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STUDY PROTOCOL

Efficacy of mesenchymal stromal cells in preclinical models of necrotizing enterocolitis: a systematic review protocol

[version 1; peer review: 2 approved with reservations]

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Abstract

Introduction: Necrotizing enterocolitis is an acute inflammatory disease of the intestine that can lead to necrosis and bowel perforation. It is a severe complication of preterm birth. It's mortality rate is up to 50% and survival after necrotizing enterocolitis leads to long-term complications. The current treatment is supportive and includes bowel rest and decompression and antibiotics. Thus, new treatments are necessary to reduce mortality and morbidity. Mesenchymal stromal cells are known to have anti-inflammatory properties and might be a promising option for treatment. Here we present a protocol for a systematic review with the aim to explore the efficacy of cell therapies with mesenchymal stromal cells in animal models of necrotizing enterocolitis. The primary outcome is histological signs of necrotizing enterocolitis. Additional outcomes include survival, bowel perforation, gut permeability, gut motility, levels of inflammatory markers, cytokine levels and adverse events.

Methods: We will conduct a systematic search of MEDLINE, Embase, and Web of Science databases. The retrieved records will be screened individually by two investigators. We will include all preclinical in vivo animal models of experimentally induced necrotizing enterocolitis that evaluate the efficacy of mesenchymal stromal cells or other cell therapy treatments. Outcome data will be extracted from each article

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and risk of bias assessment performed. Funnel plots and SYRCLE's risk of bias tool for animal studies will be used. Data will be reported as ratios, divided in predefined subgroups where relevant.

Conclusions: This systematic review aims to examine the efficacy of mesenchymal stromal cells in preclinical models of necrotizing enterocolitis and whether there is sufficient evidence to support a clinical trial of efficacy and safety of the treatment with mesenchymal stromal cells in infants with necrotizing enterocolitis.

Keywords

necrotizing enterocolitis, neonatal enterocolitis, mesenchymal stromal cells, stem cells, preclinical, animal models, systematic review protocol

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List of abbreviations

MSC: mesenchymal stromal cell

NEC: necrotizing enterocolitis

Introduction

Necrotizing enterocolitis (NEC) is an acute inflammatory disease of the intestine.^{1,2} It was first described by Mizrahi *et al.* in 1965.³ This disease is characterized by inflammation and injury to the wall barrier of the intestine that can cause necrosis and that can lead to bowel perforation.^{2,4,5} It is a major complication of preterm birth.^{6,7} In Canada, the preterm birth rate is 7.7%⁸ and, on the basis of large databases, the mean prevalence of NEC in Canada and the United States is about 7% among infants with birth weight between 500 g and 1500 g^{1,9,10} with a mortality rate as high as 50% in neonates with a birth weight less than 1000 g.^{11,12} The pathogenesis of NEC is multifactorial.¹³ The most common known risk factors of NEC include low gestational age, low birth weight, exposure to infant formula-feeding and microbial dysbiosis.^{2,4,13,14} The classic NEC pathophysiological process of intestinal injury appears to be related to a basic immaturity of host defences, culminating in an excessive inflammatory response, with serum cytokines and chemokines, especially IL-8 significantly elevated^{15–17} and imbalance of the healthy microbiome.

Survivors of a NEC episode are at increased risk of long-term adverse outcomes including gastrointestinal failure and neurodevelopmental complications.^{4,12} Because the disease occurs during an important time of brain development, the long-term NEC-associated neurodevelopment outcomes include cerebral palsy and visual, cognitive and psychomotor impairment.^{4,18–23} NEC also significantly increases hospital length of stay and health care costs. Infants with NEC needing medical treatment are hospitalized on average 22 days longer in the neonatal intensive care unit than neonates without NEC, and those who require surgical treatment are hospitalized 60 days longer than infants without NEC.²⁴ In the USA, the estimated average cost for medical and surgical treatment of NEC is \$500 000 per patient.^{24,25}

Current treatment strategies depend on the severity of the disease and remain supportive only. They include bowel rest and decompression, fluid support, vasopressors and systemic broad-spectrum-antibiotics.^{4,13,26} Surgical treatment of NEC is recommended if bowel perforation occurs or in some cases of bowel necrosis.¹³ In order to reduce the morbidity and mortality associated with this inflammatory disease, evaluation of new therapies is required. Several treatments have been studied in preclinical trials, including the utilization of mesenchymal stromal cells (MSCs).

