Cochrane Library

Cochrane Database of Systematic Reviews

Stem cell-based interventions for the prevention of morbidity and mortality following hypoxic-ischaemic encephalopathy in newborn infants (Protocol)

Bruschettini M, Romantsik O, Moreira A, Ley D, Thébaud B

Bruschettini M, Romantsik O, Moreira A, Ley D, Thébaud B.

Stem cell-based interventions for the prevention of morbidity and mortality following hypoxic-ischaemic encephalopathy in newborn infants.

Cochrane Database of Systematic Reviews 2018, Issue 11. Art. No.: CD013202.

DOI: 10.1002/14651858.CD013202.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

HEADER 1
ABSTRACT 1
BACKGROUND 1
OBJECTIVES 4
METHODS 4
ACKNOWLEDGEMENTS 7
REFERENCES 8
ADDITIONAL TABLES 15
APPENDICES 17
CONTRIBUTIONS OF AUTHORS 19
DECLARATIONS OF INTEREST 19
SOURCES OF SUPPORT 19

[Intervention Protocol]

Stem cell-based interventions for the prevention of morbidity and mortality following hypoxic-ischaemic encephalopathy in newborn infants

Matteo Bruschettini[1,2], Olga Romantsik[1], Alvaro Moreira[3], David Ley[1], Bernard Thébaud[4,5,6]

[1]Department of Paediatrics, Lund University, Skåne University Hospital, Lund, Sweden. [2]Cochrane Sweden, Skåne University Hospital, Lund, Sweden. [3]Pediatrics, Division of Neonatology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA. [4]Department of Pediatrics, Children's Hospital of Eastern Ontario, Ottawa, Canada. [5]Ottawa Hospital Research Institute, Sprott Center for Stem Cell Research, Ottawa, Canada. [6]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada

Contact address: Matteo Bruschettini, Department of Paediatrics, Lund University, Skåne University Hospital, Lund, Sweden. matteo.bruschettini@med.lu.se, matbrus@gmail.com.

Editorial group: Cochrane Neonatal Group.
Publication status and date: New, published in Issue 11, 2018.

Citation: Bruschettini M, Romantsik O, Moreira A, Ley D, Thébaud B. Stem cell-based interventions for the prevention of morbidity and mortality following hypoxic-ischaemic encephalopathy in newborn infants. *Cochrane Database of Systematic Reviews* 2018, Issue 11. Art. No.: CD013202. DOI: 10.1002/14651858.CD013202.

ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To determine the efficacy and safety of stem cell-based interventions for the treatment of hypoxic-ischaemic encephalopathy (HIE) in newborn infants.

BACKGROUND

Description of the condition

Hypoxic-ischaemic encephalopathy (HIE) is a leading cause of mortality and long-term neurological sequelae, affecting thousands of children in low-, middle-, and high-income countries. Severity of HIE is scored in three stages: Stage I (Sarnat 1) - mild, Stage II (Sarnat 2) - moderate, Stage III (Sarnat 3) - severe (Sarnat 1976). Standard management in neonatal intensive care units consists of providing supportive care to maintain cerebral perfusion and metabolic balance. The only effective intervention is therapeutic hypothermia, which reduces mortality and improves neurocognitive outcome of newborns at 18 month of age (Edwards 2010; Jacobs 2013). The lower brain temperature induced by therapeutic hypothermia may decrease brain baseline metabolism and energy demand (Owji 2017; Pfister 2010; Polderman 2008). The mechanisms include reduction of apoptosis, mitochondrial dysfunction, and free radical production; decreased permeability of blood-brain barrier; and mitigation of reperfusion injury and neuroinflammation (Goss 1995; Gunn 1997; Gunn 1998; Haaland 1997; Iwata 2007; Rothwell 1995; Silverstein 1997; Thoresen 1995). However, treatment should to be started during the latent phase of brain injury, which extends up to six hours from the primary brain insult (Gluckman 1992), to avoid reperfusion injury (Gunn 1997; Zhao 1996). Furthermore, the duration of treat-

ment should be long enough to mitigate a secondary phase of neural injury with cytotoxic cell oedema, apoptosis, accumulation of extracellular excitotoxins, and delayed seizures due to reperfusion (Sirimanne 1996; Tan 1996; Williams 1991). Thus, hypothermia should cover the whole reperfusion phase of brain injury and last 72 hours (Wyllie 2015). Additional therapies for HIE are under investigation and include erythropoietin, allopurinol, xenon, topiramate, and magnesium sulphate. These strategies will be described briefly. Erythropoietin has anti-inflammatory, antioxidative, antiapoptotic, and antiexcitotoxic properties (Villa 2003). In addition, it may promote neurogenesis and angiogenesis (Wang 2004). In animal models of HIE, erythropoietin attenuates brain damage, and improves learning memory (Gonzalez 2009; Kumral 2004; Wu 2012). These data seem to be confirmed in small clinical trials (Elmahdy 2010; Rogers 2014; Zhu 2009). Allopurinol may be neuroprotective through direct scavenging of hydroxyl radicals and neutralizing non-protein -bound iron (Peeters-Scholte 2003). Animal studies showed that allopurinol might be neuroprotective (Palmer 1993). Furthermore, its administration to term infants affected by HIE might improve neurocognitive outcome at four to eight years of age (Kaandorp 2012). Xenon has been reported to decrease brain injury in animal studies (David 2008; Ma 2005), with additional efficacy when combined with hypothermia (Hobbs 2008; Thoresen 2009). In one phase II clinical trial, xenon seemed to be safe (Dingley 2014). Topiramate may reduce brain damage, decrease the rate of apoptosis, diminish the infarcted area, and improved neurological outcome in animal models of HIE (Noh 2006; Ozyener 2012; Sfaello 2005). One ongoing trial aims to investigate the effects of topiramate on full-term neonates with HIE undergoing hypothermia (NCT01765218). There are controversies regarding the neuroprotective role of magnesium sulphate in brain damage in term and late preterm infants (Galinsky 2014; Tagin 2013). The data from animal studies show lack of beneficial neuroprotective effect following HIE (de Haan 1997; Greenwood 2000; Penrice 1997). There is an ongoing clinical trial on magnesium sulphate during therapeutic hypothermia in term neonates with HIE (NCT01646619).

