy (ISCT) [19] consensus statement. Noninterventional studies, studies without controls, and non-neonatal models of lung injury were excluded.

Search Strategy and Selection Criteria

In brief, MEDLINE including In-process and other Non-Indexed Citations (1946 to June 1, 2015), EMBASE (1947 to 2015 Week 22) using the Ovid interface and Science Citation Index Expanded (SCI-EXPANDED), and Conference Proceedings Citation Index-Science, 1990 to present using the Web of Science were searched, without language restrictions, for the search term "mesenchymal stromal cells," "bronchopulmonary dysplasia," and "animals".

The MEDLINE search strategy was developed by a librarian experienced in systematic review searching using the PRESS standards [20] and reviewed by the investigators. The MEDLINE search included a focused search for MSCs and BPD without restriction to preclinical studies or age groups, followed by an expanded search for BPD and MSC and limited to preclinical studies [21] in the neonatal period. The MEDLINE search was then adapted for the other database. No language or study design limits were applied. A simple PubMed search was then run against the PubMed subsets *pubstatusaheadofprint, publisher,* and pubmednotmedline to find material unlikely to be included in other sources [22]. The search strategies are presented in Supporting Information Appendix 1.

The titles-abstracts of the search results were screened, and the full text of all potentially eligible studies was retrieved and reviewed for eligibility, independently, by three members of the team working in pairs (S.A., B.H., and T.L.) and data extracted from each study using standardized forms (S.A., B.H.). Disagreements between reviewers were resolved by consensus or by a third member (M.A.).

As most of the data were available in figures and not in numerical form, we used a validated graphical digitizer (WebPlotDigitizer, version 3.10; Ankit Rohatgi), an open source program, that can work with a variety of plot types and images. First, the images of the figures for relevant outcome from all included studies were saved as screenshots, since WebPlotDigitizer supports .jpeg, .png, .bmp, and .gif. Then, these images were uploaded to the application. The first step of the analysis consisted of defining the type of graph analyzed, which was typically a two-dimensional Bar plot and calibrating the axis by assigning four points of known values on the axis.

Then, the data points were extracted. A manual and an automatic mode were available. We used the manual method. In the manual mode, data points were added by directly clicking on the graph, and WebPlotDigitizer would calculate the precise coordinates of each point, which in turn was used to calculate the mean and standard deviation for each graph.

Primary and Secondary Outcomes

Our primary outcome was lung alveolarization on histology. Secondary outcomes included lung inflammation, pulmonary hypertension, pulmonary artery remodeling, pulmonary vascular density, lung fibrosis, oxidative stress, lung function, exercise capacity, safety, weight gain, long-term outcome, and survival.

Risk of Bias and Study Validity

Risk of bias was assessed by two reviewers (S.A., M.A.), for each included study, using SYRCLE's Risk of Bias tool (an adaptation of Cochrane Risk of Bias tool) for animal studies [23]. We extracted study characteristics that were related to the construct and external validity [24]. For construct validity, we included: age, sex, strain and animal species, comorbidities, type of BPD model, timing, dose and mode of MSC administration, and the use of any cointerventions.

Data Analysis

Data were analyzed with OpenMetaAnalyst. We calculated standard deviations from standard errors and *n* values. For continuous data, we used standardized mean difference because different measurements scales were reported for the same outcomes. We used DerSimonian and Laird random-effects meta-analysis model

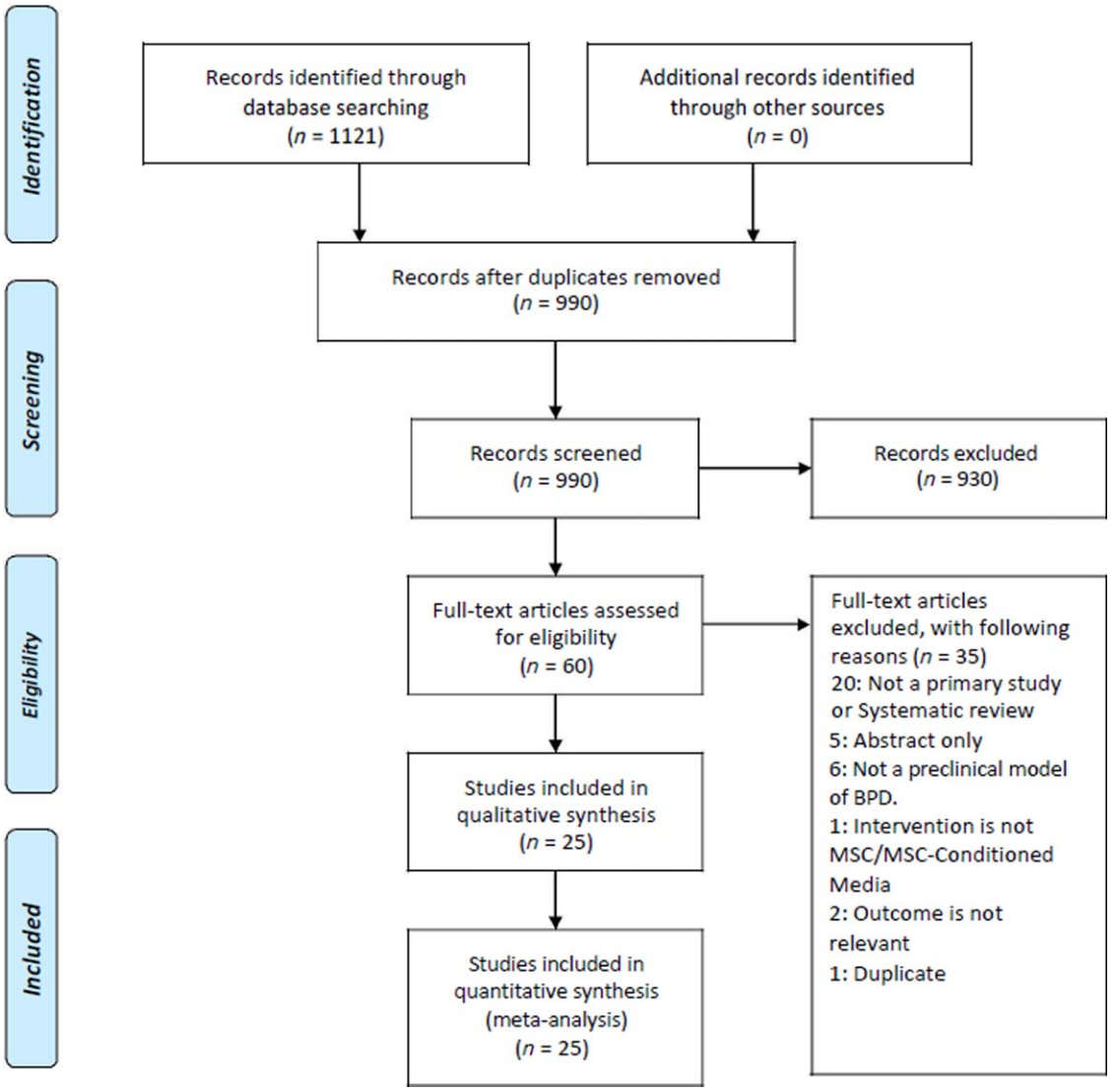


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 flow diagram. Abbreviations: BPD, bronchopulmonary dysplasia; MSC, mesenchymal stromal cell.

to account for heterogeneity (i.e., both within and between study variance) [25]. The unit of analysis for the meta-analyses were the individual extracted experiments. For dichotomous data (mortality) we calculated odd ratios. We assessed statistical heterogeneity with the I^2 statistic with 95% confidence intervals, and data were visualized using forest plots. Statistical heterogeneity was assessed as very low (0%–25%), low (25%–50%), moderate (50%–75%), and high (>75%) using the I-statistic [25]. We assessed for publication bias using a funnel plot and adjusted our results for it using the trim and fill method (Comprehensive Meta-Analysis) [26].