MSCs were described in 1976 as fibroblast precursor cells with a role in the regulation of murine hematopoietic stem cells in bone marrow.²⁷ Following this, multiple preclinical studies have shown a repair ability of MSCs in multiple injured organs and tissues of neonatal models such as in the brain,^{28–32} heart³³ lungs^{34,35} gut^{36,37} and eyes.³⁸ MSCs are known to have anti-inflammatory properties³⁹ and considering that inflammation plays a key role in the pathogenesis of NEC, they may represent a promising therapeutic option for this disease. In 2020, a systematic review and meta-analysis of preclinical studies using animal models of NEC with stem cells products was published.⁴⁰ This study suggests a benefit of stem cells in rodent models. However, the research in stem cells is a rapid-growing field in medicine and we believe that an update review with broad inclusion criteria is needed.

Objectives

We aim to conduct a systematic review of the benefits and harms of MSC therapy in preclinical models of NEC. We seek to look for any clinical effect of this therapy in animal models. We will specifically address the question: In controlled preclinical studies of necrotizing enterocolitis, can MSCs reduce the histological signs of NEC in NEC-induced animal models? Our population of interest is all animal studies of NEC and we are interested in any such studies that use MSCs or any other cell therapy as an intervention. We are interested in all comparisons in the literature. Our primary outcome is histology showing NEC. The following additional outcomes will be measured: survival, bowel perforation, gut permeability, gut motility, levels of inflammatory markers, cytokine levels and adverse events.

Protocol

Methods and analysis

This study protocol was developed through discussion between our research team which includes clinical and preclinical research experts (CMB, EH, BT, EF), an information specialist (BS), and research methodologists with expertise in knowledge synthesis (NA, KDC). The protocol was prepared using the PRISMA-P reporting guidelines⁴¹ and the protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies published by de Vries *et al.*⁴² This protocol was registered using the Open Science Framework (osf.io/5rc6t). A completed PRISMA-P checklist for this protocol is provided. Post-hoc modifications to the current protocol incurred during the review process will be documented in the publication of the final report to ensure transparency.

Eligibility criteria

We will include all study designs of research describing preclinical NEC models that evaluate the efficacy of MSCs or other cell therapy treatments that are published in English. Potentially relevant non-English materials will be provided in the appendix section but will not be included in the analysis. We will not limit our eligibility criteria by year of publication or publication status. Details on the study PICOS are provided below.

Population

The population will include all preclinical in vivo animal models of experimentally induced NEC. To enhance the generalizability of our findings, NEC will be defined as any histological intestinal tissue injury. Various mechanisms of NEC have been reported in animal models in the literature.^{43,44} These include gavage formula or hyperosmolar formula gavage feeds, exposure to hypoxia and/or hypothermia and administration of bacteria.

Intervention

The intervention of interest is all MSCs cell types, independent of the source, in animal models. MSCs will be defined by using the International Society for Cellular Therapy consensus statement. The minimal criteria to meet to define human MSC are: (1) adhesion to plastic; (2) expression of the cell surface markers CD105, CD73 and CD90 and lack of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules expression and (3) differentiation to osteoblasts, adipocytes and chondroblasts in vitro.⁴⁵

All types of MSCs will be considered in the review to enhance the generalizability of our study findings. Any therapeutic MSCs regimen (prophylactic – before or during induction of NEC, or rescue after induction of NEC) will be considered.

Comparison

In our population study of experimental animal models of NEC, we will consider all types of reported comparator. We will also consider groups with no comparator.

Outcome

The primary outcome is histological signs of NEC measured at any time after the administration of MSCs. The secondary outcomes measured are: survival, bowel perforation, gut permeability (e.g. disruption of tight junction proteins, liver fatty acid-binding protein level and intestinal fatty acid binding protein level), gut motility, levels of inflammatory markers (e.g. white blood cells count, C-reactive protein level and fecal calprotectin) cytokine levels (e.g., interleukine-8 and toll-like receptor 4) and any describe adverse events.