Description of the intervention

Mesenchymal stem/stromal cells (MSCs) have emerged as exciting and new therapeutic agents that could potentially ameliorate HIE (Nabetani 2018). MSCs are the most commonly used regenerative cells in clinical trials due to their relatively safe profile, ease of isolation/propagation, ability to reduce inflammation and oxidative stress, restore energy failure, and decrease cell apoptosis (Trounson 2015). The International Society for Cellular Therapy defines cells as MSCs by the following criteria: adhere to plastic in standard culture conditions, express specific surface antigen markers, and have the capacity for multipotent differentiation (Dominici 2006). Although MSCs are ubiquitously used in regenerative studies, additional stem cell-based therapies (collectively referred to as 'regenerative cells') are also under consideration. For instance, mononuclear cells, oligodendrocyte progenitor cells, neural stem cells, hematopoietic stem cells, endothelial cells, and inducible pluripotent stem cells have demonstrated efficacy in animal models of brain injury (Pimentel-Coelho 2012). Furthermore, one ongoing clinical trial is evaluating the efficacy of autologous umbilical cord blood (which contains a combination of MSCs, hematopoietic stem cells, and other mononuclear cells) as a treatment for HIE (NCT02612155). While inducible pluripotent stem cells are typically obtained from skin cells, they can also be retrieved from blood or MSCs to become a specialized cell, such as a neural stem cell or oligodendrocyte (Cai 2010).

Several factors must be taken into account when optimizing regenerative cells. For example, the tissue source, laboratory processing, passage number, dose, frequency, timing, and route of administration are all variables that may affect efficacy (Möbius 2015). The perinatal period offers an opportune time to collect umbilical cord tissue/blood, amniotic fluid or placental tissue (Garcia 2014; Parolini 2014; Sanberg 2014; Taghizadeh 2014). These sources, once considered medical waste, offer a vast supply of regenerative cells that are obtained non-invasively and recognized for their high proliferative rates/differentiation potential, paracrine release of biological factors and their low likelihood of mounting an immune response after transplantation (Batsali 2013; Möbius 2015; Moreira 2017; Parolini 2008). The therapeutic potential of regenerative cells may also be influenced by different laboratory cell processing techniques (e.g. oxygen tension, cell expansion media, passage number, fresh versus cryopreserved cells) (Frey 2006; Kaplan 2017; Parody 2013). Fewer passages is preferred, as multiple passages may impair cell function (Bellayr 2014; Wagner 2008). Interestingly, larger amounts of immature hematopoietic progenitors and MSCs with high proliferative potential have been detected in the cord blood of preterm as compared with term infants (Podesta 2015). In one systematic review of animal models of HIE, neuroprotective dosages of MSCs ranged between 200,000 to 3,500,000 cells, with most of the studies administering a one-time intracerebral/intranasal dose of cells 72 hours or less after injury (Archambault 2017).

Though regenerative cells are characterized by low immunogenicity (Gebler 2012), autologous transplantation is likely to be associated with lower risks for infections and immune rejection. However, allogeneic transplantation may offer significant practical advantages such as rapid availability of disease-free products that are generated in a cost-effective manner (Hare 2017).

How the intervention might work

It is currently unknown which regenerative cell(s) promotes the therapeutic effects in HIE; however, we will present a brief overview of the potential role(s) each cell has after neurological injury (see also Table 1).

Mesenchymal stem cells: the beneficial effects of MSCs occurs at the cellular and functional level. The proposed mechanism of action is through the paracrine release of factors known to improve neurogenesis, such as basic fibroblast growth factor, insulin-like growth factor-1, and anti-inflammatory cytokines (Li 2002; Murphy 2013; Qu 2007; van Velthoven 2009; van Velthoven 2011; van Velthoven 2012). MSCs can also modulate the local immune response by regulating the function of immune cells, such as T-cells and B-cells, macrophages, and dendritic cells (Iyer 2008). Furthermore, in injured brain tissue, MSCs upregulate the expression of genes associated with cell proliferation (e.g. Spp1 and interleukin (IL) 17 (IL-17)), neurogenesis (neural cell adhesion molecule and nerve growth factor), migration (CXCR4), neuronal survival (glial-derived neurotrophic factor), and downregulation of genes involved in inflammation (i.e. IL-1β) (van Velthoven 2011). In addition, MSCs may inhibit apoptosis by transporting mitochondria through tunnelling nanotubules in efforts to rescue aerobic respiration (Liu 2014).

At the functional level, MSC treatment, given within the first 10 days after injury, prevents neuroinflammation and apoptosis (Bonestroo 2015; Donega 2013). The administration of umbilical cord-derived MSCs resulted in nerve fibre remyelination and axonal regeneration, diminished loss of white and grey matter and improved sensorimotor function, and better long-term neurological recovery (Donega 2013; Donega 2015; Liu 2010; Morán 2017).

Mononuclear cells: in a newborn lamb model of HIE, treatment with umbilical cord blood mononuclear cells showed a decrease in neuronal apoptosis and inflammation, along with a trend towards a reduction in seizures (Aridas 2016). Mononuclear cells also reduce motor deficits and cortical brain loss by decreasing CD4+ T-cell and microglial infiltration to the injury site (McDonald 2018).

Oligodendrocyte progenitor cells: administration of oligodendrocyte progenitor cells to a small animal model of cervical spinal injury increased myelination and enhanced motor performance (Manley 2017). Differentiation into myelin-producing cells and expression of brain-derived neurotrophic factor and bcl-2 appear to be mechanisms by which oligodendrocyte progenitor cells encourage learning and memory in neonatal asphyxia (Chen 2015).

Neural stem cells: Mine and colleagues showed that intrastriatal transplantation of neural stem cells slows inflammation (microglia/macrophage activation) and promotes axonal connections (Mine 2013). Moreover, it is posited that neural stem cell therapy inhibits IL-1β expression and improves neural plasticity by upregulating nuclear factor kappa β signalling (Ji 2015).

Hematopoietic stem cells: human umbilical cord 34+ cells given 48-hours after middle cerebral artery occlusion in mice transiently increased cerebral blood flow and blood vessel diameter in the peri-infarct area (Tsuji 2014). These findings can be attributed to the release of growth factors (i.e. vascular endothelial growth factor and glial-derived neurotrophic factor) known to stimulate neurogenesis and angiogenesis (Verina 2013).

Endothelial cells: the neuroprotective effects of endothelial cells are ascribed to their ability in decreasing neuroinflammation and cell apoptosis (Grandvuillemin 2017). Intraperitoneal injection of human umbilical vein endothelial cells preserved microvessels and lessened apoptosis in the cortex of treated animals, via regulation of stromal cell-derived factor 1 and CXC chemokine receptor 4 (Wu 2013).

Inducible pluripotent stem cells: using human skin-derived inducible pluripotent stem cells, Tornero and colleagues produced cortical progenitor cells that survived and differentiated into functional neurons that improved performance of impaired limb movement in a rat stroke model (Tornero 2013). Furthermore, inducible pluripotent stem cell treatment reduced the number of inflammatory cells and glial scar formation in a preclinical study of hemorrhagic stroke (Qin 2015).

