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Concise Review

Cell-based therapies in preclinical models of necrotizing enterocolitis: a systematic review and meta-analysis

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Abstract

Necrotizing enterocolitis (NEC) remains an incurable gut complication of prematurity with significant morbidity and mortality. Cell therapies, including mesenchymal stromal cells (MSCs), may be a promising treatment given their anti-inflammatory and regenerative potential. We assessed the effect of MSCs and other cell therapies (not classified as MSCs) on incidence, severity, and mortality in preclinical models of NEC. Bibliographic and gray literature searches yielded 17 371 records with 107 full-text articles assessed and ultimately 16 studies were included. These studies featured only rodents NEC models via combination of hyperosmolar feeds, hypoxia, hypothermia, or lipopolysaccharides. Ten studies used interventions with MSCs. Only 2 met the minimal criteria to define MSCs proposed by the International Society for Cell & Gene Therapy (ISCT). The overall risk of bias was assessed as high partly due to paucity of data with important gaps in reporting, reinforcing the importance of rigorous research framework, appropriate cell-therapy and outcome reporting in preclinical research. A reduction in the incidence of NEC (odds ratio [OR] 0.32, 95% CI [0.17, 0.62]), severe NEC (OR 0.30, 95% CI [0.18, 0.50]), and mortality (OR 0.30, 95% CI [0.16, 0.55]) was noted with MSCs treatment, seemingly more pronounced for ISCT-defined (ISCT$^+$) MSCs. Amniotic fluid stem cells, neural stem cells, and placenta stem cells also showed a reduction in these measures. Given their accessibility (ie, umbilical cord) and proven safety profile in extremely preterm infants, our analysis provides a foundation for considering MSCs as promising candidate that requires further evaluation for the treatment of NEC.

Key words: necrotizing enterocolitis; intestinal injury; umbilical cord; bone marrow; mesenchymal stromal cells; stem cells; rodent models; preclinical model; premature infants.

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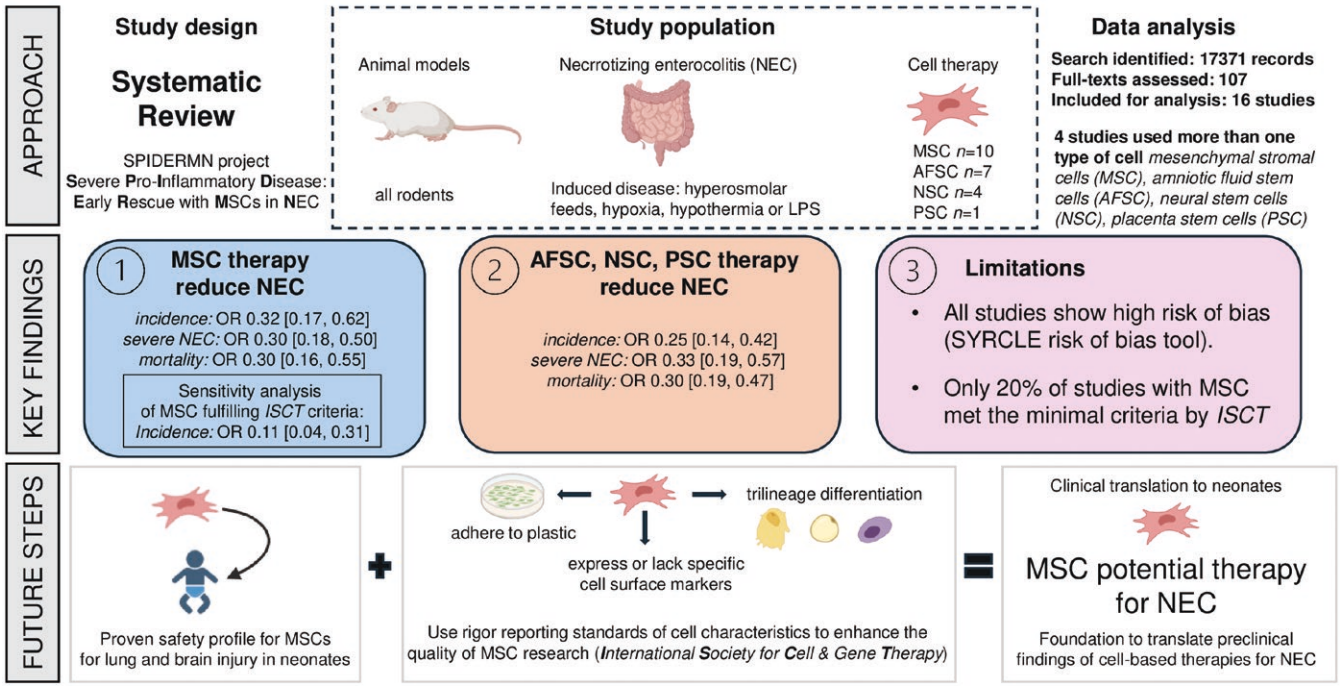
Graphical Abstract

Cell-based therapies in preclinical models of necrotizing enterocolitis: a systematic review and meta-analysis



Significance Statement

Necrotizing enterocolitis (NEC) is a severe disease of the newborn's gut with significant mortality and morbidity. To date, there is no cure. Mesenchymal stromal cells (MSCs) and other cell therapies reduce the incidence and severity of NEC in preclinical models. These results suggest MSCs as a promising candidate that requires further evaluation for its consideration as a treatment of NEC-related morbidity and mortality in preterm infants.