We performed prespecified subgroup analyses to examine heterogeneity of the treatment of MSCs on alveolarization where there was sufficient data reported. The prespecified subgroups included pairwise meta-analyses based on: MSC dose, route of injection, source of MSCs, timing of treatment post-natal, and timing of assessment post-natal.

Effect size was interpreted based on Cohen's *d* as small effect (≤0.2), medium effect (0.5), and large effect size (≥0.8) [27].

Results

Study Characteristics

In total, 1,121 records were identified (Fig. 1) and duplicate references were removed, resulting in 990 records for screening. Preliminary screening excluded 930 records. Sixty records were further examined by reviewing the full text articles where, based on our eligibility criteria, a further 35 papers were excluded leaving 25 included studies [28–52] (Fig. 1). Two studies were excluded post hoc because the outcomes did not meet inclusion criteria. One study described Bronchoalveolar stem cell number in response to MSC treatment [53]; the other study used surfactant protein-C expression as endpoint [54].

Rodents exposed to hyperoxia were the exclusive animal BPD model (Table 1) [28–52]. The O_2 concentrations used to induce lung injury ranged from 60% to 95%.

Alveolarization was the primary outcome in 42 experiments from 21 studies [28–43, 46–48, 51, 52]. We looked at different

Table 1. General characteristics of all included studies

Author (year); Sample size; Country	Animal model, gender	BPD model	Hyperoxia duration	Control group	MSC/MSC-CM source, type; fresh/frozen	MSC/MSC-CM dose, delivery time (hours), and method of delivery	Control, amount, time	Time of assessment	Outcomes
Anh et al. (2013) [28]; $N = 33$; South Korea	Sprague Dawley rat pups	Hyperoxia (90%)	<10 hours–P14 RA from P14 to P70	Hyperoxia; Normoxia	Xenogeneic, Human UCB; Unclear	5×10^5 cells in 0.05 ml PBS, P5, IT	PBS, 50 μl, P5	P70	Alveolarization (MLI), Lung inflammation (Alveolar Macrophages, lung inflammatory foci), Angiogenesis (vWF), safety (Hematoma, hemorrhage, Tumor), weight, survival rate
Ahn et al. (2015) [29]; $N = 95$; South Korea	Sprague Dawley rat pups	Hyperoxia (90%)	Birth–P14	Hyperoxia	Xenogeneic, Human UCB and AT; Unclear	Three arms (a) Human UCB MSC 5×10^5 cells in 0.05 ml PBS, (b) AT MSC 5×10^5 cells in 0.05 ml PBS, (c) Human UCB MNC 5×10^5 cells in 0.05 ml PBS; P5, IT	PBS, 50 μl;	P7, 14	Alveolarization (MLI), Lung inflammation (IL-1α, IL-1β, IL-6, TNF-α), lung angiogenesis (VEGF, HGF)
Aslam et al. (2009) [30]; $N = NR$; U.S.	Newborn FVB mice pups, Female	Hyperoxia (75%)	P1–P14	Hyperoxia; Normoxia	Syngeneic, Bone marrow;	Two arms (a) BMSC 5×10^4 cells in 0.05 ml PBS, (b) BMSC-CM 50 μl P4, IV	Two arms (a) PBS, unclear (assumed 50 μl), (b) PASMC 5×10^4 cells in 0.05 ml PBS	P5 or P14	Alveolarization (VDawt), Lung fibrosis (Mean alveolar septal thickness), Pulmonary hypertension (Fulton index), Pulmonary artery remodeling (α-SMA), Lung Inflammation (Alverolar macrophages, BALF Macrophages, BALF PMN)
Chang et al. (2014) [31]; $N = NR$; South Korea	Newborn Sprague Dawley rat pup, NR	Hyperoxia (90%)	Birth–P14	Hyperoxia, Normoxia; None	Xenogeneic, Human UCB; Unclear	Three arms (a) UCB MSC 5×10^5 (b) Scrambled siRNA-transfected MSCs 5×10^5, (c) VEGF siRNA-transfected MSCs 5×10^5, P5 P5, IT	PBS, unclear,	P7, P10, P14	Alveolarization (MLI, MAV), Lung inflammation (TUNEL positive, ED-1 positive, IL-1α, IL-1β, IL-6, TNF-α), Lung angiogenesis (vWF), VEGF
Chang et al. (2013) [32]; $N = NR$; South Korea	Newborn Sprague Dawley rat pup, NR	Hyperoxia (90%)	Birth–P14: 90% P14–P21: 60%	Hyperoxia	Xenogeneic, Human UCB; Unclear	5.0×10^5, Three arms (a) P3 (b) P10, (c) P3 + P10 IT	PBS, 50 μl	P1, P3, P5, P7, P10, P14, P21	Weight, Survival, Alveolarization (MLI, MAV), Apoptosis (TUNEL positive) Lung inflammation (ED-1 positive, IL-1α, IL-1β, IL-6, TNF-α, TIMP, CXCL7, RANTES, L-Selectin, sICAM-1, MPO activity), Lung fibrosis (Collagen) VEGF, HGF, Oxidative stress (Cytosol/Membrane NADPH oxidase P47phox)
Chang et al. (2011) [33]; $N = NR$ in methods; South Korea	Newborn Sprague Dawley rat pup, NR	Hyperoxia (95%)	<10 hours–P14	Hyperoxia (95%); Normoxia	Xenogeneic, Human UCB; Unclear	Three arms (a) 5.0×10[3] (b) 5.0×10^4, (c) 5.0×10^5, P5, IT	PBS, 50 μl, P5	P14	Weight, Survival, Alveolarization (MLI, MAV), Apoptosis (TUNEL positive), Lung inflammation (ED-1 positive, IL-1β, IL-6, TNF-α, TGF-β, MPO activity, Lung fibrosis (Collagen), Oxidative stress (Cytosol/Membrane NADPH Oxidase P47phox)