Taken together, regenerative cells may exert their therapeutic benefit through multiple routes to establish a favourable environment for tissue regeneration, which ultimately leads to better functional outcomes following hypoxic-ischaemic damage.

Why it is important to do this review

It is important to conduct this review as the use of regenerative cells in neonatology continues to grow. To date, US clinical trials studying the safety, feasibility, efficacy (or a combination of these) of regenerative cells have been registered for bronchopulmonary dysplasia, HIE, hypoplastic left heart syndrome, and intraventricular haemorrhage. The most widespread tissue used to derive regenerative cells is the umbilical cord (Mitsialis 2016; Yoon 2016). While MSCs dominate as cell type, a few trials are evaluating neural progenitor cells, mononuclear cells, and placenta/cord blood cells (NCT02434965; NCT02854579; NCT02999373).

The efficacy and safety of use of regenerative cell administration has been assessed in several systematic reviews and meta-analyses. For instance, the safety of MSCs has been evaluated in a meta-analysis across different disciplines (eight studies including 321 adults): there was no association between acute infusional toxicity, organ system complications, infection, death or malignancy (Lalu 2012). However, the risk of potential tumourigenicity related to MSC-based interventions needs to be further elucidated (Barkholt 2013). The Cochrane review "Stem cell transplantation for ischaemic stroke" included three small trials in adults (Boncoraglio 2010). In newborns, one Cochrane Review has been conducted on MSC for the prevention and treatment of bronchopulmonary dysplasia in preterm infants (Pierro 2017). Early phase trials have been conducted (or are underway) on the use of MSCs or cord blood cells (or both) for bronchopulmonary dysplasia, severe IVH (NCT02274428), and HIE (Chang 2014; Cotten 2014).

Regenerative cells might be an effective intervention for the prevention of morbidity and mortality following HIE, which is one of the most severe morbidities in newborn infants.

OBJECTIVES

To determine the efficacy and safety of stem cell-based interventions for the treatment of hypoxic-ischaemic encephalopathy (HIE) in newborn infants.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomized controlled trials (RCTs), quasi-RCTs, and cluster trials.

Types of participants

- Term infants (37 weeks or greater) and late preterm infants (34+0 to 36+6 weeks' gestation) 10 days of age or less.
- Evidence of peripartum asphyxia, characterized by evidence of neonatal or foetal distress with each enrolled infant satisfying at least one of the following criteria:
    - cord gas or postnatal blood gas (within the first hour of life) with pH 7.0 or less or base deficit 12 mEq/L or greater;
    - Apgar score 5 or less at five minutes;
    - need for mechanical ventilation or resuscitation at 10 minutes of life;
    - with or without evidence of encephalopathy (moderate or severe) according to Sarnat staging (Sarnat 1976):
        - Stage 1 (mild): hyperalertness, hyper-reflexia, dilated pupils, tachycardia, absence of seizures;
        - Stage 2 (moderate): lethargy, hyper-reflexia, miosis, bradycardia, seizures, hypotonia with weak suck and Moro;
        - Stage 3 (severe): stupor, flaccidity, small-to-mid position pupils that react poorly to light, decreased stretch reflexes, hypothermia, and absent Moro.
- No major congenital abnormalities recognizable at birth.

Types of interventions

Comparison 1: stem cell-based interventions (any type) compared to control (placebo or no treatment).
Comparison 2: use of MSCs of type (e.g. number of doses or passages) or source (e.g. autologous versus allogeneic, or bone marrow versus cord) versus MSCs of other type or source.
Comparison 3: use of stem cell-based interventions other than MSCs of type (e.g. mononuclear cells, oligodendrocyte progenitor cells, neural stem cells, hematopoietic stem cells, and inducible pluripotent stem cells) or source (e.g. autologous versus allogeneic, or bone marrow versus cord) versus stem cell-based interventions other than MSCs of other type or source.
Comparison 4: MSCs versus stem cell-based interventions other than MSCs.
We will include all types of transplantation regardless of cell source (bone marrow, cord blood versus Wharton's jelly, placenta, adipose tissue, peripheral blood), type of graft (autologous or allogeneic), and dose. We will exclude stem cell-derived cerebral organoids (Di Lullo 2017).
Characteristic of the interventions and cointerventions (e.g. cooling) are specified in Subgroup analysis and investigation of heterogeneity.

Types of outcome measures

Primary outcomes

- All-cause neonatal mortality (mortality less than 28 days of age).
- Major neurodevelopmental disability: cerebral palsy, developmental delay (Bayley Mental Developmental Index (Bayley 1993; Bayley 2006) or Griffiths Mental Development Scale (Griffiths 1954) assessment greater than two standard deviations (SD) below the mean), intellectual impairment (intelligence quotient (IQ) greater than two SDs below the mean), blindness (vision less than 6/60 in both eyes), or sensorineural deafness requiring amplification (Jacobs 2013). We will separately assess data on children aged 18 to 24 months and aged three to five years.
- Death or major neurodevelopmental disability assessed at 18 to 24 months of age (defined as cerebral palsy, developmental delay (Bayley or Griffith assessment more than two SD below the mean) or intellectual impairment (IQ more than two SD below mean), blindness (vision less than 6/60 in both eyes), or sensorineural deafness requiring amplification) (Bayley 1993; Bayley 2006; Griffiths 1954; Jacobs 2013).

Secondary outcomes

- All-cause mortality prior to first hospital discharge.
- Each component of major neurodevelopmental disability (these components of long-term outcome will be reported for all studies that have evaluated children after 18 months' chronological age; separate analyses will be performed for children aged 18 to 24 months and three to five years):
    - cerebral palsy;
    - developmental delay:
        - Bayley or Griffith assessment more than two SD below the mean;
        - neuromotor development (Bayley Scales of Infant Development - Psychomotor Development Index (BSID PDI)) assessed in survivors;

◇ cognitive development (Bayley Scales of Infant Development - Mental Development Index (BSID MDI)) assessed in survivors;

○ intellectual impairment (IQ more than two SD below mean);

○ blindness (vision less than 6/60 in both eyes);

○ sensorineural deafness requiring amplification.

- Seizures (suspected clinically or identified by electroencephalogram (EEG) or amplitude-integrated electroencephalogram (aEEG)):

○ seizures during neonatal period (after MSC administration);

○ seizures or need for anticonvulsants at follow-up 12 to 14 months of age.