Introduction

Necrotizing enterocolitis (NEC) is a severe inflammatory disease of the newborn's intestine,[1,2] characterized by bowel wall inflammation and necrosis. It leads to consequent bowel perforation and death in some cases.[3] In infants less than 1500 g, 7.6% of infants will develop NEC.[1,4] In susceptible low gestational age, low birth weight infants, its etiology is hypothesized to culminate from host-defense immaturity, inflammation, impaired intestinal barrier integrity, and altered microbiome.[5,6] Long-term adverse outcomes include intestinal failure and neurodevelopmental complications.[7,8]

Management strategies are limited to bowel rest, hemodynamic support, and systemic broad-spectrum-antibiotics.[9,10] When perforation occurs, surgical treatment remains controversial.[11,12] A clear therapeutic gap exists. Mesenchymal stromal cells (MSCs) may be a potential strategy for NEC. MSCs have immunomodulatory effects with repair capacity shown in preclinical models for different neonatal diseases[13-20] including the gut. They appear to protect the intestinal barrier and limit inflammation.[21] Other cells *not classified as MSCs* may also be beneficial. Amniotic fluid stem cells (AFSCs), placenta stem cells (PSCs), and neural stem cells (NSCs) are multipotent cells with varied differentiation capacity with potential to affect inflammatory response or gut function.[22-26]

Given their theoretical effect, early cellular treatment for neonates susceptible to NEC is appealing. However, it is unclear whether there is sufficient preclinical data supporting cell therapies for NEC. Additionally, therapeutic cell dosage, mode, and timing of delivery need further clarification. Thus, we conducted a systematic review (SR) with broad inclusion criteria to evaluate the effects of MSCs and other cell therapies (AFSC, PSC, and NSC) in preclinical models of NEC. We aim to identify whether cell therapies reduce the incidence and mortality of NEC in animal models with the indirect collection of data informing preferable administration modalities.

Methods

Open science statement

The study protocol followed standardized methods for SR including animal intervention studies[27] and reporting (PRISMA-P[28]). The SR was registered using the Open Science Framework[29] a priori (osf.io/5rc6t)[30] of data extraction and reported following PRISMA guidelines.[31]

Inclusion criteria

Studies of all types with the following features:

- Language: English
- Population: animal models of NEC
- Intervention: effects of MSCs or other cell therapies
- Control: placebo/sham/vehicle in animals with NEC
- Outcome: incidence of histological NEC

The vocabulary used in stem cell research is varied and lacks consensus. To ensure consistent nomenclature, we categorized cell therapies from each study based on characteristics provided by the authors (Supplementary Table S1) and (re-)labeled cells used (Supplementary Table S2) adhering to the minimal criteria proposed by the ISCT to define human "multipotent MSCs":

- Adhesion to plastic surfaces under culture conditions
- Expression of the cell surface markers CD105, CD73, and CD90
- Lack of hematopoietic markers CD45, CD34, CD14, or CD11b, CD79 or CD19, and HLA-DR surface molecules expression
- The ability to differentiate into osteoblasts, adipocytes, and chondroblasts[32]

Given that ISCT-defined human MSCs, we used a modified ISCT scheme including plastic adherence, trilineage differentiation, and at least 1 positive (expressed) and 1 negative (lack of) surface marker to label the cells (Supplementary Table S1). They were labeled ISCT^{+} MSCs when criteria were fullfiled and ISCT^{-} MSCs when the criteria were not fullfiled or missing information. AFSC, AFSC-derived NSC, enteric NSC, and PSC were referred to as "other cells." To ensure MSCs were not missed due to mislabeling, all cells' characteristics reported for these cell types were reanalyzed via (Supplementary Table S1) the modified ISCT criteria stated above. Unlike MSCs, there is no minimal criteria for these cell types, hence the inability to ensure their accurate reporting/labeling. We defined "preventive therapy" as given prior to NEC induction or at the time of injury, and "treatment" when given after the induction of NEC.

Exclusion criteria

- Any studies not presenting primary data
- Studies using a non-neonatal model of NEC
- Any therapy with "knock-out," "knock-down," and "loss-of-function" as experimental framework
- Extracellular vesicles, exosomes, condition media, and microvesicles derived from cells (outside of the scope of this study)

Information sources and search strategy

The full search strategy and methods can be found in Supplementary Appendix S1.

Selection process

The records of the search were independently reviewed for eligibility by 2 reviewers (C.M.-B., E.H.) using Distiller Systematic Review (DSR) Software (Evidence Partners Inc., Ottawa, Canada) in 2 stages as per standard SR methodology. Disagreements between the reviewers were resolved by consensus with a third investigator (B.T.).

Data collection

Data collection was performed by the 2 investigators independently using piloted forms in DSR (Supplementary Appendices S2 and S3). Primary data were collected from numerical value published or extrapolated from figures using the online tool Webplotdigitizer (Version 4.5, A. Rohatgi, 2021, Pacifica, CA) independently by both reviewers. Data where insufficient reporting occurred (including *N* number) lead to exclusion from the meta-analysis.

Outcomes

The primary outcome:

- Incidence of NEC based on histological findings (score of grade 2 or higher on the standard histologic scoring system,[33,34] described in Supplementary Appendix S4).

Secondary outcomes:

- Incidence of severe NEC (histology findings of grade 3 or higher)
- Mortality rate
- Clinical sickness score
- Weight change
- Clinical or histological signs of bowel perforation
- Gut permeability and motility
- Engraftment or homing capacity
- Levels of inflammatory markers

Data analysis

Data were analyzed using Review Manager (RevMan) Version 5.4 from the Cochrane Collaboration 2020[35] and Microsoft Excel.[36] We compared NEC animals receiving intervention with cell-based therapies (MSCs or other cells, Table 1) versus no therapeutic intervention (controls—with or without vehicle such as phosphate-buffered saline [PBS], normal saline, or cell medium). Standardized mean difference (SMD) was used for continuous data and odds ratio (OR) for dichotomous data, together with inverse variance and random effects model. Standard error of mean has been converted to SD. When a control group served different treatment groups, we divided the number of control animals with the number of treatment groups, to ensure the same animal was not calculated twice. When 2 control groups were evaluated for the same outcome, we grouped them together to create a single control group using a previously published protocol.[53] Statistical heterogeneity between studies was assessed using the I^2 statistic: very low (0%-25%); low (25%-50%); moderate (50%-75%); and high (>75%)[53] heterogeneity. Effect size was interpreted based on Cohen's *d* as small effect (<0.2), medium effect (0.5), and large effect (>0.8).[54] Publication biases were assessed by visual inspection using a funnel plot when more than 10 studies reported the outcome. Clinical and methodological diversity was explored by prespecified subgroup analysis including animal models, experimental model of NEC, origin of cells, route of administration, dose, and timing of NEC therapy. An additional sensitivity analysis was performed for MSCs fitting the ISCT criteria, which offers a more stringent definition of MSCs.[32]