Table 1. *Continued*

Author (year); Sample size; Country	Animal model, gender	BPD model	Hyperoxia duration	Control group	MSC/MSC-CM source, type; fresh/ frozen	MSC/MSC-CM dose, delivery time (hours), and method of delivery	Control, amount, time	Time of assessment	Outcomes
Chang et al. (2009) [34]; *N* = 158; South Korea	Newborn Sprague Dawley rat pup, NR	Hyperoxia (95%)	<10 hours–P14	Hyperoxia (95%); Normoxia	Xenogeneic, Human UCB; Unclear	Two arms (a) 5.0×10^6, IT, (b) 2.0×10^6, IP P5	Human fibroblast 5.0×10^6 P5	P14	Weight, Survival, Alveolarization (MLI, MAV), Apoptosis (TUNEL positive), Lung inflammation (IL-6, TNF-α, TGF-β, MPO activity) Lung Fibrosis (Collagen, α-SMA)
Fritzell et al. (2009) [35] *N* = NR U.S.	Newborn C57BL/6J mice	Hyperoxia (95%)	Birth–P7 Re-exposure: P66–p68	Disease, Sham;	Syngeneic, Bone marrow; Unclear	2× (5.0×10^6), P5, IN	NS, 25 μl, P5	P7, P12, P19, P66	Weight, Alveolarization (MCL, Lung volume, Air exchanging parenchyma, Aa (ae/lu), Ki-67 labeling index)
Gulasi et al. (2016) [36]; *N* = 66; Turkey	Newborn Wistar albino rats, Not reported	Hyperoxia (85–95%)	Birth–P10	Hyperoxia, Normoxia, Sham	Syngeneic, Bone marrow; Unclear	Three arms (a) MSC 1.0×10^5, (b) Culture medium 25 μl, (c) Remaining Medium 25 μl, P11, IT	NS 25 μl, P11	P10, P60	Weight,[a] Lung weight,[a] Lung weight/Body weight,[a] Alveolarization (# of alveoli, Alveolar diameter),[a] Lung fibrosis (α-SMA)[b], Inflammation score[b]
Hansmann et al. (2012) [37]; *N* = NR in methods; U.S.	FVB mice pups, Not reported	Hyperoxia (75%)	P1–P14	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	MSC-CM 50 μl, P14, IV	MLF-CM 50 μl, P14, IV	P28, P42	Alveolarization (MLI), Lung fibrosis (MAST, Collagen), Lung Function (Airway resistance, Dynamic Lung compliance), Pulmonary Hypertension (PAAT, PAAT/PAET, RVWT), Pulmonary Artery Remodeling (medial thickness index), Angiogenesis (#pulmonary blood vessel small/ Moderate)
Liu et al. (2014) [38]; *N* = NR in methods; U.S.	Fox Chase SCID beige,	Hyperoxia (90%)	Birth–P7	Disease, Sham; None	Xenogeneic, Human Umbilical cord tissue; Frozen	Six arms (a) Low 0.1×10^6, IN (b) Medium 0.5×10^6, IN (c) High 1.0×10^6, IN (d) Low 0.1×10^6, IP (e) Medium 0.5×10^6 IP (f) High 1.0×10^6 IP P5	Two arms: (a) PBS, 20 μl IN (b) PBS, 25 μl IP	P60	Alveorization (MLI/MCL, Inflated lung volume, Volume of air exchanging parenchyma, Areal density of air-exchanging parenchyma), Lung fibrosis (Mean Septal Wall thickness)[c] Lung function (Inspiratory capacity, Resistance, Compliance, Elastance, Tissue damping, Tissue hysteresitivity, Quasi-Static Compliance, Total lung capacity, K, Area of PV loop).[a] Lung weight, Weight
Luan et al. (2015) [39]; *N* = 15 People's Republic of China	Neonatal C57BL/6 mice, Not reported	Hyperoxia (60%)	24 hours–P14	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Fresh	1.0×10^6, 24 hours, IV	Unclear		Weight, Alveolarization (RAC), Lung Inflammation (TGF-β1), Lung angiogenesis (VEGF)

Table 1. *Continued*

Author (year); Sample size; Country	Animal model, gender	BPD model	Hyperoxia duration	Control group	MSC/MSC-CM source, type; fresh/frozen	MSC/MSC-CM dose, delivery time (hours), and method of delivery	Control, amount, time	Time of assessment	Outcomes
Pierro et al. (2013) [40]; $N = NR$ Canada	Rat pups, Not reported	Hyperoxia (95%)	Birth–P14	Hyperoxia, Normoxia	Xenogeneic, Human Umbilical cord blood and Wharton Jelly; Fresh Three arms: (a) MSC (b) MSC-CdM (c) PC	Four arms: (a) MSC Prevention 3×10^5, P4, IT (b) MSC Regeneration 6×10^5, P14, IT (c) CdM Prevention 7 μl/g, P4–21, IP (d) CdM Prevention 7 μl/g, P14–28, IP	HNDF Prevention 3×10^5, P4, IT	Prevention P22 Regeneration P35 Long-term P6mo	Alveolarization (MLI, Septal count), Pulmonary artery remodeling (Medial wall thickness), Pulmonary hypertension (Fulton index), Lung angiogenesis (Vessels/hpf), Lung function (Compliance), Exercise capacity
Sutsko et al. (2013) [41]; $N = NR$ U.S.	Sprague Dawley, Not reported	Hyperoxia (90%)	P2–P16	Hyperoxia (90%), Normoxia	Allogeneic, Bone marrow; Frozen	Two arms: (a) MSC:2×10^6, P9, IT (b) MSC-CM: 50 μl IT	PBS, (50 μl), P9, IT	P16, P30, P100	Alveolarization (MLI, Average alveolar area), Lung angiogenesis (Vessels/HPF, VEGF), Lung inflammation (IL-6, IL-1β, TTF), Pulmonary hypertension (RVSP, RV/LV+S)
Tian et al. (2007) [42]; $N = 32$; People's Republic of China	Sprague-Dawley rat, Not reported	Hyperoxia (95%)	P3–P10	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	5×10^4, P10, IV	PBS, 50 μl, P10	P13	Alveolarization (RAC), Lung inflammation (TGF-β, TNF-α)
Tian et al. (2008/10) [43]; $N = 32$; People's Republic of China	C57BL/6 mouse, Male	Hyperoxia (95%)	P3–P10	Disease; None	Xenogeneic, Bone marrow; Fresh#	5.0×10^4, P10, IP	PBS, unclear likely 50 μl, P10	P13	Alveolarization (RAC), Lung inflammation (TNF-α, IL-1β, BAL WCC, BAL Neutrophil)
Tian et al. (2012) [44]; $N = 24$; People's Republic of China	Sprague-Dawley rat, Not reported	Hyperoxia (95%)	Birth–P7	Hyperoxia, Normoxia	Xenogeneic, Bone marrow; Unclear	5×10^4, P10, IV	PBS, 50 μl, P10, SC	P13	Lung inflammation (NF-kB, TGF-β, TNF-α)
Tian et al. (2013/2) [45]; $N = 30$; People's Republic of China	Sprague-Dawley rat, Not reported	Hyperoxia (95%)	Birth–P7	Hyperoxia, Normoxia	Xenogeneic, Bone marrow; Unclear	5×10^4, P7, IV	PBS, 50 μl, P7, SC	P10	Lung inflammation (NF-kB, RAGE, TNF-α, Lung injury score)
Tian et al. (2008/1) [46]; $N = 32$; People's Republic of China	Sprague Dawley, Not reported	Hyperoxia (95%)	P3-P10	Hyperoxia, Normoxia	Xenogeneic, Bone marrow; Unclear	$1.0x10^5$, P10, IP	PBS, 30 μl, P13	Unclear	Alveolarization (RAC), Lung inflammation (TGF-β1, TNF-α)
Van Haaften et al (2009) [47]; $N = NR$; Canada	Sprague-Dawley rat, Not reported	Hyperoxia (95%)	Birth–P14	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	Two arms (a) Prevention: MSC 1×10^5, P4, IT (b) Regeneration MSC 1×10^5, P14, IT	Two arms (a) Prevention: PASMC 1×10^5, P4, IT (b) Regeneration PASMC 1×10^5, P14, IT	Prevention: P21 Regeneration: P45	Alveolarization (MLI), Lung angiogenesis (vessels/hpf), Pulmonary hypertension (RVH, PAAT), Exercise capacity, Survival rate
Waszak et al. (2012) [48]; $N = NR$; Canada	Sprague-Dawley rat, Not reported	Hyperoxia (95%)	P0–P14	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	Two arms (a) MSC-CM 1 ml/kg, P0–P20, IP (b) Preconditioned MSC-CM 1 ml/kg, P0–P20, IP	Two arms (a) DMEM 1 ml/kg, P0–P20, IP b) Preconditioned RLF-CM 1 ml/kg, P0–P20, IP	P21	Alveolarization (MLI), Pulmonary hypertension (PAAT/RVET, Fulton Index), Pulmonary artery remodeling (Medial wall thickness)