Study design

We will include any study design in the search including both comparative and non-comparative studies as well as empirical research.

Public involvement

We have identified one parent of a preterm infant who suffered from NEC and its complications who engaged in the design of the project and provided feedback after reading the protocol manuscript. The engagement of this parent will be an important addition to our team for subsequent research leading to clinical trial preparation and conduct.

Information sources

Search strategy

The search strategies will be developed and tested through an iterative process by an experienced medical information specialist in collaboration with the study team members. The MEDLINE strategy will be peer-reviewed by another senior information specialist prior to execution using the Peer Review of Electronic Search Strategies (PRESS) guidelines.⁴⁶ Proposed databases include MEDLINE, Embase, and Web of Science. Searches will utilize a combination of controlled vocabulary (e.g., “Stem cells”, “Mesenchymal Stem Cells”, “Stromal Cells” and “Enterocolitis, Necrotizing”) and keywords (e.g., “MSCs”, “stromal cell”, “neonatal enterocolitis”). Vocabulary and syntax will be adjusted across the databases. To enhance search efficacy, we will use animal filters validated for Pubmed/MEDLINE and Embase,^{42,47,48} amended as appropriate to accommodate new developments in vocabulary. There will be no language or date restrictions on any of the

searches. We will perform a grey literature search of conference websites and animal research organizations' websites to identify literature not included in the aforementioned databases, and also perform general searches using Google Scholar.

Study records

Data management

DistillerSR (Evidence Partners Inc, Ottawa, Canada) software will be used for records and data management throughout the review. The resulting search citations will be deduplicated and then imported into DistillerSR. Study screening and data extraction forms will be created on the platform to facilitate review of citations obtained from the search.

Selection process

Two reviewers, independently and in duplicate, will carry out citation screening in two stages, reviewing records against the a priori eligibility criteria using an online systematic review software program (Distiller Systematic Review (DSR) Software; Evidence Partners Inc, Ottawa, Canada). At Stage 1, the titles and abstracts of all records obtained from the search will be screened in duplicate independently by CMB and EH while Stage 2 screening will encompass full-text review of the articles deemed potentially relevant at Stage 1. If there are disagreements between the reviewers, it will be resolved by a consensus or by a third member of the study team (EF or BT). Both stages of screening will begin with a calibration exercise to ensure consistent application of eligibility criteria between reviewers. This will include pilot screening of titles and 25 citations at Stage 1 and 25 full-text articles at Stage 2. In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,⁴¹ the exclusion reasons of potentially eligible studies will be recorded.

Data collection process

The two independent investigators (CMB and EH) conducting screening and extraction will use standardized forms (See drafts, [Table 1](#)). These forms will be piloted on a set of 10 documents and then discussed. If necessary, changes to the

Table 1. Data collection items.

Data category	Specific items
Study characteristics	Title, name of corresponding author, number of authors, publication date, journal of publication, study design, single vs. multi-centre trial, funding and country of publication of the corresponding author
Study population	Animal species, sex, age at the time of experimentally induced NEC, age at time of intervention, comorbidity, duration of follow-up, inclusion and exclusion criteria, relevant baseline data.
Experimental model of necrotizing enterocolitis	Hypercaloric stress, exposure to hypoxia, exposure to hypothermia or other mechanism.
Intervention	Type of MSCs administered, MSCs's preparation method before administration (e.g. cells allowed or not to recover for a definite period of time before being administered), time of treatment after the diagnosis of NEC, route given and dosage of MSCs used, unit, duration, frequency, strategy of administration, and co-intervention, etc.
Comparison	Type and preparation of control suspension, time of treatment after the diagnosis of NEC, unit, duration, frequency, route of administration, strategy of administration, and co-intervention, etc.
Co-interventions	Fluids, bowel rest, antibiotics, etc.
Preclinical endpoints	Histological description of intestinal tissue injury, survival, evidence of gut perforation, gut permeability (e.g., disruption of tight junction proteins, liver fatty acid-binding protein level and intestinal fatty acid binding protein level), gut motility, level of inflammatory markers (e.g., white blood cells count, C-reaction protein and fecal calprotectin), level of cytokines (e.g., interleukine-8 and toll-like receptor 4) and any reported adverse events.
Risk of bias assessments	In accordance with the elements of the SYRCLE's risk of bias tool for animal studies ⁵⁰ : Random sequence generation, description of baseline animal characteristics, allocation concealment, random animal housing blinding of personnel, blinding of outcome assessment, completeness of outcome data and statement of selective reporting.
Quality of reporting of individual preclinical studies	In accordance with the elements of the ARRIVE guidelines ⁵¹