Risk of bias assessment

The risk of bias was evaluated independently (CMB and EH) using Systematic Review Center for Laboratory Animal Experimentation (SYRCLE)'s risk of bias tool for animal studies[55] (low, high, or unclear risk, Supplementary Appendix S3). Disagreements were resolved by consensus. If the risk of bias of one domain was evaluated as high, the overall risk of bias was judged at high risk for the study.

Protocol deviations

The search was extended to include the analysis of other cell therapies beyond MSCs as well as the additional exclusion for

Table 1. Characteristics of the included studies.

Author (year); Country; Sample size	Type of animal, preterm or term	NEC model	Age (in days) at induction of NEC	Type of cell-based therapy, source	Control group	Comparator group	Dosage, timing, frequency, and route of cell administration	Primary outcome	Outcomes reported and/or extracted
Preventive therapy, prior NEC induction									
Li et al. (2022)[37]; Canada; *N* = 64	C57BL/6 mice, term	Hypercaloric stress, hypoxia, and LPS	5	(1) AFSC, rat (2) BMMSC, rat	Breastfed	NEC and PBS	2×10^6; daily at day 3, 4; IP	Microscopic histology	Microscopic histology, Incidence of NEC, Incidence of severe NEC, Inflammation, Intestinal injury.
Provitera et al. (2023)[38]; Italy; *N* = 95	C57BL/6 mice, term	Hypoxia, hypothermia, and LPS	3	BMMSC, human	Breastfed	NEC and PBS	2 regimens (1) 0.5×10^6; once at day 2; IP (2) 1×10^6; once at day 2; IP	Microscopic histology	Incidence of NEC, Incidence of severe NEC, Inflammation, Survival, Intestinal injury, Weight change.
Preventive therapy, at time of NEC induction									
Drucker et al. (2019)[39]; United States; *N* = 30	C57BL/6 mice, NR	Hypercaloric stress, hypoxia, hypothermia, and LPS	5	UCMSC, human	NEC and administration of saline vehicle	NEC and administration of negative control siRNA USMSCs	80 000 cells/g; once at day 5; IP	Incidence of severe NEC	Microscopic histology, Incidence of NEC, Incidence of severe NEC, Macroscopic changes, Clinical sickness score.
McCulloh et al. I (2017)[40]; United States; *N* = 169	Sprague Dawley rat pups, preterm	Hypercaloric stress, hypoxia, hypothermia, and LPS	0	(1) AFSC, rat (2) BMMSC, rat (3) AFNSC, rat (4) NSC, rat	Breastfed	NEC and PBS	2×10^6; once at day 0; IP	Permeability	Intestinal injury.
McCulloh et al. II (2017)[41]; United States; *N* = 235	Sprague Dawley rat pups, preterm	Hypercaloric stress, hypoxia, hypothermia, and LPS	0	(1) AFSC, rat (2) BMMSC, rat (3) AFNSC, rat (4) NSC, rat	Breastfed	NEC and PBS	2×10^6; once at day 0; IP	Incidence of NEC	Incidence of NEC, Incidence of severe NEC.
Rager et al.[a] (2016)[42]; United States; *N* = 188	Sprague Dawley rat pups, preterm	Hypercaloric stress, hypoxia, and hypothermia	0	(1) BMMSC, mouse (2) BMMSC EV, mouse	Breastfed	NEC	2 regimens (1) For MSC: 3×10^5; once at day 0; IP (2) For EV: 2.5×10^9; once at day 0; IP	Incidence of NEC	Incidence of NEC, Incidence of severe NEC, Intestinal injury.
Wei et al. (2015)[43]; United States; *N* = 278	C57BL/6 mice, NR	Hypercaloric stress, hypoxia, and hypothermia	0	NSC, mouse	Breastfed	NEC	3 regimens (1) For NSC: 20 000 cells; once at day 0; IP (2) For scramble transfected NSC: 20 000 cells in 30 µL HBSS; once at day 0; IP (3) For scramble transfected NSC rat: 50 000 cells in 50 µL HBSS; once at day 0; IP	Incidence of NEC	Microscopic histology, Incidence of NEC, Incidence of severe NEC, Intestinal injury, Engraftment.