Table 1. *Continued*

Author (year); Sample size; Country	Animal model, gender	BPD model	Hyperoxia duration	Control group	MSC/MSC-CM source, type; fresh/frozen	MSC/MSC-CM dose, delivery time (hours), and method of delivery	Control, amount, time	Time of assessment	Outcomes
Yao et al (2013) [49]; $N = NR$; People's Republic of China	Sprague-Dawley rat, Not reported	Hyperoxia (95%)	P1–Unclear	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	Two arms (a) MSC 8×10^5, P3, IP (b) Precondition MSC-KGF 8×10^5, P3, IP	PBS, unclear, P3	P17	Lung fibrosis (Area of Masson trichome staining, collagen, Hydroxyproline)
Zhang et al (2013/6) [50]; $N = NR$; People's Republic of China	Sprague Dawley, Not reported	Hyperoxia (95%)	P3–P10	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	1×10^5, P10, IV	PBS, Unclear, assumed to be 100 μl, P10	P13, P17, P24	Lung angiogenesis (VEGF, HIF), Lung apoptosis (TUNEL, BCL2, BAX),
Zhang et al (2012) [51]; $N = NR$; People's Republic of China	Sprague Dawley, Not reported	Hyperoxia (95%)	P3–P10	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	1×10^5, P10, IV	PBS, Unclear, assumed to be 100 μl, P10	P13, P17, P24	Weight, Alveolarization (RAC), Lung tissue cytokine (TNF-α, TGF-β, IL10)
Zhang et al (2012) [52]; $N = 60$; People's Republic of China	Kumming, Not reported	Hyperoxia (60%)	Birth–P45	Hyperoxia, Normoxia	Syngeneic, Bone marrow; Unclear	1×10^5, P7, IP	PBS, Unclear, Not reported assumed to be P7	P45	Alveorization (RAC), Lung fibrosis (TGF-β1, TIMP1, Collagen), Lung tissue cytokine (TNF-α, IL-1β), Survival rate

[a]Reported as median.
[b]Reported as prevalence.
[c]Reported as a measure of alveolarization.
Abbreviations: Aa, alveolar area; α-SMA, α-smooth muscle actin; AT, adipose tissue; BALF, bronchoalveolar lavage fluid; BASC, broncoalveolar stem cell; CM, conditioned media; DMEM, Dulbecco's modified Eagle's medium; FCM, fibroblast conditioned media; HGF, hepatocyte growth factor; HNDF, human neonatal dermal fibroblast; IL, interleukin; IM, intramuscular; IN, intranasal; IP, intraperitoneal; IP, intraperitoneal; IT, intratracheal; IV, intravenous; K, curvature of upper portion of deflation PV loop; KGF, kerationocyte growth factor; LV, left ventricle; MAST, mean alveolar septal thickness; MAV, mean alveolar volume; MCL, mean chord length; MLF, mouse lung fibroblast; MLI, mean linear intercept, MNC, mononuclear cell; MSC, mesenchymal stromal cell; MPO, myeloperoxidase; NADPH, nicotinamide adnine dinucleotide phosphate; NF, nuclear factor; P, post-natal; PAAT, pulmonary artery acceleration time; PAET, pulmonary artery ejection time; PASMC, pulmonary artery smooth muscle cell; PMN, polymorphonuclear cell; PV, pressure-volume; NR, not reported; NS, normal saline; PBS, phosphate buffered saline; RAC, radial alveolar count; RAGE, receptor for advanced glycation end products; RVET, right ventricular ejection time; RVWT, right ventricular wall thickness; RVSP, right ventricular systolic pressure; RLF, rat lung fibroblast; S, septum; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; TTF, thyroid transcription factory; VEGF, vascular endothelial growth factor; VDawt, volume density of alveolar tissue; vWF, von Willebrand factor; WCC, white cell count; UCB, umbilical cord blood.

Table 2. Construct and external validity of the hyperoxia-control versus hyperoxia-mesenchymal stromal cells (MSCs) comparison for the primary outcome: alveolarization

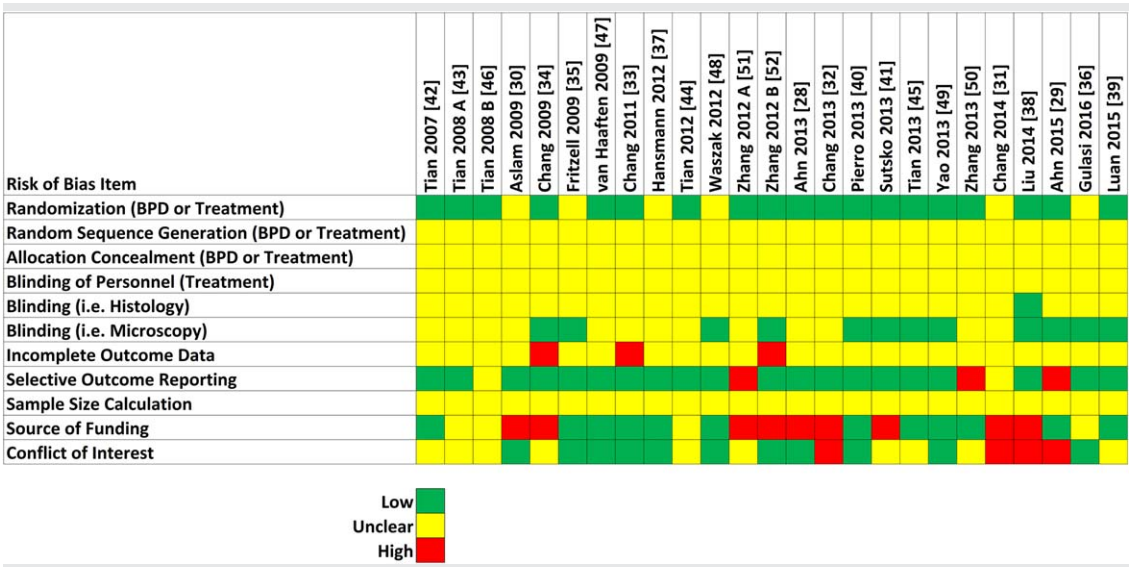
Sample characteristics	N	%
Number of papers	18	100%
Number of experiments	33	100%
Number of animals hyperoxia-control	212	44%
Number of animals hyperoxia-MSCs	271	56%
Number of animals in total	483	100%
Median # of animals hyperoxia-control	6 animals	—
Median # of animals hyperoxia-MSCs	6 animals	—
Result reproducibility		
# of experiments with positive result	18	55%
# of experiments with neutral result	15	45%
# of experiments with negative result	0	0%
Construct validity characteristics		
Species		
Mouse	8	25%
Rat	25	76%
Strain		
Sprague-Dawley	22	67%
C57BL/6J	4	12%
Kumming	1	3%
FVB	1	3%
Fox Chase SCID Beige	2	3%
Unclear	3	9%
Sex		
Female	NR	
Male	NR	
Experiment type/timing of treatment		
Prevention–Treatment ≤P5	22	77%
Rescue–Treatment >P5	11	33%
Age (Postnatal Day) at sampling		
≤P14	17	52%
P15–P28	7	21%
>P28	9	27%
Model type		
Hyperoxia %: ≥90%	30[a]	91%
Hyperoxia %: < 90%	3	9%
Most frequent hyperoxia %: 95%	16	48%
Hyperoxia start: ≤P1	22[b]	67%
Hyperoxia start: > P1	5	15%
Unclear	6[c]	18%
Hyperoxia duration: ≤7 days	9	27%
Hyperoxia duration: 8–14 days	19[d]	58%
Hyperoxia duration: ≥15 days	3[e]	9%
Unclear	2	6%
Median duration of hyperoxia	14 days	—
Minimum duration of hyperoxia	7 days	—
Maximum duration of hyperoxia	45 days	—
MSCs route of administration		
Intranasal	4	12%
Intraperitoneal	5	15%
Intratracheal	19	58%
Intravenous	5	15%
MSCs dose (# of cells)		
≤100,000	8	24%
100,000–1,000,000	15	46%
≥1,000,000	10	30%
External validity characteristics		
Prevention experiments		
Rat + Hyperoxia ≥90%	15	68%
Rat + Hyperoxia < 90%	0	—
Mouse + Hyperoxia ≥90%	5	23%
Mouse + Hyperoxia < 90%	2	9%
Rescue Experiments		
Rat + Hyperoxia ≥90%	10	91%
Rat + Hyperoxia < 90%	0	—
Mouse + Hyperoxia ≥90%	0	—
Mouse + Hyperoxia < 90%	1	9%