- Cystic periventricular leukomalacia on brain ultrasound in the first month of life.
- Brain magnetic resonance imaging (MRI) abnormalities (yes/no), defined as white matter lesions (i.e. cavitations; Rutherford 2010) and punctate lesions (Cornette 2002); germinal matrix-intraventricular haemorrhage (GM-IVH) (Parodi 2015); or cerebellar haemorrhage (Limperopoulos 2007).
- Duration of hospital stay (days).
- Tumour formation, any type, any location, detected by MRI or computed tomography (to assess the risk of tumourigenicity of donor MSCs).
- Immune-rejection or any serious adverse event (certain, probable or possible according to the World Health Organization probability scale). We will consider post-hoc analyses for any unexpected adverse effects reported by the studies.

Search methods for identification of studies

We will use the criteria and standard methods of Cochrane and Cochrane Neonatal (see the Cochrane Neonatal search strategy for specialized register).We will search for errata or retractions from included studies published in full-text on PubMed (www.ncbi.nlm.nih.gov/pubmed) and report the date this was done within the review.

Electronic searches

We will conduct a comprehensive search including: Cochrane Central Register of Controlled Trials (CENTRAL, current issue) in the Cochrane Library; MEDLINE via PubMed (1996 to current); Embase (1980 to current); and CINAHL (1982 to current) using search terms detailed in Appendix 1 for the full search strategies for each database). We will apply no language restrictions. We will search clinical trials registries for ongoing or recently completed trials (clinicaltrials.gov; the World Health Organization's International Trials Registry and Platform, and the ISRCTN Registry).

Searching other resources

We will review the reference lists of all identified articles for relevant articles not identified in the primary search.

Data collection and analysis

We will use the standard methods of the Cochrane Neonatal Review Group, as described below.

Selection of studies

Two review authors (MB, OR) will independently search for and identify eligible trials that meet the inclusion criteria. We will screen the titles and abstracts to identify potentially relevant citations, and will retrieve the full texts of all potentially relevant articles; we will independently assess the eligibility of studies by filling out eligibility forms designed in accordance with the specified inclusion criteria. We will exclude studies published only in abstract form unless the final results of the trial are reported and all necessary information can be ascertained from the abstract or authors, or both. We will review studies for relevance by assessing study design, types of participants, interventions provided, and outcome measures reported. We will resolve disagreements by discussion and, if necessary, by consultation with a third review author (DL). We will provide details of excluded studies in the 'Characteristics of excluded studies' table, along with reasons for exclusion. We will contact trial authors if details of primary trials are unclear.

Data extraction and management

Two review authors (MB, OR) will independently extract data using a data extraction form integrated with a modified version of the Cochrane Effective Practice and Organisation of Care Group data collection checklist (Cochrane EPOC Group 2017).
We will extract the following characteristics from each included study.

- Administrative details: study author(s); published or unpublished; year of publication; year in which study was conducted; presence of vested interest; details of other relevant papers cited.
- Details of the study: study design; type, duration, and completeness of follow-up (e.g. greater than 80%); country and location of study; informed consent; ethics approval.
- Details of participants: sex, birth weight, gestational age, number of participants.
- Details of interventions: initiation, dose, and duration of MSCs administration; cointervention such as cooling.
- Details of outcomes as mentioned above under Types of outcome measures.

We will resolve disagreements by discussion. We will describe ongoing studies identified by our search, when available, detailing

the primary author, research question(s), methods, and outcome measures, together with an estimate of the reporting date.
Should any queries arise, or in cases for which additional data are required, we will contact study investigators/authors for clarification. Two review authors (MB, OR) will use Cochrane software for data entry (Review Manager 2014). We will replace any standard error of the mean (SEM) by the corresponding SD.

Assessment of risk of bias in included studies

Two review authors (OR, MB) will independently assess the risk of bias (low, high, or unclear) of all included trials using the Cochrane 'Risk of bias' tool for the following domains (Higgins 2017).

- Sequence generation (selection bias).
- Allocation concealment (selection bias).
- Blinding of participants and personnel (performance bias).
- Blinding of outcome assessment (detection bias).
- Incomplete outcome data (attrition bias).
- Selective reporting (reporting bias).
- Any other bias.

We will resolve any disagreements by discussion or by consultation with a third review author (AM). See Appendix 2 for a more detailed description of risk of bias for each domain.

Measures of treatment effect

We will use risk ratios (RRs), risk differences (RDs), numbers needed to treat for an additional beneficial outcome (NNTB) or numbers needed to treat for an additional harmful outcome (NNTH) for categorical variables, and mean differences (MDs) for continuous variables. We will replace any within-group SEM reported in a trial by its corresponding SD using the formula SD = SEM $\times \sqrt{n}$, where n is the number of participants. We will report 95% confidence intervals (CIs) for each statistic.

Unit of analysis issues

We will include all RCTs and quasi-RCTs in which the unit of allocation was the individual infant. If we find any cluster-RCTs, we will adjust analysis for the designed effect using the method stated in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2017).

Dealing with missing data

We will obtain a dropout rate for each study. If we find a significant dropout rate (e.g. greater than 20%), we will contact study author(s) to request additional data. We will perform a sensitivity analysis to evaluate the overall results with and without inclusion of studies with a significant dropout rate. If a study reports outcomes only for participants completing the trial or only for participants who followed the protocol, we will contact study author(s) to ask them to provide additional information to facilitate an intention-to-treat analysis; in instances when this is not possible, we will perform a complete-case analysis.

Assessment of heterogeneity

We will assess clinical heterogeneity by comparing the distribution of important participant factors between trials and trial factors (randomization concealment, blinding of outcome assessment, loss to follow-up, treatment type, cointerventions). We will assess statistical heterogeneity by examining the I^2 statistic (Higgins 2017), a quantity that describes the proportion of variation in point estimates that is due to variability across studies rather than to sampling error.
We will interpret the I^2 statistic as follows, as described by Higgins 2003.

- Less than 25%: no (none) heterogeneity.
- 25% to 49%: low heterogeneity.
- 50% to 74%: moderate heterogeneity.
- 75% or greater: high heterogeneity.

We will consider statistical heterogeneity to be substantial when the I^2 statistic is 50% or greater. In addition, we will employ the Chi^2 test of homogeneity to determine the strength of evidence that heterogeneity is genuine. We will explore clinical variation across studies by comparing the distribution of important participant factors among trials and trial factors (randomization concealment, blinding of outcome assessment, loss to follow-up, treatment types, and cointerventions). We will consider a threshold of P value less than 0.1 as an indicator of whether heterogeneity (genuine variation in effect sizes) is present.

Assessment of reporting biases

We will examine the possibility of within-study selective outcome reporting for each study included in the review. We will search for trial protocols of included trials on electronic sources such as PubMed, ClinicalTrials.gov, and the WHO ICTRP to assess whether outcome reporting seems to be sufficiently complete and transparent. We will investigate publication by using funnel plots if we include 10 or more clinical trials in the systematic review (Egger 1997; Higgins 2017).