Table 1. Continued

Author (year); Country; Sample size	Type of animal, preterm or term	NEC model	Age (in days) at induction of NEC	Type of cell-based therapy, source	Control group	Comparator group	Dosage, timing, frequency, and route of cell administration	Primary outcome	Outcomes reported and/or extracted
Yang et al. (2012)[44]; United States; *N* = 197	Sprague Dawley rat pups, preterm	Hypercaloric stress, hypoxia, and hypothermia	0	BMMSC, mouse	Breastfed	NEC	2 regimens (1) 300×10^3 in 40 µL vehicle; once at day 0; IP (2) 300×10^3 in 40 µL vehicle; once at day 0; IV	Incidence of NEC	Incidence of NEC, Incidence of severe NEC, Macroscopic changes, Survival, Intestinal injury, Engraftment.
Preventive therapy, prior NEC induction and treatment intervention									
Li et al.[a] (2020)[45]; Canada; *N* = 60	C57BL/6 mice, NR	Hypercaloric stress, hypoxia, and LPS	5	(1) AFSC, rat (2) AFSC EV, rat	Breastfed	NEC and PBS	3 regimens (1) For AFSC: 2×10^6; once daily at day 6, 7; IP (2) For EV: 200 µL of media from 2×10^6 AFSC; once at day 6, 7; IP (3) For EV: 200 µL of media from 2×10^6 AFSC; once at day 3, 4; IP	Microscopic histology	Microscopic histology, Inflammation, Intestinal injury.
Treatment intervention									
Lee et al. (2024)[46]; Korea; *N* = 118	ICR mice, term	Hypothermia and LPS	2	BMMSC, mice	Breastfed	NEC and PBS	5 regimens (1) 1×10^5; once daily at day 5; IP (2) 3.3×10^4; once daily at day 5, 6, 7; IP (3) 1×10^6; once daily at day 5; IP (4) 3.3×10^5; once daily at day 5, 6, 7; IP (5) 1×10^5; once at day 5; PO	Incidence of NEC	Incidence of NEC, Inflammation, Intestinal injury.
Li et al. (2021)[47]; Canada; *N* = 30	C57BL/6 mice, NR	Hypercaloric stress, hypoxia, and LPS	5	AFSC, rat	Breastfed	NEC and PBS	2×10^6; daily at day 6, 7; IP	Permeability	Microscopic histology, Intestinal injury.
Tayman et al. (2011)[48]; Turkey; *N* = 36	Sprague Dawley rat pups, NR	Hypercaloric stress, hypoxia, and hypothermia	0	BMMSC, human	Breastfed and administration of MSC	NEC	6×10^5 BMMSC; once at day 3; IP	Survival and clinical sickness score	Microscopic histology, Survival, Clinical sickness score, Weight change, Engraftment.
Weis et al. (2021)[49]; United States; *N* = 33	Sprague Dawley rat pups, term	Hypercaloric stress, hypoxia, and LPS	0	PSC, human	Breastfed and administration of saline vehicle	NEC and administration of saline	2×10^6; daily at day 1, 2; IP	Macroscopic changes	Microscopic histology, Macroscopic changes, Inflammation, Survival, Intestinal injury, Clinical sickness score, Weight change, Engraftment.

Table 1. Continued

Author (year); Country; Sample size	Type of animal, preterm or term	NEC model	Age (in days) at induction of NEC	Type of cell-based therapy, source	Control group	Comparator group	Dosage, timing, frequency, and route of cell administration	Primary outcome	Outcomes reported and/or extracted
Zani et al., I (2014)[50]; United Kingdom; *N* = 221	Sprague Dawley rat pups, NR	Hypercaloric stress, hypoxia, and LPS	0	AFSC, rat	Breastfed	NEC and PBS	2×10^6 in 50 µL PBS; once at day 1; IP	Body weight and presence of ascites	Survival, Clinical sickness score, Weight change.
Zani et al., II[a] (2014)[51]; United Kingdom; *N* = 223	Sprague Dawley rat pups, NR	Hypercaloric stress, hypoxia, and LPS	0	(1) BMMSC, rat (2) AFSC, human (3) AFSC EV, human	Breastfed	NEC and PBS	2 regimens (1) 2×10^6 cells in 50 µL PBS; once at day 1, 2; IP (2) For AFSC EV: 50 µL once at day 1, 2; IP	Survival	Microscopic histology, Macroscopic changes, Inflammation, Survival, Intestinal injury, Clinical sickness score.
Zhou et al. (2013)[52]; United States; *N* = 24	Sprague Dawley rat pups, preterm	Hypercaloric stress, hypoxia, hypothermia, and LPS	0	NSC, mouse	Breastfed	NEC and administration of cell free medium	50 000 cells in 50 µL DMEM/F12; once at day 3; IP	Microscopic histology and cellular engraftment	Microscopic histology, Survival, Intestinal injury.

[a]The study is included with its experiments using cells. The experiments using EVs are excluded for analysis in this systematic review.
Abbreviations: AFNSC: amniotic fluid-derived neuronal stem cells, AFSC: amniotic fluid stem cells, BMMSC: bone marrow-derived mesenchymal stromal cells, DMEM-F12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, EV: extracellular vesicles, ICR: Institute of Cancer Research, IP: intraperitoneal, IV: intravenous, LPS: lipopolysaccharide, MSC: mesenchymal stromal cells, microL: microlitre, NEC: necrotizing enterocolitis, NR: not reported, NSC: neural stem cells, PBS: phosphate-buffered saline, PO: per os, PSC: placental stem cell, siRNA: small interfering ribonucleic acid, UCMSC: umbilical cord-derived mesenchymal stromal cells.

empirical research or noncomparative studies. Authors were not contacted for missing data given the frequent occurrence.

Results

Study characteristics

A total of 17 371 records were identified from bibliographic and gray literature searches. After removing duplicates, 12 489 records were screened, and 107 full-text articles were assessed for eligibility and 16 studies were included[37–52] (Supplementary Figure S1). All studies used rodents, induced NEC with a combination of at least 2 interventions (Supplementary Figure S2) and 3 research groups (based in Canada, USA, UK) produced 13 of the 16 studies (Table 1). Studies assessed therapeutic efficacy of MSCs from bone-marrow or umbilical cord ($N = 10$),[37–42,44,46,48,51] AFSC ($N = 7$),[37,40,41,45,47,50,51] NSC ($N = 4$),[40,41,43,52] and PSC ($N = 1$)[49] (Supplementary Table S2) of which 4 studies included more than one type of cell.[37,40,41,51] One study could include several experiments (each type of cells, route of administration, or dose of cells included as a separate experiment). Logistics of administration such as the timing, frequency, dose, and route of therapy are provided in Table 1.

Risk of bias assessment

The overall judgment was evaluated as high risk of bias for all included studies using the SYRCLE[55] (Supplementary Table S3 and Appendix S3).