[a]Experiments from Chang et al. 2013 [32] A and B used 90% oxygen for 2 weeks followed by 60% oxygen for 1 week.
[b]Most experiments just stated birth without specific timing of treatment start (e.g., within 10 hours of birth).
[c]Not reported in Fritzell et al. 2009 [35], and not reported in English language abstract of Tian et al. 2007 [42], 2008 [43, 46].
[d]Only Luan et al. 2015 [39] and Sutsko et al. 2013 [41], indicated that exposure to hyperoxia was not continuous because of animal care interruptions of less than 10 minutes per day.
[e]Fritzell et al. 2009 [35] exposed neonates to hyperoxia for 7 days and then re-exposed them at P66 to P68. Abbreviations: –, not applicable; MSC, mesenchymal stromal cells.

subgroups for the primary outcome of lung alveolarization. For source of MSC, 38% ($n = 8$) of the studies used MSC from umbilical cord (blood [28, 29, 31–34, 38, 40] and tissue [38, 40]) while 62% ($n = 13$) used bone marrow [30, 35–37, 39, 41–43, 46–48, 51, 52]. Two studies used preconditioned MSCs with either oxygen [48] or keratinocyte growth factor [49] to test for strategies enhancing the therapeutic efficacy. Thirty-three experiments examined treatment effects based on dose of MSC. The dose of MSCs in the intervention group varied widely (Table 2). Hence, we subdivided, a priori, dosage of MSCs into low ($<10^5$ cells), medium ($>10^5$–10^6 cells), and high ($>10^6$ cells). Low dose was used in 24% ($n = 8$) of these experiments [30, 33, 42, 43, 46, 47, 52], 46% ($n = 15$) used medium [28, 29, 31, 32, 34, 40, 51], and less than one-third (30%, $n = 10$) used high dose [34, 35, 38, 39, 41]. Fifty-eight percent ($n = 19$) used intratracheal route of administration [28, 29, 31–34, 40, 41, 47], while 12% ($n = 4$) used intranasal [35, 38] and 15% ($n = 5$) each used intraperitoneal [34, 38, 43, 46, 51] and intravenous routes (Table 2) [30, 39, 42, 52].

Lung alveolarization in rodents starts on postnatal day 5 (P5). Hence timing of treatment was subdivided, a priori, into Prevention (≤P5) and Rescue (>P5). Also, timing of assessment was subdivided into Early (≤P14), Mid (>P14 to ≤P28), and Late (>P28).

Table 3. Risk of bias



Two-thirds of the experiments were preventive where 39% ($n = 13$) [29–31, 33–35, 39], 12% ($n = 4$) [32, 35, 40, 47], 15% ($n = 5$) [28, 35, 38, 40] were early, mid, and late assessments, respectively. Among rescue experiments, 9% ($n = 3$) [42, 43], 12% ($n = 4$) [32, 41, 52], and 12% ($n = 4$) [40, 41, 51] were early, mid, and late assessments, respectively.

Risk of Bias Assessments

Risk of Bias was assessed for the primary outcome of alveolarization in 25 included studies using 11 domains (see Table 3). The SYRCLE'S Risk of Bias contains 10 entries related to selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. We adapted the SYRCLE's Risk of Bias to include sample size calculation, source of funding and conflict of interest. None of the studies met the criteria for low risk of bias across all 11 domains. While a large majority, 75% ($n = 19$) [28, 29, 32–34, 38–47, 49–52] were considered low risk of bias under the general heading of randomization to BPD model or treatment, the risk of bias was unclear as to sequence generation and allocation concealment. In all 25 included studies, it was unclear how the sample size was calculated. Half of the included studies [29, 34–36, 38–41, 45, 48, 49, 52] reported a low risk of bias under blinded assessment of outcome by microscopy though it was unclear whether the personnel were blinded to the treatment group or during processing of tissue for histology. Under the domain for "incomplete outcome data," three studies were considered high risk [33, 34, 52] of bias while the rest were assessed as unclear risk of bias. For selective outcome reporting, a large majority, (80%, $n = 21$) [28, 30, 32–45, 47–49, 52] had low risk of bias while 3 had high risk of bias [29, 50, 51]. Under the domain for "source of funding" almost half (46%, $n = 12$) [29, 33, 35, 37, 39, 40, 42, 45, 47–50] had low risk of bias while more than a third (35%; $n = 9$) [28, 30–32, 34, 38, 41, 51, 52] had high risk of bias and 4 studies did not report any information on funding [36, 43, 44, 46]. For "conflict of interest" domain, again less than half (42%, $n = 11$) [28, 30, 35–37, 40, 47–49, 51, 52] had low risk of bias and 15% ($n = 4$) were considered high risk [29, 31, 32, 38] while 38% ($n = 10$) [34, 39, 41–46, 50, 51] did not report any risk of bias.

Meta-Analysis: Primary Outcome

MSCs. Overall, the treatment effect favored MSC compared with controls for the primary outcome of alveolarization [28–35, 38–43, 46, 47, 51, 52] with a Standardized mean difference (SMD) of −1.33, 95% Confidence interval (CI) (–1.72, −0.94; moderate heterogeneity $I^2 = 69\%$; Fig. 2A).

Funnel plot analysis revealed asymmetry suggesting potential missing studies. Subsequent trim and fill analysis resulted in the addition of six imputed experiments and a small reduction in estimated effect size (–1.19), 95% CI (–1.62, −0.72) (Fig. 3).

Treatment effect was further examined in pre-specified subgroups for dose (three subgroups: low [$<10^5$ cells], medium [10^5–10^6 cells], and high [$>10^6$ cells]), route (four subgroups: Intranasal, Intraperitoneal, Intratracheal, Intravenous), source (two subgroups: Bone marrow and Umbilical Cord), treatment timing (two subgroups: Prevention [$\leq$P5] and Rescue [$>$P5)], and assessment timing (two subgroups: Early [$\leq$P14], Mid [$>$P14 to $\leq$P28], and Late [$>$P28]); (Fig. 4). In all subgroups, the treatment effect favored MSCs compared with controls with the exception of the intranasal route (SMD 0.28, 95% CI [–0.43, 1.00], I^2 66), which was dominated by three experiments from one study. We noted that Bone marrow MSCs were more commonly used than umbilical cord. Although both sources showed similar statistically significant large effect size, there was high heterogeneity in the former group and moderate in the latter (SMD −1.47, 95% CI [–2.22, −0.72], I^2 =78 vs. SMD −1.21, 95% CI [–1.63, −0.78], I^2 56).

We did not find any difference in effect based on dose of MSC, with low, medium, and large dose producing similar statistically significant large effect size (SMD −1.50, 95% CI [–2.29, −0.72], I^2 77, SMD −1.52, 95% CI [–2.04, −0.99], I^2 58, SMD −0.86, 95% CI [–1.72, −0.01], I^2 73 respectively), though heterogeneity was moderate with medium and high dose and considerable with low dose. However, with respect to route of administration, we found a statistically significant larger effect size with intravenous (SMD −2.23, 95% CI [–3.65, −0.81], I^2 83) than the intra tracheal route (SMD −1.62, 95% CI [–2.09, −1.15], I^2 60), though the heterogeneity was moderate in the latter.