Note: Included data collection items related to key study items.

forms will be made and they will be re-piloted on 10 further documents. This process will be repeated until 80% agreement or higher is reached between the reviewers. We will contact the first author of the included studies once, if necessary, for any missing data.

Data items

Once all studies are identified, data extraction will be performed using a standardized data extraction form in Microsoft Excel (Microsoft Corporation, Seattle, Washington, USA). We will collect data related to key study items that include but not limited to variables in [Table 1](#) below. If means and measures of dispersion are reported via figures in the primary studies, they will be approximated using online tools such as Webplotdigitizer (A. Rohatgi, 2020, Pacifica, California, USA).

Outcomes and prioritization

The primary outcome is histological signs of NEC measured at any time after the administration of MSCs. The histological signs can be tissue inflammation, bacterial invasion, tissue necrosis and signs of micro-perforation. If applicable, we will use the standard histologic scoring previously described in the literature to assess the severity of histological changes in NEC.^{43,49} Histopathology of the lesions will be categorized in four classes: grade I focal, mild injury confined to villous types, grade II, partial loss of vili, grade III, necrosis extending to submucosa and grade IV, transmural necrosis.

The secondary outcomes measured will be: survival, bowel perforation, gut permeability, gut motility, levels of inflammatory markers, cytokine levels and any reported adverse events. Survival will be measured in number of days between the diagnosis of NEC and unintentional death. Bowel perforation will be defined as any free air in the abdominal cavity on imaging or visualization of a hole in the wall of the intestinal barrier by direct visualization or by histology. Any possible parameters to assess gut permeability (including but not restricted to disruption of tight junction proteins, liver fatty acid-binding protein level and intestinal fatty acid binding protein level) and motility will be considered as a valid measure for these outcomes. Any kind of inflammatory markers (including but not restricted to white blood cells count (cells $\times 10^9/L$), C-reactive protein (mg/L) and fecal calprotectin (mcg/g)) and cytokine levels (including but not restricted to interleukin-8 (pg/mL) and presence of toll-like receptor 4) will be recorded. All reported adverse events will be considered.

Risk of bias in individual studies

Two independent investigators (CMB and EH) will evaluate the risk of bias for each study included in the review. We will use the SYRCLE's risk of bias tool for animal studies.⁵⁰ This tool was created to establish consistency and avoid discrepancies in the assessment of risk of bias in systematic reviews of animal intervention studies. Each item will be assigned a value of low, high or unclear. Elements of the SYRCLE's tool include assessment of method used for the sequence generation, description of baseline animal characteristics, allocation concealment, random animal housing, blinding of outcome assessment, random outcome assessment, completeness of outcome data and statement of selective outcome reporting.