Cell therapies reduced NEC incidence and severity

Mesenchymal stromal cell therapy reduced the incidence of NEC (OR 0.32, 95% CI [0.17, 0.62], $I^2 = 49\%$ Figure 1A, Supplementary Figure S3A) and severe NEC (OR 0.30, 95% CI [0.18, 0.50], $I^2 = 0\%$, both outcomes from the same 6 studies, 8 experiments, 502 animals—Supplementary Figure S3C).[37–39,41,42,44] Other cell interventions showed similar findings (OR 0.25, 95% CI [0.14, 0.42], 3 studies, 5 experiments, 346 animals, Supplementary Figures S4 and S5).[37,41,43] To further evaluate aspects of cell therapy processing and administration, we evaluated different subgroups of relevant factors that may impact the efficacy of the cells, such as the therapy (origin of cells, single or multiple doses, timing, route of administration), animal model (species, preterm, or term), and the disease model (timing and number of interventions for NEC induction). All subgroups that were analyzed did not reveal statistical differences among them except if MSCs met the ISCT criteria or not (Figure 1A). The subgroup analysis of other cells was similar (Supplementary Figure S6). Reduction of incidence of NEC for a single injection of any cell type in relation to dose of cells given is shown in Supplementary Figure S7.

Cell therapies reduce mortality, improve mucosal integrity, and decrease inflammation

Mortality was reduced using MSCs therapy (OR 0.30, 95% CI [0.16, 0.55], $I^2 = 24\%$, 4 studies, 6 experiments, 327 animals—Figure 1B, Supplementary Figure S8A)[38,44,48,51] and other cells (AFSC and PSC, OR 0.30, 95% CI [0.19, 0.47], $I^2 = 0\%$, 3 studies, 3 experiments, 448 animals—Supplementary Figure S8B).[49–51] No statistical difference was observed in subgroup analysis performed (Figure 1B). Clinical sickness score, weight change, and macroscopic signs of NEC[38,39,44,48–51] were riddled with incompatible reporting data across the studies, limiting meta-analysis. Individual studies found an amelioration of those outcomes with treatment (UCMSCs,[39] PSCs,[49] AFSCs,[51,50] and BMMSCs[38,44,48]). Twelve studies reported on intestinal injury assessing morphological aspects (apoptosis, villous height, epithelial proliferation, mucosal evaluation), intestinal motility, permeability, inflammation, and regenerative capacity of intestinal stem cells (including engraftment and homing), but only permeability for intestinal injury allowed for appropriate pooling of the results[40,42–44,47,51] (other outcomes lacked rigor, consistent reporting, or paucity of data limiting analysis). Cell therapies restored the intestinal permeability with BMMSC (SMD −1.27, 95% CI [−1.68, -0.86], $I^2 = 0\%$, 3 studies, 4 experiments, 130 animals, Supplementary Figure S9 and Table S4),[40,42,44] AFSC (SMD −0.88, 95% CI [−1.31, −0.46], $I^2 = 0\%$, 3 studies, 3 experiments, 131 animals),[40,47,51] and NSC (SMD −1.10, 95% CI [−1.51, −0.69], $I^2 = 0\%$, 2 studies, 3 experiments, 122 animals—Supplementary Figure S9).[40,43] Therapy with MSCs and other cells had a subjective anti-inflammatory effect, though pooling was not appropriate in the context of different techniques, sample size, and paucity of data (quantitative polymerase chain reaction of interleukin-1b,[38,49] interleukin-6,[37,45] nuclear factor kappa-light-chain-enhancer of activated B cells,[49] tumor necrosis factor-alpha[45,49]), toll-like-4-receptor,[46] or neutrophil-produced myeloperoxidase[51] in intestinal tissue.

ISCT$^+$ MSCs show potentially more robust therapeutic effect compared with ISCT$^-$ MSCs on various NEC measures

Given the poor reporting in MSCs research,[56] it is critical to ensure that the results obtained are pertinent to MSCs' potential for NEC. ISCT provides minimal criteria identifying cells as MSC (see Methods). From the 10 studies using MSCs in this SR, 7 studies did not show trilineage differentiation[37,38,40,41,44,48,51] and 4 lacked sufficient cell surface marker characterization.[37,44,46,51] Only 2 studies using MSCs (UC- and BM-)[39,42] met the ISCT minimal criteria for MSCs (labeled as ISCT$^+$ MSCs, Supplementary Tables S1 and S4). Out of 10 studies using MSCs, 7 studies reported on incidence of NEC of which one study could not be included in the meta-analysis due to insufficient reported number of animals.[46] For the remaining 6 studies, the incidence of NEC appear more prominently reduced for ISCT$^+$ MSCs (OR 0.11, 95% CI [0.04, 0.31], $I^2 = 0\%$, 2 studies, 2 experiments, 160 animals) compared with all MSCs (OR 0.32, 95% CI [0.17, 0.62] $I^2 = 49\%$, 6 studies, 8 experiments, 502 animals—Supplementary Table S4 and Figure S10A and S3A). Severe NEC and intestinal permeability were similarly improved in the ISCT$^+$ MSCs (Supplementary Table S4 and Figure S10B and S3C) compared with ISCT$^-$ MSCs. No studies with ISCT$^+$ MSCs reported on mortality.