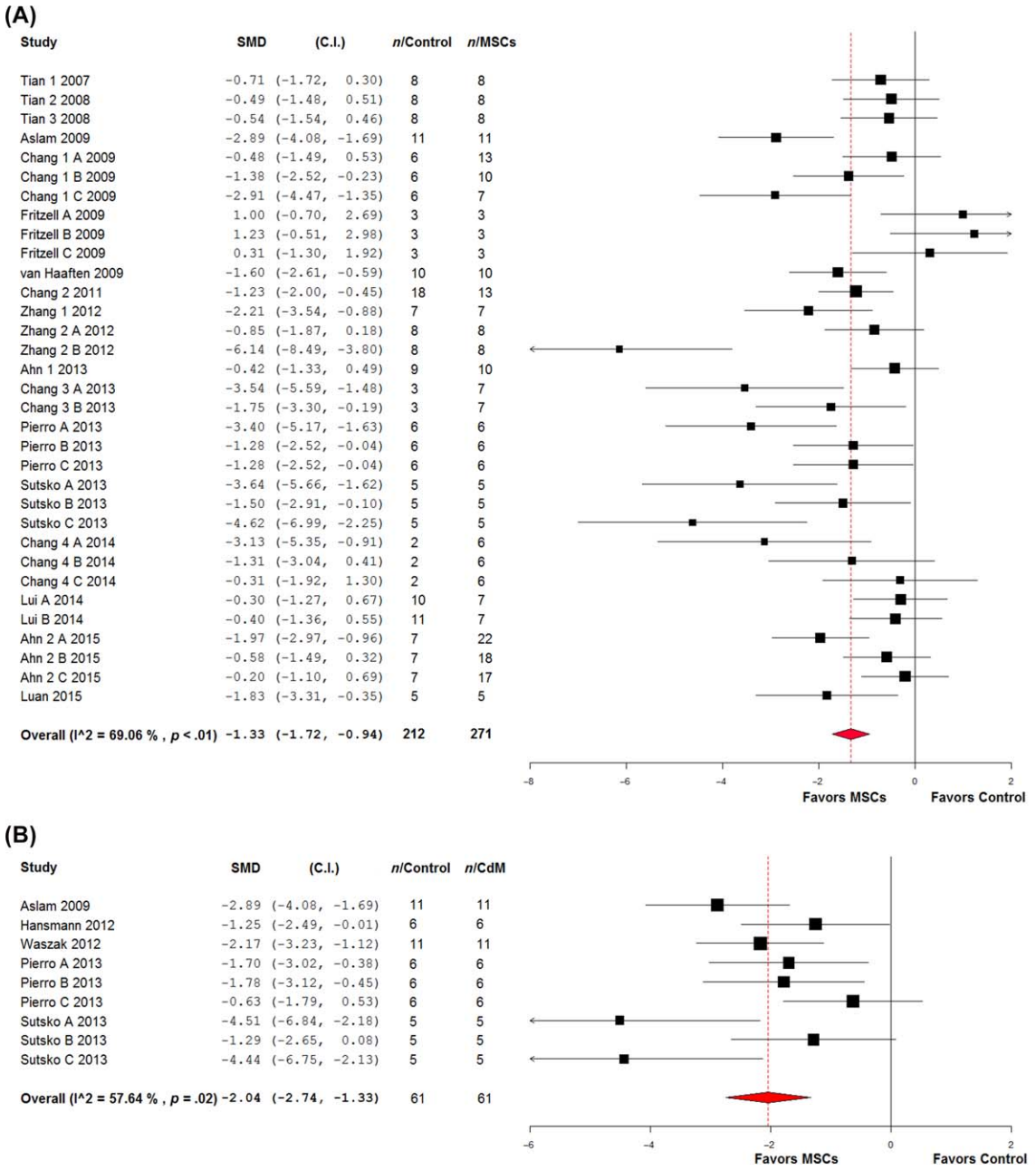


Figure 2. Meta-Analysis of all included studies for the primary outcome of alveolarization. Forest plot of therapeutic potential of **(A)** MSCs and **(B)** MSC-Conditioned media in animal model of BPD for the primary outcome of alveolarization. Black squares indicates the actual effect size of primary/individual studies. Red diamond indicates the overall or average effect size of all the primary studies. Abbreviations: MSC, mesenchymal stromal cell; SMD, standardized mean difference.

Further, the treatment effect favored MSCs compared with controls, irrespective of timing of treatment and assessment (Fig. 4). MSCs when administered preventively or as rescue (>P5), produced a statistically significant large effect size (SMD −1.13, 95% CI [–1.58, −0.67], SMD −1.82, 95% CI [–2.61, −1.03] respectively), though with moderate heterogeneity. Similarly, MSC treatment resulted in a statistically significant large effect size at early (≤P14), mid (>P14 to ≤P28), and late assessment (>P28) assessment with SMD −1.08, 95% CI (–1.51, −0.65), SMD −2.24, 95% CI (–3.62, −0.85) and SMD −1.3, 95% CI (–2.1, −0.51), respectively, (Fig. 4). Again, the heterogeneity ranged from moderate to high.

Conditioned Media. Overall conditioned media conferred a large treatment effect size ($p = .02$) on alveolarization [30, 37, 40, 41, 48] (SMD of −2.04, 95% CI [–2.74, −1.33]) compared with controls though with moderate heterogeneity (I^2 58%; Fig. 2B).

Meta-Analysis for Secondary Outcomes

Lung Inflammation. MSC. The treatment effect favored MSCs in reducing seven of the 19 markers of inflammation compared with controls where there was sufficient data to analyze (Supporting Information Fig. 1). While treatment with MSCs resulted in a statistically significant reduction in Alveolar macrophages (SMD −1.90, 95% CI[–2.94, −0.86] I^2 77, IL-1α SMD

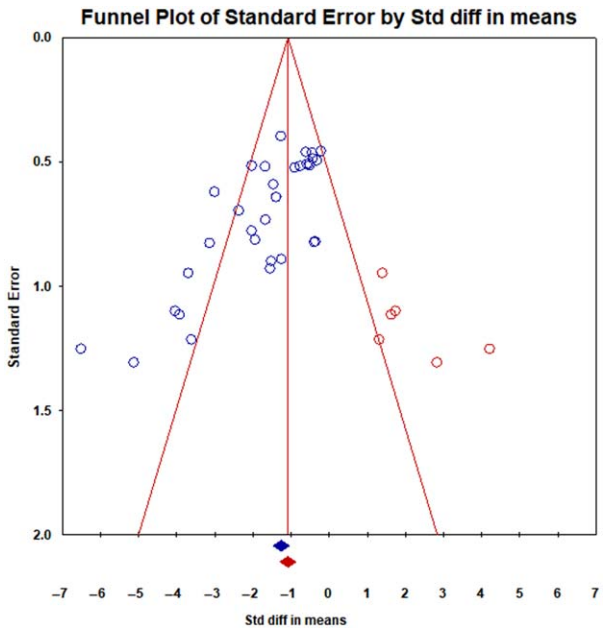


Figure 3. Funnel plot. Blue circles indicate studies included in the meta-analysis for the primary outcome of alveolarization. Red circles suggest potentially missing studies for the same outcome.

−0.88, 95% CI[–1.73, −0.04] I^2 63, IL-1β SMD −3.17, 95% CI[–4.47, −1.87] I^2 84, TNF-α SMD −1.26, 95% CI[–1.94, −0.58] I^2 80, TGF-β SMD −1.55, 95% CI[–2.55, −0.55] I^2 77, IL-6 SMD −2.28, 95% CI[–3.55, −1.02] I^2 85, and myeloperoxidase SMD −2.77, 95% CI [–4.71, −0.84] I^2 82), albeit with moderate to substantial heterogeneity, there was no increase in the anti-inflammatory cytokine IL-10 (SMD 0.51, 95% CI [–0.52, 1.55] I^2 52).

Conditioned Media. Aslam et al. [30] reported suppression of polymorphonuclear cells and macrophages in the bronchoalveolar lavage fluid, lung tissue macrophages, TNF-α, IL-5, and IL-17. Sutsko et al. [41] reported decreased gene expression of pro inflammatory cytokines IL-6 and IL-1β.

Pulmonary Hypertension

MSCs. MSCs improved pulmonary hypertension [30, 41, 47] with a large effect size compared with controls ($p = .02$; SMD −1.57, 95% CI [–2.21, −0.92]) with moderate heterogeneity (I^2 64%) (Supporting Information Fig. 2A).