Data synthesis

We will perform statistical analyses according to the recommendations of Cochrane Neonatal (neonatal.cochrane.org/en/index.html) using Review Manager 5 (Review Manager 2014). We will analyse all infants randomized on an intention-to-treat basis. We will analyse treatment effects in the individual trials. We will use a fixed-effect model to combine the data. For any meta-analyses, we will synthesize data using RR, RD, NNTB, NNTH,

MD, and 95% CI. We will analyse and interpret individual trials separately when we judge meta-analysis to be inappropriate.

Quality of evidence

We will use the GRADE approach to assess the quality of evidence for the following (clinically relevant) outcomes (Schünemann 2013): 1) All-cause neonatal mortality (mortality less than 28 days of age); 2) Major neurodevelopmental disability: cerebral palsy, developmental delay (Bayley Mental Developmental Index or Griffiths Mental Development Scale assessment greater than two standard deviations (SD) below the mean), intellectual impairment (intelligence quotient (IQ) greater than two SDs below the mean), blindness (vision less than 6/60 in both eyes), or sensorineural deafness requiring amplification; 3) Death or major neurodevelopmental disability assessed at 18 to 24 months of age (defined as cerebral palsy, developmental delay (Bayley or Griffith assessment more than two SD below the mean) or intellectual impairment (IQ more than two SD below mean), blindness (vision less than 6/60 in both eyes), or sensorineural deafness requiring amplification; 4) All-cause mortality prior to first hospital discharge; 5) cognitive development (Bayley Scales of Infant Development - Mental Development Index (BSID MDI)) assessed in survivors; 6) seizures or need for anticonvulsants at follow-up 12 to 14 months of age; 7) Immune-rejection or any serious adverse event (certain, probable or possible according to the World Health Organization probability scale).

Two review authors will independently assess the quality of the evidence for each of the seven ? ? ? ? ? outcomes above. We will consider evidence from RCTs as high quality but downgrade the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and presence of publication bias. We will use the GRADEpro GDT Guideline Development Tool to create a 'Summary of findings' table to report the quality of the evidence.

The GRADE approach results in an assessment of the quality of a body of evidence in one of four grades.

- High: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- Low: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
- Very low: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Subgroup analysis and investigation of heterogeneity

We will perform the following subgroup analyses.

For MSCs trials:

- gestational age: term infants (37 weeks or greater), late preterm infants (34 to 36+6 weeks' gestation);
- HIE severity stage: mild, moderate, and severe (Sarnat);
- chronological age: less than three days, three days or greater;
- cointervention: with/without cooling;
- MSCs source: bone marrow, cord blood versus Wharton's jelly, placenta, adipose tissue, peripheral blood;
- type of graft: autologous or allogeneic;
- preconditioned (yes, no);
- fresh or frozen and thawed;
- MSCs dose: less than 2×10^7/kg; 2×10^7/kg or greater;
- number of doses: multiple or single administration;
- passage number (i.e. removing cells from a culture flask and plating them into more culture flasks, see Description of the intervention): less than three; three to six; greater than six.

For other cell-based interventions:

- gestational age: term infants (37 weeks or greater), late preterm infants (34 to 36+6 weeks' gestation);
- HIE severity stage: mild, moderate and severe (Sarnat);
- chronological age: less than three days, three days or greater;
- cointervention: with/without cooling;
- cell source: bone marrow, cord blood, peripheral blood, placenta;
- type of graft: autologous or allogeneic;
- fresh or frozen and thawed;
- number of doses: multiple or single administration.

Sensitivity analysis

We will conduct sensitivity analyses to explore the effect of the methodological quality of trials, checking to ascertain whether studies with a high risk of bias will overestimate the effect of treatment. Differences in study design of included trials might affect the results of the systematic review. We will perform a sensitivity analysis to compare the effects of MSCs in truly randomized trials as opposed to quasi-randomized trials.

ACKNOWLEDGEMENTS

We thank Colleen Ovelman, Jennifer Spano, and Roger Soll for the editorial support.

The methods section of this protocol is based on a standard template used by Cochrane Neonatal.

REFERENCES

Additional references

Ahn 2016

Ahn SY, Chang YS, Park WS. Stem cells for neonatal brain disorders. *Neonatology* 2016;**109**(4):377–83. [PUBMED: 27251746]

Archambault 2017

Archambault J, Moreira A, McDaniel D, Winter L, Sun L, Hornsby P. Therapeutic potential of mesenchymal stromal cells for hypoxic ischemic encephalopathy: a systematic review and meta-analysis of preclinical studies. *PloS One* 2017;**12**(12):e0189895. [PUBMED: 29261798]

Aridas 2016

Aridas JD, McDonald CA, Paton MC, Yawno T, Sutherland AE, Nitsos I, et al. Cord blood mononuclear cells prevent neuronal apoptosis in response to perinatal asphyxia in the newborn lamb. *Journal of Physiology* 2016;**594**(5):1421–35.

Barkholt 2013

Barkholt L, Flory E, Jekerle V, Lucas-Samuel S, Ahnert P, Bisset L, et al. Risk of tumorigenicity in mesenchymal stromal cell-based therapies - bridging scientific observations and regulatory viewpoints. *Cytotherapy* 2013;**15**(7):753–9. [PUBMED: 23602595]

Batsali 2013

Batsali AK, Kastrinaki MC, Papadaki HA, Pontikoglou C. Mesenchymal stem cells derived from Wharton's Jelly of the umbilical cord: biological properties and emerging clinical applications. *Current Stem Cell Research & Therapy* 2013;**8**(2):144–55. [PUBMED: 23279098]

Bayley 1993

Bayley N. *Bayley Scales of Infant Development*. 2nd Edition. San Antonio (TX): The Psychological Corporation, 1993.

Bayley 2006

Bayley N. *Bayley Scales of Infant and Toddler Development*. San Antonio (TX): Harcourt Assessment, 2006.

Bellayr 2014

Bellayr IH, Catalano JG, Lababidi S, Yang AX, Lo Surdo JL, Bauer SR, et al. Gene markers of cellular aging in human multipotent stromal cells in culture. *Stem Cell Research & Therapy* 2014;**5**(2):59. DOI: 10.1186/scrt448; PUBMED: 24780490

Boncoraglio 2010

Boncoraglio GB, Bersano A, Candelise L, Reynolds BA, Parati EA. Stem cell transplantation for ischemic stroke. *Cochrane Database of Systematic Reviews* 2010, Issue 9. DOI: 10.1002/14651858.CD007231.pub2

Bonestroo 2015

Bonestroo HJ, Heijnen CJ, Groenendaal F, van Bel F, Nijboer CH. Development of cerebral gray and white matter injury and cerebral inflammation over time after inflammatory perinatal asphyxia. *Developmental Neuroscience* 2015;**37**(1):78–94. DOI: 10.1159/000368770; PUBMED: 25634435

Boshuizen 2018

Boshuizen MC, Steinberg GK. Stem cell-based immunomodulation after stroke: effects on brain repair processes. *Stroke* 2018;**49**(6):1563–70.