Data synthesis

Descriptive statistics and tables of evidence summary will be reported. Study characteristics will be summarized using frequencies and percentages. As a rule, heterogeneity that may be explained by clinical or methodological differences between studies will preclude any planned meta-analyses. If so, study results will be synthesized narratively. Outcomes with dichotomous endpoints (e.g., survival and presence of intestinal perforation) from each included study will be pooled and described using risk ratio and/or odds ratios and 95% confidence intervals that incorporate a random effect modelling approach. We will use forest plots to visualize the data. Statistical heterogeneity between studies will be quantified with I-squared statistics. Sparse data will not be meta-analyzed but described narratively. Outcomes with continuous endpoints (e.g., gut permeability, gut motility, levels of inflammatory markers and cytokine levels) will be pooled using mean difference or standardized mean difference. Meta-regression with multiple study level covariates will be attempted when there are at least 10 studies in the body of evidence. Otherwise, clinical and methodological diversity in studies will be explored in pre-specified subgroup and sensitivity analyses. We will analyze subgroups for the following: animal models, sex, experimental model of NEC (e.g., hypercaloric stress, exposure to hypoxia or exposure to hypothermia), administration route of MSC (e.g., intravenous or intraperitoneal), dose of MSC, timing of MSC administration after induction of NEC (less or equal to one hour, greater than one hour to less or equal to four hours, more than four hours or multiple doses), other treatment used and control group. To assess the potential meta-bias and publication bias, we will use the funnel plot as a graphical tool. The quality of reporting of individual preclinical studies will be assessed in accordance with the elements of the ARRIVE (Animal Research: Reporting of in vivo Experiments) guidelines.⁵¹

Ethics and dissemination

This study is a systematic review of animal models and there will be no direct interaction with animals or patients. The systematic review is planned to be conducted between March and September 2021. The results of this study will be presented to the scientific community at pertinent national and international meetings. We will also publish the results of the systematic review in peer-reviewed scientific journals. The general public will be informed through various local and national associations and media.

Study status

Our systematic review has been started in March 2021 with the literature search and the screening of the searched abstracts. We are currently working on the quality assessment of the included studies. At the time of writing this protocol, no data collection has been started.

Discussion and conclusions

NEC is an important inflammatory disease that affects infants and the current treatment is supportive. In order to reduce the mortality and the morbidity associated with this disease, there is a need for new treatment targeting the inflammation process and MSCs may be beneficial. To our knowledge, no systematic review of the use of MSCs in preclinical models for NEC has been reported. The data from this review will provide information on whether there is sufficient rigorous evidence to support a clinical trial testing the feasibility and safety of MSC therapy in neonates with NEC. If not, this study will illustrate any gaps in the field which can guide the design of future research studies to address those issues.

Data availability

No data are associated with this article.

Reporting guidelines

Open Science Framework: PRISMA-P checklist for 'Efficacy of mesenchymal stromal cells in preclinical models of necrotizing enterocolitis: a systematic review protocol', <https://doi.org/10.17605/OSF.IO/YJNXP>.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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Maltai-Bilodeau et al propose to carry out a systematic review of the efficacy of mesenchymal stromal cells in preclinical models of necrotizing enterocolitis. While a similar review was recently published in 2020, they suggest that since then further research has been done and there is a need for a more comprehensive review of these promising potential therapies for NEC. The authors state in their objectives that they will focus on studies that utilize MSCs or any other cell therapy as a preclinical intervention in animal models. It is suggested that they change their title to "cell therapies" in preclinical models of NEC to broadly include any cell, or stick with MSCs as they define them.

Overall, the study proposal is very well planned and written, and the methods and outcomes are clear. Several points to address are noted below.

1. It would be helpful to estimate how many new studies could be included under the MSC category in the broader review they are proposing in comparison to the prior 2020 review on stem cell therapies for NEC, or describe more clearly how their review will be different. (See Villamor-Martinez et al 2020)¹.
2. Findings of the previous review are not commented on other than to say the prior meta-analysis suggested a benefit of stem cells. Given that the authors will address one primary outcome (histological) and several secondary outcomes (survival, bowel perforation, gut permeability, etc) in the proposed review, it is suggested that the prior study results in relation to these outcomes be briefly mentioned, or describe why the proposed study may provide further useful perspective than was reviewed in that study.
3. Given that histological scoring is their primary outcome, it might be helpful to include a description of how they will reconcile potentially different scoring schemes used by different authors, or will they exclude authors that do not use the standard histological scoring scheme they indicate on pg 6?
4. Given the many possible ways to experimentally induce NEC as described, do the authors

consider one or more of these better representations of NEC, or will all findings with all models be given equal weight in terms of potential clinical benefit?