Conditioned Media. MSC-Conditioned media did not improve pulmonary hypertension ($p = .07$) [30, 37, 40, 41, 48] compared with controls though effect size was large (SMD −0.73, 95% CI [–1.21, −0.26]) with low heterogeneity [I^2 47%]) (Supporting Information Fig. 2B).

Lung Fibrosis

MSCs. MSC reduced lung fibrosis with a large effect size ($p < .01$), SMD −2.55, 95% CI (–3.95, −1.14) compared with controls, with high heterogeneity (I^2 80%) [32–34, 49, 52] (Supporting Information Fig. 3).

Conditioned Media. Hansmann et al. [37] found a 50% decrease in alveolar septal collagen deposition when compared with hyperoxia-exposed/Mouse Lung Fibroblast-conditioned media-treated animals.

Lung Angiogenesis

MSCs. Overall MSCs produced a large effect size ($p = .01$) (SMD −1.55, 95% CI [–1.95, −1.16]) with low heterogeneity (I^2 46%) [28, 29, 31, 39, 41, 47, 50] compared with controls (Supporting Information Fig.4A).

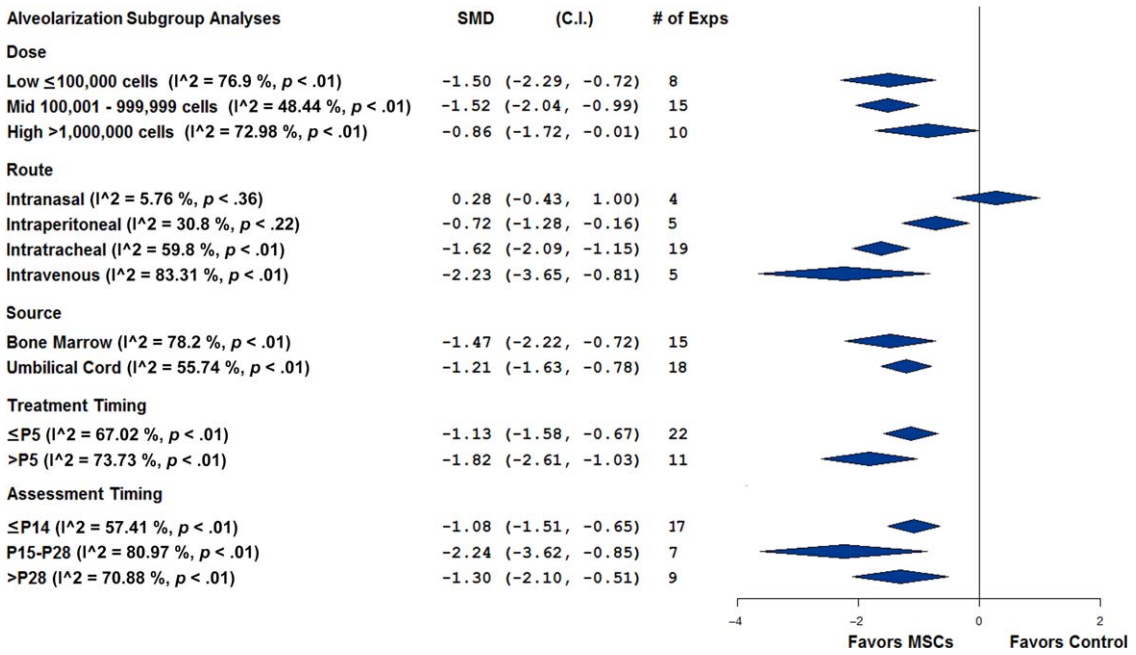


Figure 4. Subgroup analyses of MSCs in animal model of bronchopulmonary dysplasia for the primary outcome of alveolarization. Abbreviations: MSC, mesenchymal stromal cells; SMD, standardized mean difference.

Conditioned Media. MSC-conditioned media caused a large effect size ($p < .01$) on lung angiogenesis [37, 40, 41] (SMD −3.17, 95% CI [–4.72, −1.62]) with high heterogeneity (I^2 83%) compared with controls (Supporting Information Fig. 4B).

Apoptosis

MSCs. MSCs significantly ($p < .01$) reduced apoptosis, (SMD −1.01, 95% CI [–1.79, −0.22]) with high heterogeneity (I^2 71%) [32–34, 50] compared with controls (Supporting Information Fig. 5).

Conditioned Media. We did not find any studies using conditioned media for this outcome.

Pulmonary Artery Remodeling

MSCs. One study [30] found that hyperoxia-induced muscularization of intrapulmonary arterioles decreased significantly with Bone marrow derived MSC treatment compared with the PBS-injected controls.

Conditioned Media. MSC-conditioned media resulted in a large effect size ($p < .01$) on pulmonary artery remodeling [30, 37, 40, 48] (SMD −2.16, 95% CI [–3.98, −0.33]) with high heterogeneity (I^2 87%) compared with controls (Supporting Information Fig. 6).

Lung Function

MSCs. Liu et al. [38] reported a significant dose-dependent effect of intraperitoneal MSC in restoring total lung capacity, inspiratory capacity, compliance, elastance, and area of PV loop while having no effect on airway resistance. In contrast, intranasal MSC had no obvious effect on lung function. Pierro et al. [40] found that MSC prevented the decrease in lung compliance when given at postnatal day 4.

Conditioned Media. Hansmann et al. [37] reported complete reversal of airway hyper responsiveness to inhaled methacholine and restoration of dynamic lung compliance. Pierro et al. [40] reported that conditioned media prevented and restored significant deterioration in lung compliance.

Oxidative Stress

MSC. Three experiments from two studies [32, 33] reported on oxidative stress. Although treatment with MSCs had a nonsignificant effect on oxidative stress (SMD −1.48, 95% CI [–2.29, −0.67]; $p = .72$). (Supporting Information Fig. 7)

Conditioned Media. We did not find any studies using conditioned media for the outcome of oxidative stress.

Exercise Capacity

MSC. Only two studies [40, 47] looked at exercise capacity as a result of use of MSCs in preclinical BPD. Van Haaften et al. [47] found improved exercise tolerance in both Prevention (P4) and Rescue (P14) experiments. However, it was unclear as to when the assessment was done. Pierro et al. [40] found a similar benefit at 6 months when a hyperoxic BPD model was preventively treated with MSC on P4 compared with hyperoxic controls.

Conditioned Media. Pierro et al. [40] reported improved exercise capacity 6 months following treatment with MSC derived conditioned media.

Survival

MSCs. Six studies [28, 32–34, 47, 51] examined the effect of MSCs on survival. Overall, there was a nonsignificant effect ($p = .48$) of MSCs compared with controls (Odd Ratio 0.58, 95% CI[0.36, 0.94]), with very low heterogeneity (I^2 0%) (Supporting Information Fig. 8).

Conditioned Media. We did not find any studies reporting on this outcome.

Safety

Ahn et al. [28] and Pierro et al. [40] were the only studies which reported on safety. While the former examined hypertrophy, tumor, hemorrhage and hematoma by histopathology in brain, heart, lung, liver, and spleen on postnatal day 70, the latter study looked at tumor formation at 6 months by whole body CT scan. Both studies did not report any adverse events.

Discussion

Our systematic review shows that MSCs in preclinical hyperoxic rodent models of BPD resulted in a statistically significant large treatment effect (Cohen's $d \geq 0.8$) for the primary outcome of lung alveolarization and secondary outcomes, including inflammation, pulmonary hypertension, lung fibrosis, apoptosis, and lung angiogenesis. Likewise, MSC-derived conditioned media conferred therapeutic benefit for alveolarization, pulmonary artery remodeling, and angiogenesis.