Cai 2010

Cai J, Li W, Su H, Qin D, Yang J, Zhu F, et al. Generation of human induced pluripotent stem cells from umbilical cord matrix and amniotic membrane mesenchymal cells. *Journal of Biological Chemistry* 2010;**285**(15):11227–34.

Chang 2014

Chang YS, Ahn SY, Yoo HS, Sung SI, Choi SJ, Oh WI, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. *Journal of Pediatrics* 2014;**164**(5):966–72.e6. DOI: 10.1016/j.jpeds.2013.12.011; ClinicalTrials.gov NCT01297205; PUBMED: 24508444

Chen 2015

Chen LX, Ma SM, Zhang P, Fan ZC, Xiong M, Cheng GQ, et al. Neuroprotective effects of oligodendrocyte progenitor cell transplantation in premature rat brain following hypoxic-ischemic injury. *PloS One* 2015;**10**(3):e0115997.

Chopp 2002

Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurology* 2002;**1**(2):92–100. [PUBMED: 12849513]

Cochrane EPOC Group 2017

Cochrane Effective Practice, Organisation of Care (EPOC). Screening, data extraction and management. EPOC Resources for Review Authors, 2017. epoc.cochrane.org/epoc-specific-resources-review-authors (accessed prior to 20 September 2018).

Cornette 2002

Cornette LG, Tanner SF, Ramenghi LA, Miall LS, Childs AM, Arthur RJ, et al. Magnetic resonance imaging of the infant brain: anatomical characteristics and clinical significance of punctate lesions. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2002;**86**(3):F171–7. [PUBMED: 11978747]

Cotten 2014

Cotten CM, Murtha AP, Goldberg RN, Grotegut CA, Smith PB, Goldstein RF, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. *Journal of Pediatrics* 2014;**164**(5):973–9.e1.

Daadi 2016

Daadi MM, Klausner JQ, Bajar B, Goshen I, Lee-Messer C, Lee SY, et al. Optogenetic stimulation of neural grafts enhances neurotransmission and downregulates the inflammatory response in experimental stroke model. *Cell Transplantation* 2016;**25**(7):1371–80. [PUBMED: 26132738]

David 2008

David HN, Haelewyn B, Rouillon C, Lecoq M, Chazalviel L, Apiou G, et al. Neuroprotective effects of xenon: a

therapeutic window of opportunity in rats subjected to transient cerebral ischemia. *FASEB Journal* 2008;**22**(4): 1275–86. [PUBMED: 18024836]

de Haan 1997
de Haan HH, Gunn AJ, Williams CE, Heymann MA, Gluckman PD. Magnesium sulfate therapy during asphyxia in near-term fetal lambs does not compromise the fetus but does not reduce cerebral injury. *American Journal of Obstetrics and Gynecology* 1997;**176**(1 Pt 1):18–27. [PUBMED: 9024083]

Di Lullo 2017
Di Lullo E, Kriegstein AR. The use of brain organoids to investigate neural development and disease. *Nature Reviews. Neuroscience* 2017;**18**(10):573–84. [PUBMED: 28878372]

Dingley 2014
Dingley J, Tooley J, Liu X, Scull-Brown E, Elstad M, Chakkarapani E, et al. Xenon ventilation during therapeutic hypothermia in neonatal encephalopathy: a feasibility study. *Pediatrics* 2014;**133**(5):809–18. [PUBMED: 24777219]

Dominici 2006
Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;**8**(4):315–7. DOI: 10.1080/14653240600855905; PUBMED: 16923606

Donega 2013
Donega V, van Velthoven CT, Nijboer CH, van Bel F, Kas MJ, Kavelaars A, et al. Intranasal mesenchymal stem cell treatment for neonatal brain damage: long-term cognitive and sensorimotor improvement. *PloS One* 2013;**8**(1): e51253. DOI: 10.1371/journal.pone.0051253; PUBMED: 23300948

Donega 2015
Donegal V, Nijboer CH, van Velthoven CT, Youssef SA, de Bruin A, van Bel F, et al. Assessment of long-term safety and efficacy of intranasal mesenchymal stem cell treatment for neonatal brain injury in the mouse. *Pediatric Research* 2015; **78**(5):520–6. DOI: 10.1038/pr.2015.145; PUBMED: 26270577

Edwards 2010
Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, et al. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ (Clinical Research Ed.)* 2010;**340**: c363. [PUBMED: 20144981]

Egger 1997
Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**(7109):629–34. [PUBMED: 9310563]

Elmahdy 2010
Elmahdy H, El-Mashad AR, El-Bahrawy H, El-Gohary T, El-Barbary A, Aly H. Human recombinant erythropoietin in asphyxia neonatorum: pilot trial. *Pediatrics* 2010;**125**(5): e1135–42. [PUBMED: 20385632]

Fan 2005
Fan CG, Zhang QJ, Tang FW, Han ZB, Wang GS, Han ZC. Human umbilical cord blood cells express neurotrophic factors. *Neuroscience letters* 2005;**380**(3): 322–5. [PUBMED: 15862910]

Frey 2006
Frey NV, Lazarus HM, Goldstein SC. Has allogeneic stem cell cryopreservation been given the 'cold shoulder'? An analysis of the pros and cons of using frozen versus fresh stem cell products in allogeneic stem cell transplantation. *Bone Marrow Transplantation* 2006;**38**(6):399–405. [PUBMED: 16892075]

Galinsky 2014
Galinsky R, Bennet L, Groenendaal F, Lear CA, Tan S, van Bel F, et al. Magnesium is not consistently neuroprotective for perinatal hypoxia-ischemia in term-equivalent models in preclinical studies: a systematic review. *Developmental Neuroscience* 2014;**36**(2):73–82. [PUBMED: 24854050]

Garcia 2014
Garcia O, Warburton D. Amniotic fluid stem cell therapy for lung disease. In: Atala A, Murphy S editor(s). *Perinatal Stem Cells*. New York (NY): Springer, 2014:59–66. DOI: 10.1007/978-1-4939-1118-9_6

Gebler 2012
Gebler A, Zabel O, Seliger B. The immunomodulatory capacity of mesenchymal stem cells. *Trends in Molecular Medicine* 2012;**18**(2):128–34. DOI: 10.1016/ j.molmed.2011.10.004; PUBMED: 22118960