5. In their outcomes, I understand they mean fecal calprotectin and interleukin-8. (pg 4 and Table 1 Preclinical endpoints)
6. Table 1 preclinical endpoints - tight junction proteins, C-reactive protein. (spelling)
7. Adverse events are mentioned as a secondary outcome. Are there any potential adverse events from MSCs that are likely to be missed or unreported in NEC preclinical models (for example cancer risks)? Might these risks need further consideration in their literature review? (see Lee and Hong 2017)²

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Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: intestinal inflammation, ER stress, microbiome

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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In this Study Protocol manuscript, the authors describe their proposed systematic review that “aims to examine the efficacy of mesenchymal stromal cells in preclinical [animal] models of necrotizing enterocolitis and whether there is sufficient evidence to support a clinical trial of efficacy and safety of the treatment with mesenchymal stromal cells in infants with necrotizing enterocolitis.”

Overall, the rationale and approaches proposed for the systematic review of MSC use in animal models of so called NEC should provide a comprehensive review of the literature that might provide evidence for justifying potential clinical trials of MSCs for reducing incidence and severity and adverse outcomes of human preterm infant NEC.

- The rationale for, and objectives of, the Systematic Review are clearly stated.
- Sufficient details of the methods and analysis are provided that would allow replication by others.
- The statistical analysis and its interpretation are appropriate.

The conclusions drawn are supported by the results presented in the review, but there are a number of issues that should be further considered and revised, as noted below:

1. The authors justify their systematic review and potential for clinical trials of anti-inflammatory mesenchymal stromal cells to prevent or treat NEC with an average reported prevalence of 7% in North America and a mortality rate of about 50% in more preterm infants with lower birth weight. These rates may be higher than in many centers today, as preventative measures of exclusive mother's milk feeding, including donor milk and human milk-derived fortifiers, are more commonly used, as well as in some centers the addition of prebiotics and probiotics.
2. The authors note that a similar systematic review was published only last year, but argue that the field is rapidly expanding, justifying the need for a new systematic review. It would help to show the authors' evidence for how expanded the literature is in the past year, if it is, from the one published last year, to better justify such a short time between these reviews.
3. Some evidence from the similar systematic review published last year should be included to help justify the potential efficacy of MSCs for the purposes of the proposed review and study. What did this previous review find?
4. The authors note that “To enhance the generalizability of our findings, NEC will be defined as any histological intestinal tissue injury.” This should be better defined and justified, as some of the approaches to inducing so called NEC in animal models might cause gut

injuries that are not specifically characteristic of human preterm infant NEC.

5. The authors also should discuss the strengths but particularly the limitations of the animal models used to produce so called NEC, as they note that “various mechanisms of NEC have been reported in animal models in the literature, including... gavage formula or hyperosmolar formula gavage feeds, exposure to hypoxia and/or hypothermia and administration of bacteria”. Many have criticized these experimental methods as not that relevant to human preterm infant NEC, which clearly is multifactorial in its origins and pathogenesis (which the current authors acknowledge). For example, very few cases of true NEC are caused by hyperosmolar formulas, hypoxia, hypothermia, or inoculation with pathogenic bacteria, even though associated anemia might produce gut enterocyte “hypoxia”, pathogenic bacteria are commonly associated with NEC, and there appears to be a higher incidence of NEC in infants who are fed exclusive or mixed diets of bovine-derived formulas primarily with intact bovine protein.
6. The authors also should consider that many so called cases of NEC have been variably diagnosed, sometimes erroneously, e.g., including early postnatal spontaneous intestinal perforation which is not NEC, and signs of gut inflammation which may or may not be related to true NEC. The animal models therefore may be of less certain and potentially limited value for determining whether mesenchymal stromal cells would be appropriate for clinical trial in human preterm infants to prevent or treat true NEC.
7. There should be some expected number of studies to be considered from the literature for the different times related to NEC noted--prophylactic (before or during induction of NEC), during NEC, or rescue after induction of NEC, and since a previous systematic review was conducted only a year ago, that review might provide some insight into whether there are enough studies in each of these periods.
8. Evidence for whether there might be benefit to MSCs once NEC is established vs. prophylactic use should be considered.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neonatal nutrition and metabolism

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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