DISCUSSION

Cell therapy as a potential candidate for further exploration in NEC

MSCs have been safely used in neonatal clinical trials for bronchopulmonary dysplasia (BPD),[57-62] intraventricular hemorrhage,[63] and hypoxic-ischemic encephalopathy.[64] To date, one clinical trial has been registered using cell therapy for NEC, but the results have not been published (https://clinicaltrials.gov/study/NCT05138276). One known case report showed modest improvement in an NEC patient.[65] We

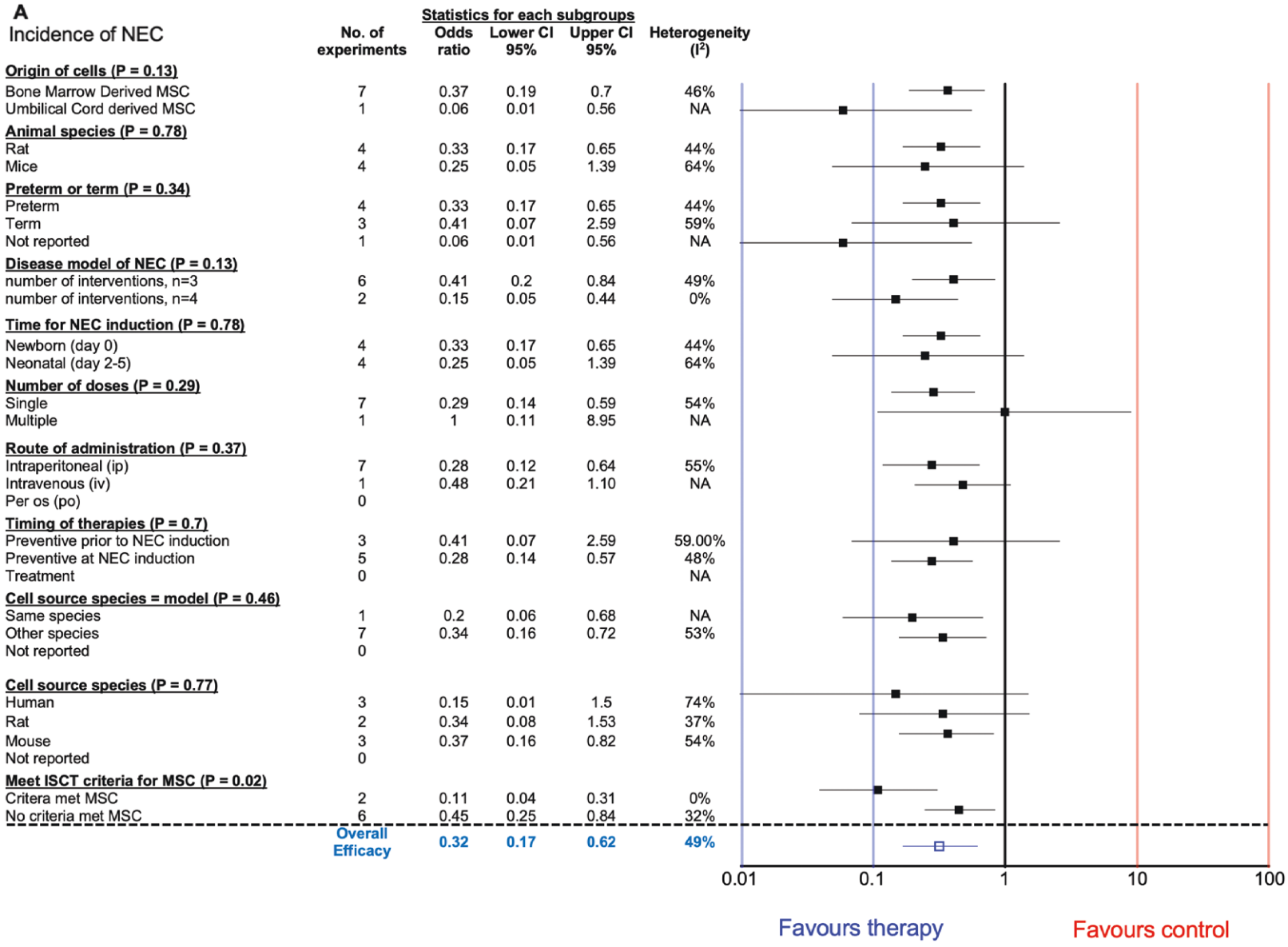


Figure 1. Subgroup analysis of all studies that reported on the outcome incidence of NEC (A) and for mortality (B) for intervention with MSCs. Each row represents the pooled estimate for studies included in each subgroup for 11 different experiment characteristics evaluated (addressing origin of and characteristics of cells, species of animal model and cells, disease model, time for disease induction, therapy characteristics with dose, route of administration, and timing in relation to disease induction). Data are presented as a schematic forest plot with odds ratio and 95% CIs. The statistical heterogeneity within each subgroup is represented as the I^2 value. Effect sizes <1 favors therapy and >1 favors control. The *P*-value indicates if there is any subgroup difference for the studies within that experiment characteristic. "Overall Efficacy" is the pooled estimate effect of therapy for reducing incidence of NEC in the 8 experiments from 6 primary studies (A) and the reduced mortality in 6 experiments from 4 primary studies (B) included in the meta-analysis of these outcomes.