Gluckman 1992
Gluckman PD, Williams CE. When and why do brain cells die?. *Developmental Medicine and Child Neurology* 1992;**34** (11):1010–4. [PUBMED: 1358734]

Gonzalez 2009
Gonzalez FF, Abel R, Almli CR, Mu D, Wendland M, Ferriero DM. Erythropoietin sustains cognitive function and brain volume after neonatal stroke. *Developmental Neuroscience* 2009;**31**(5):403–11. [PUBMED: 19672069]

Gopagondanahalli 2016
Gopagondanahalli KR, Li J, Fahey MC, Hunt RW, Jenkin G, Miller SL, et al. Preterm hypoxic-ischemic encephalopathy. *Frontiers in Pediatrics* 2016;**4**:114. [PUBMED: 27812521]

Goss 1995
Goss JR, Styren SD, Miller PD, Kochanek PM, Palmer AM, Marion DW, et al. Hypothermia attenuates the normal increase in interleukin 1 beta RNA and nerve growth factor following traumatic brain injury in the rat. *Journal of Neurotrauma* 1995;**12**(2):159–67. [PUBMED: 7629862]

GRADEpro GDT [Computer program]
McMaster University (developed by Evidence Prime). GRADEpro GDT. Version accessed 09 January 2018. Hamilton (ON): McMaster University (developed by Evidence Prime), 2015.

Grandvuillemin 2017
Grandvuillemin I, Garrigue P, Ramdani A, Boubred F, Simeoni U, Dignat-George F, et al. Long-term recovery

after endothelial colony-forming cells or human umbilical cord blood cells administration in a rat model of neonatal hypoxic-ischemic encephalopathy. *Stem Cells Translational Medicine* 2017;**6**(11):1987–96. [PUBMED: 28980775]

Greenwood 2000

Greenwood K, Cox P, Mehmet H, Penrice J, Amess PN, Cady EB, et al. Magnesium sulfate treatment after transient hypoxia-ischemia in the newborn piglet does not protect against cerebral damage. *Pediatric Research* 2000;**48**(3): 346–50. [PUBMED: 10960501]

Griffiths 1954

Griffiths R. *The Abilities of Babies: a Study in Mental Measurement*. New York (NY): McGraw-Hill Book Co. Inc, 1954.

Gunn 1997

Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *Journal of Clinical Investigation* 1997;**99**(2):248–56. [PUBMED: 9005993]

Gunn 1998

Gunn AJ, Gunn TR. The 'pharmacology' of neuronal rescue with cerebral hypothermia. *Early Human Development* 1998;**53**(1):19–35. [PUBMED: 10193924]

Haaland 1997

Haaland K, Loberg EM, Steen PA, Thoresen M. Posthypoxic hypothermia in newborn piglets. *Pediatric Research* 1997; **41**(4 Pt 1):505–12. [PUBMED: 9098852]

Hare 2017

Hare JM, DiFede DL, Rieger AC, Florea V, Landin AM, El-Khorazaty J, et al. Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM trial. *Journal of the American College of Cardiology* 2017;**69**(5):526–37. [PUBMED: 27856208]

Higgins 2003

Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**(7414):557–60. DOI: 10.1136/bmj.327.7414.557; PUBMED: 12958120

Higgins 2017

Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.2.0 (updated June 2017). The Cochrane Collaboration, 2017. Available from training.cochrane.org/handbook (accessed prior to 20 September 2018).

Hobbs 2008

Hobbs C, Thoresen M, Tucker A, Aquilina K, Chakkarapani E, Dingley J. Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. *Stroke* 2008;**39**(4):1307–13. [PUBMED: 18309163]

Hsu 2016

Hsu YC, Wu YT, Yu TH, Wei YH. Mitochondria in mesenchymal stem cell biology and cell therapy: from cellular differentiation to mitochondrial transfer. *Seminars in Cell & Developmental Biology* 2016;**52**:119–31. [PUBMED: 26868759]

Huang 2018

Huang L, Zhang L. Neural stem cell therapies and hypoxic-ischemic brain injury. Progress in Neurobiology 2018 May 21 Epub ahead of print]. DOI: 10.1016/ j.pneurobio.2018.05.004; PUBMED: 29758244

Islam 2012

Islam MN, Das SR, Emin MT, Wei M, Sun L, Westphalen K, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine* 2012;**18**(5):759–65.

Iwata 2007

Iwata O, Iwata S, Thornton JS, De Vita E, Bainbridge A, Herbert L, et al. "Therapeutic time window" duration decreases with increasing severity of cerebral hypoxia-ischaemia under normothermia and delayed hypothermia in newborn piglets. *Brain Research* 2007;**1154**:173–80. [PUBMED: 17475224]

Iyer 2008

Iyer SS, Rojas M. Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. *Expert Opinion on Biological Therapy* 2008;**8**(5):569–81. DOI: 10.1517/14712598.8.5.569; PUBMED: 18407762

Jacobs 2013

Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database of Systematic Reviews* 2013, Issue 1. DOI: 10.1002/14651858.CD003311.pub3

Ji 2015

Ji G, Liu M, Zhao XF, Liu XY, Guo QL, Guan ZF, et al. NF-kappaB signaling is involved in the effects of intranasally engrafted human neural stem cells on neurofunctional improvements in neonatal rat hypoxic-ischemic encephalopathy. *CNS Neuroscience & Therapeutics* 2015;**21**(12):926–35. [PUBMED: 26255634]

Kaandorp 2012

Kaandorp JJ, van Bel F, Veen S, Derks JB, Groenendaal F, Rijken M, et al. Long-term neuroprotective effects of allopurinol after moderate perinatal asphyxia: follow-up of two randomised controlled trials. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2012;**97**(3):F162–6. DOI: 10.1136/archdischild-2011-300356; PUBMED: 22102633

Kaplan 2017

Kaplan A, Sackett K, Sumstad D, Kadidlo D, McKenna DH. Impact of starting material (fresh versus cryopreserved marrow) on mesenchymal stem cell culture. *Transfusion* 2017;**57**(9):2216–9. [PUBMED: 28653392]

Kidani 2016

Kidani Y, Miki Y, Nomimura N, Minakawa S, Tanaka N, Miyoshi H, et al. The therapeutic effect of CD133(+) cells derived from human umbilical cord blood on neonatal mouse hypoxic-ischemic encephalopathy model. *Life Sciences* 2016;**157**:108–15. [PUBMED: 27287679]