Internal and External Validity

First, we found potential publication bias for our primary outcome. The Trim and Fill statistical adjustment resulted in a minor reduction in the treatment effect although this effect remained statistically significant and clinically important. Regardless, the high prevalence of publication bias in animal research and inflation of effect sizes is a cause for concern, which could potentially bias conclusions [55]. Furthermore, our analyses revealed that poor reporting was prevalent. None of the 25 studies met the criteria for "low risk of bias." While almost three quarters of the included studies mentioned randomization, there was no attempt made to report the important specifics about sequence generation or allocation concealment. Similarly, the reporting of other fundamental information, such as sample size calculation, was also lacking. This appears to be consistent with other studies in animal research [56, 57] and stem cell research is no exception. In all likelihood, such auxiliary data exist yet remaining unpublished, thereby making it challenging to precisely replicate and validate these experiments and encourage a robust understanding and characterization of evolving stem cell research [58]. Notwithstanding the existence of ARRIVE guidelines on reporting standards, we found many key aspects largely ignored, calling for rigorous enforcement of these guideline [59]. (Supporting Information Table 1; Supporting Information Fig. 9)

Second, we found a moderate to high degree of heterogeneity in our meta-analyses. Although this is not uncommon in animal studies—given their diversity of species, heterogeneous design, intervention protocols, and different outcomes [60]—heterogeneity abounds in animal studies of BPD because of the wide range of oxygen concentration used, variable periods of exposure resulting in differing degrees of severity of the BPD model, compounded by

varying cell dosage, different time points of intervention, assessment and multiple methods of assessment (Supporting Information Table 2). Hence, there is a need for standardization of oxygen concentration and duration of oxygen exposure for induction of lung injury in future preclinical studies. Also, it is our speculation that the heterogeneity between dosages of MSCs could be potentially diminished by standardization of dose by indexing it to the body weight.

Implications for Research

To the best of our knowledge, this is the first systematic review using quantitative methods (i.e., meta-analyses) examining the therapeutic potential of MSCs and MSC-conditioned media in preclinical models of BPD. The study is timely considering the fact that MSCs are currently being tested in numerous clinical trials for their safety and therapeutic potential for regenerative purposes including neonatal diseases in an effort toward evidence generation as mandated by the Food and Drug administration (FDA). However, the value of the promising preclinical data should be treated with caution, because of the historic failure of apparently exciting therapies in animal models to translate from the bench to the clinic, partly related to some short-comings in the design and reporting of preclinical studies [61].

Rodents were the only animal model of BPD found in our systematic review. Clearly rodents are a well-established model, with lungs in the late canalicular/early saccular stage, equivalent to the lung developmental stage of extreme preterm infants, providing excellent insights into lung developmental events [62]. Nonetheless, the disparity between rodent and human physiology remains so great that the direct translation to clinical trials may fall short. It may, therefore, be imperative that other species or newer models that more closely resemble human biology be explored to bridge the translational gap to clinical trials. Larger and arguably better animal models of BPD exist, such as the preterm lamb [63] and nonhuman primate models [64]. We speculate that the high costs and ethical considerations with these models might have precluded them from preclinical studies of MSCs in BPD so far. Indeed, as with studies in stroke, the effect size may diminish as different animal models representing different clinical characteristics and comorbidities are explored [65]. Hence there is a need for multi-species testing in preclinical BPD to ensure that the promising results from the rodent model can be translated to humans before commencing expensive and protracted clinical trials. Large animal models have played a pivotal role in dispelling safety concerns from regulatory agencies and establishing pharmacokinetics and pharmacodynamics with novel therapeutic agents in adult regenerative medicine [66].

Implications for Clinical Trials

MSCs in small animal models have lent a thorough understanding of the therapeutic mechanism in preclinical BPD thereby setting the stage for multiple ongoing early clinical trials. MSCs are potent immune modulatory cells capable of decreasing inflammation in experimental BPD (Supporting Information Figure 1). Even though there was no difference in effect size between timing of intervention, the main mechanism of action of MSCs would imply clinical benefit during the inflammatory phase of BPD, the period when postnatal steroids are being carefully considered around 10 to 21 days of age in current clinical practice. It is difficult to answer this question based on preclinical studies in rodents. Another limitation of this model is the ability to explore whether a single or repeated injections may yield superior benefit. The first phase I trial was designed to administer MSCs after the first week of life [67].

Interestingly, our systematic review suggests a larger effect size of intravenous versus intratracheal administration of MSCs, although this was not adjusted for cell dose and only one study directly compared these two routes of administrations. Since the advent of surfactant and inhaled nitric oxide, neonatologists are comfortable with airway delivery of medications. Local administration may confine the therapeutic effect to the lung and reduce potential adverse effects to other organs. Logistical aspects also need to be taken into account for clinical trial design including the timing of administration and whether an endotracheal tube is still in place at this time. The phase I trial in BPD used the intratracheal route while a phase I trial for Acute Respiratory Distress Syndrome administered MSC intravenously [68] Carefully designed animal studies in other species and models as well as clinical trials will need to address the magnitude of differential therapeutic effects between intravenous versus intratracheal administration of MSCs.

The heterogeneity of effect size in the low and high dose group highlights the need for dose-escalation design in early phase clinical trials [67, 68] to detect the safest effective dosing regimen.

Finally, our systematic review was not able to provide guidance for clinical trial design on a crucial aspect specific to cell-based therapies: the manufacturing process and thus quality of the MSC product [69]. With regard to cell source, perinatal tissue (including placenta, umbilical cord, and cord blood) appears as the clinically more relevant source for the treatment of neonatal diseases and may provide MSCs with greater repair potential than older adult sources (bone marrow, adipose tissue) [70] although this requires more investigations. Preclinical studies analyzed in our systematic review used mostly bone marrow-derived MSCs ($n = 17$) compared with perinatal sources (umbilical cord blood, $n = 6$; Wharton Jelly $n = 2$), and no difference in therapeutic benefit were found. However, knowing that even small variation in the processing methods (enzymatic digestion, plating density, culture media and devices, supplements or growth factors, oxygen concentration, passage number, cryopreservation method, fresh vs. cryopreserved product) may change the efficacy of the final MSC product [48] reporting of these crucial parameters should be mandatory to allow appropriate interpretation of the results to provide useful guidance for clinical trial design.

The strengths of our systematic review include a rigorous peer reviewed search strategy in accordance with the PRESS standards [20] and use of international guidance and standards to conduct our systematic review and meta-analysis. However, our review was also limited by the fact that we did not perform an updated search as we had registered a protocol on CAMARADES website for an expanded review of all cell types [71]. Also, a large number of published data were available only in the form of figures and not in an easily extractable numerical form. Almost all the data were extracted from the figures in the published article using an open source program that can work with a variety of plot types and images. Minor distortion of data is possible but all groups would be equally affected.

Conclusion

In summary, this is the first systematic review of therapeutic MSC animal studies for BPD quantifying the difference in effect for important endpoints. Treatment with MSCs in preclinical

hyperoxic models of BPD in rodents resulted in statistically significant improvement in lung injury. Although this may be true in rodents, there is a need to explore this effect in different animal models and species. Overall, we noted unclear risk of bias and incomplete reporting in the primary studies. This review highlights methodological flaws and other knowledge gaps to guide clinical trial design suggesting a need to implement reporting standards such as the ARRIVE guidelines to bring more rigor in the design of preclinical studies and ultimately ensure timely, safe and effective translation of discoveries into patients.

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Author Contributions

S.A.: conception and design, collection and/or assembly of data, manuscript writing, final approval of manuscript; M.A.: design, collection and/or assembly of data, data analysis and interpretation, final approval of manuscript; B.H., T.L., and M.G.: collection and/or assembly of data, final approval of manuscript; D.M.: critical revisions to the manuscript, final approval of manuscript.; B.T.: conception and design, financial support, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interests

The authors indicated no potential conflicts of interest.

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