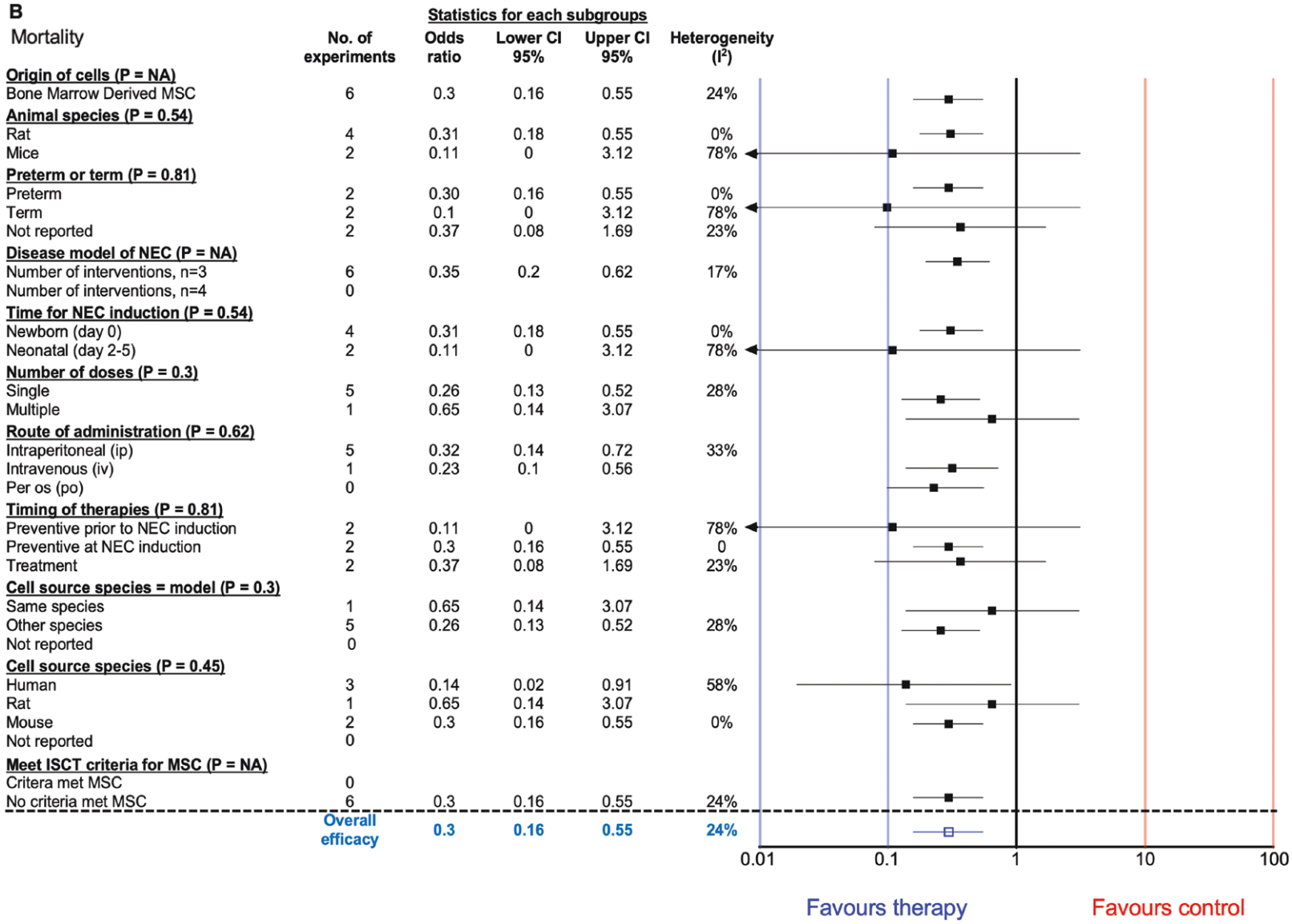


Figure 1. Continued

aimed to identify preclinical evidence to lay foundation for cell therapies, more particularly MSC, in the management of NEC. Our SR led to the analysis of 16 preclinical NEC studies with the hope to fill this gap. MSCs were the most studied cell therapy and all studies used rodents. MSCs, along with other cell therapies, decrease the incidence of NEC, attenuate its severity, and improve survival and intestinal permeability in NEC rodents model. Our findings solidified a previous review of the literature[66] that suggests a similar benefit of cell therapies for NEC. However, our review included 8 additional studies beyond this initial research[66] and relabeled the cell used in individual studies to align with ISCT to ensure rigorous evaluation of MSCs' effect in NEC.

Providing foundational data for MSC delivery in future NEC clinical trials

Aligning with the results of our SR, MSCs are an appealing cell therapy option for NEC given their anti-inflammatory properties and safety profile for preterm infants. The broad inclusion criteria allowed indirect comparisons with other cell types that are not MSCs, but that may still hold promise as potential NEC therapies (AFSC, PSC, and NSC). However, this was limited due to the vast heterogeneity of the studies. Another aim was to identify the optimal cell type, quantity, route, and time of administration to provide foundational data to propel translation of cell therapies for NEC.

Modality of administration

The route of administration tested included oral (PO), intraperitoneal (IP—not commonly used in clinic), and intravenous (IV). Oral route of administration was as effective as IP injection in one included study but did not have sufficient information to be included in the meta-anylsis.[46] Interestingly, IV administration, a clinically favored administration route, showed a trend toward favoring treatment but confidence interval crossed 1.[44] This is a surprising finding given the systemic inflammatory process underlying NEC pathogenesis.

Our results provided a clearer picture for timing and logistics of administration. Reduction of the incidence of NEC was only statistically significant for the single-dose experiments and were more beneficial if given at the time of injury rather than prior to induction of NEC in all cell types. This is consistent with the anti-inflammatory mechanism of action of MSCs once inflammation has been initiated. Regarding timing of administration of therapy in the studies, an overwhelming number of studies that report on the outcome incidence of NEC was performed in a "preventive" manner or "at the time of NEC induction" rather than once the disease is established (Table 1). While early MSCs intervention in the pathogenic course of NEC is a clinically relevant administration timepoint (unlike the preventive option), it is currently very difficult to predict when NEC will occur. Indeed, NEC can come as an acute event, without a clear chance for preventive or "at onset" treatment. No biomarker of NEC is currently widely available/used in the clinical settings.[67,68] As such, studies within our review diverge from current clinical practice. We encourage future studies to include cell administration after NEC has been induced to closely replicate current clinical scenarios.

Dosing and type of cells

The current data prevent strong conclusions about the optimal effect dosing as dose comparisons were only performed in 2 studies. Additionally, studies did not report dosing per weight in most instances but rather provided an absolute number of cells, leading to possible heterogeneity in efficacy. Lee et al.[46] found no difference using 1 million compared with 0.1 million MSCs irrespectively if given as a single dose or divided into 3 repeated doses. This contrasts with Provitera et al.[38] who showed a stronger reduction in the incidence of NEC with 1 million MSCs compared with 0.5 million MSCs (58% compared with 6%, respectively). Similarly, there appears to be a trend toward higher doses leading to lower NEC incidence as visually appreciated in Supplementary Figure S7, with doses ranging from 20 000 to 2 million cells.

All cell therapies reduced the incidence of NEC. Reporting of MSCs studies in the current research landscape has been poor, variable, and often misleading. This culminates in heterogeneity and precarious research foundations undermining advancement.[56] We expected a similar state of reporting in the preclinical studies included. Hence, we purposely re-evaluated the labeling of cell therapies of included studies with a modified standardized definition derived from ISCT[32] (see Methods). This ensured enhanced validity of our conclusions toward MSCs as a potential therapeutic candidate for NEC treatment. Only 2[39,42] out of the 10 studies using MSCs met the ISCT criteria for "multipotent MSCs," while the others failed to report enough information.[37,38,40,41,44,46,48,51] We identified that ISCT^{+} MSCs performed seemingly better than ISCT^{-} MSCs in reducing NEC, thus highlighting the importance of proper reporting to ensure accurate interpretation. Nevertheless, significant heterogeneity was present between studies. We urge authors to use rigorous reporting standards of cell characteristics, culture conditions, viability assay, cell manufacturing process, and functional assay to enhance the quality of MSC research. This conforms with an updated position statement from the ISCT[69] to enhance transparency and rigor in MSC research.

Strengths and limitations

This study has several strengths inherent to the rigorous state-of-the-art methodology with a sensitive peer-reviewed search using the PRESS guidelines,[70] SYRCLE's risk of bias tool,[55] and following PRISMA[31] standards. We also re-evaluated the results based on the latest criteria defining MSCs to ensure up-to-date reporting and validity of our conclusions. Nevertheless, SR synthesis is significantly impacted by the quality and rigor of included studies, compounded by the variability incurred in defining population, treatment (eg, defining MSCs), and outcomes. We found that there were significant differences in the NEC models and the specific interventions used (cell type, timing, route of administration, etc.) among the studies included. While we were able to relabel MSCs as per the ISCT minimal criteria, no such classification system exists for other cell types (AFSC, PSC, and NSC) included in this SR. This complicates comparisons between studies/experiments and may affect the reported results. More importantly, all studies reviewed used rodents, which impacts the strength of the findings without proper reproduction in a large animal model. The overall risk of bias was evaluated as high in all studies. We would also expect a small study bias. To note, a major fraction of extracted data was reported in figures or plots as points, which required our team to provide estimates through previously accepted published methods, thereby leading to potential imprecision. Thus, the

strength of the conclusions is modest due to inherent biases stemming from the primary data and overall low certainty of the evidence synthesized. Caution should be exercised when interpreting these results.

We also appreciate the importance of extracellular vesicles in the therapeutic scheme of MSCs. While we initially intended to include them, their inclusions would have increased the complexity of the manuscript and rendered the conclusions difficult to capture. We aim to evaluate this therapeutic approach in future research endeavors.

Conclusion

In preclinical studies of NEC, MSCs and other cell therapies reduce the incidence, severity, and mortality of NEC and improve intestinal permeability. The ISCT^{+} MSC appeared to have a stronger effect than ISCT^{-} MSC, potentially underlining the importance of appropriate characterization of cells. Our work provides an important synthesis of the preclinical landscape of cell therapies for NEC. It highlights important gaps limiting the strength of our conclusion, namely the importance of rigorous research framework, appropriate cell-therapy, and outcome reporting in preclinical research. Future efforts should incorporate studies with well-characterized cell types, thoroughly designed and in appropriate small and large animal models of NEC with the use of ISCT[32] and the ARRIVE guidelines[71,72] to further increase the quality of proof prior to considering human trials. Indeed, a framework to translate preclinical findings of MSCs into testing therapeutic effect in clinical trials has been well delineated in the context of BPD[73] and recently proposed for NEC.[74] Applying a similar framework enhances the rapidity at which cell-based therapies are considered as a therapeutic approach for NEC and accelerate clinical translation in phase I trials. Altogether, our findings, combined with the published safety and feasibility data on cellular therapies in neonates for lung and brain injury,[57-64,75] suggest MSCs as promising candidate that requires further evaluation for its consideration as a treatment of NEC.

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Authors contributions

Camille Maltais-Bilodeau (Conceptualization, Data curation, Investigation, Formal Analysis, Visualization, Methodology, Writing—original draft, Writing—review & editing), Ewa Henckel (Conceptualization, Data curation, Investigation, Formal Analysis, Visualization, Methodology, Writing—original draft, Writing—review & editing), Marc-Olivier Deguise (Visualization, Writing—review & editing), Flore Lesage (Investigation, Formal Analysis, Visualization, Writing—review & editing), Kelly D. Cobey (Conceptualization, Methodology, Writing—review & editing), Nadera Ahmadzai (Conceptualization, Methodology, Writing—review & editing), Becky Skidmore (Methodology, Data curation, Resources, Writing—review & editing), Emanuela Ferretti (Conceptualization, Supervision, Writing—review & editing), and Bernard Thébaud (Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review & editing)

Registration and protocol

The protocol of this study was registered using the Open Science Framework (osf.io/5rc6t) and a manuscript of the protocol was published as: Maltais-Bilodeau C, Henckel E, Cobey KD, *et al.* Efficacy of mesenchymal stromal cells in preclinical models of necrotizing enterocolitis: a systematic review protocol [version 1; peer review: 1 approved with reservations]. *F1000Research* 2021, 10:1011.[30]

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Conflict of interest

The authors declared no potential conflicts of interests.

Data Availability

The data underlying this article are available in the article and in its online supplementary material

Supplementary material

Supplementary material is available at *Stem Cells Translational Medicine* online.

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