Kim 2018
Kim TK, Park D, Ban YH, Cha Y, An ES, Choi J, et al. Improvement by human oligodendrocyte progenitor cells of neurobehavioral disorders in an experimental model of neonatal periventricular leukomalacia. *Cell Transplantation* 2018;**27**(7):1168–77. [PUBMED: 29978719]

Kumral 2004
Kumral A, Uysal N, Tugyan K, Sonmez A, Yilmaz O, Gokmen N, et al. Erythropoietin improves long-term spatial memory deficits and brain injury following neonatal hypoxia-ischemia in rats. *Behavioural Brain Research* 2004; **153**(1):77–86. [PUBMED: 15219709]

Lalu 2012
Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PloS One* 2012;**7**(10): e47559. DOI: 10.1371/journal.pone.0047559; PUBMED: 23133515

Li 2002
Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology* 2002;**59** (4):514–23. [PUBMED: 12196642]

Limperopoulos 2007
Limperopoulos C, Bassan H, Gauvreau K, Robertson RL Jr, Sullivan NR, Benson CB, et al. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors?. *Pediatrics* 2007;**120**(3):584–93. DOI: 10.1542/ peds.2007-1041; PUBMED: 17766532

Liu 2010
Liu AM, Lu G, Tsang KS, Li G, Wu Y, Huang ZS, et al. Umbilical cord-derived mesenchymal stem cells with forced expression of hepatocyte growth factor enhance remyelination and functional recovery in a rat intracerebral hemorrhage model. *Neurosurgery* 2010;**67**(2):357-65; discussion 365-6. DOI: 10.1227/ 01.NEU.0000371983.06278.B3; PUBMED: 20644422

Liu 2014
Liu K, Ji K, Guo L, Wu W, Lu H, Shan P, et al. Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. *Microvascular Research* 2014;**92**:10–8. DOI: 10.1016/j.mvr.2014.01.008; PUBMED: 24486322

Ma 2005
Ma D, Hossain M, Chow A, Arshad M, Battson RM, Sanders RD, et al. Xenon and hypothermia combine to provide neuroprotection from neonatal asphyxia. *Annals of Neurology* 2005;**58**(2):182–93. [PUBMED: 16049939]

Manley 2017
Manley NC, Priest CA, Denham J, Wirth ED 3rd, Lebkowski JS. Human embryonic stem cell-derived oligodendrocyte progenitor cells: preclinical efficacy and safety in cervical spinal cord injury. *Stem Cells Translational Medicine* 2017;**6**(10):1917–29. [PUBMED: 28834391]

McDonald 2018
McDonald CA, Penny TR, Paton MC, Sutherland AE, Nekkanti L, Yawno T, et al. Effects of umbilical cord blood cells, and subtypes, to reduce neuroinflammation following perinatal hypoxic-ischemic brain injury. *Journal of Neuroinflammation* 2018;**15**(1):47. [PUBMED: 29454374]

Mine 2013
Mine Y, Tatarishvili J, Oki K, Monni E, Kokaia Z, Lindvall O. Grafted human neural stem cells enhance several steps of endogenous neurogenesis and improve behavioral recovery after middle cerebral artery occlusion in rats. *Neurobiology of Disease* 2013;**52**:191–203. [PUBMED: 23276704]

Mitsialis 2016
Mitsialis SA, Kourembanas S. Stem cell-based therapies for the newborn lung and brain: possibilities and challenges. *Seminars in Perinatology* 2016;**40**(3):138–51.

Moreira 2017
Moreira A, Alayli Y, Balgi S, Winter C, Kahlenberg S, Mustafa S, et al. Upcycling umbilical cords: bridging regenerative medicine with neonatology. *Journal of Maternal-fetal & Neonatal Medicine* 2017:1–10. [PUBMED: 29132234]

Morán 2017
Morán J, Stokowska A, Walker FR, Mallard C, Hagberg H, Pekna M. Intranasal C3a treatment ameliorates cognitive impairment in a mouse model of neonatal hypoxic-ischemic brain injury. *Experimental Neurology* 2017;**290**:74–84. DOI: 10.1016/j.expneurol.2017.01.001; PUBMED: 28062175

Murphy 2013
Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Experimental & Molecular Medicine* 2013;**45**:e54. DOI: 10.1038/emm.2013.94; PUBMED: 24232253

Möbius 2015
Möbius MA, Thebaud B. Stem cells and their mediators - next generation therapy for bronchopulmonary dysplasia. *Frontiers in Medicine* 2015;**2**:50. [PUBMED: 26284246]

Nabetani 2018
Nabetani M, Shintaku H, Hamazaki T. Future perspectives of cell therapy for neonatal hypoxic-ischemic encephalopathy. *Pediatric Research* 2018;**83**(1-2):356–63. [PUBMED: 29016557]

NCT01646619
NCT01646619. Efficacy study of hypothermia plus magnesium sulphate (MgSO4) in the management of term and near term babies with hypoxic ischemic encephalopathy (MagCool). clinicaltrials.gov/ct2/show/NCT01646619 Date first received: 20 July 2012.

NCT01765218
NCT01765218. Topiramate in neonates receiving whole body cooling for hypoxic ischemic encephalopathy.

clinicaltrials.gov/ct2/show/NCT01765218 Date first received: 10 January 2013.

NCT02274428

NCT02274428. Phase 1 clinical trial of PNEUMOSTEM® treatment in premature infants with intraventricular hemorrhage. clinicaltrials.gov/ct2/show/NCT02274428 Date first received: 2 October 2014.

NCT02434965

NCT02434965. Autologous cord blood and human placental derived stem cells in neonates with severe hypoxic-ischemic encephalopathy (HPDSC+HIE). clinicaltrials.gov/ct2/show/NCT02434965 Date first received: 21 June 2018.

NCT02612155

NCT02612155. A multi-site study of autologous cord blood cells for hypoxic ischemic encephalopathy (HIE). clinicaltrials.gov/ct2/show/NCT0261215 Date first received: 23 November 2015.

NCT02854579

NCT02854579. Neural progenitor cell and paracrine factors to treat hypoxic ischemic encephalopathy. clinicaltrials.gov/ct2/show/NCT02854579 Date first received: 3 August 2016.

NCT02999373

NCT02999373. Prevention of preterm infection by autologous umbilical cord blood mononuclear cells therapy. clinicaltrials.gov/ct2/show/NCT02999373 Date first received: 21 December 2016.

Niimi 2018

Niimi Y, Levison SW. Pediatric brain repair from endogenous neural stem cells of the subventricular zone. *Pediatric Research* 2018;**83**(1-2):385–96. [PUBMED: 29028220]

Noh 2006

Noh MR, Kim SK, Sun W, Park SK, Choi HC, Lim JH, et al. Neuroprotective effect of topiramate on hypoxic ischemic brain injury in neonatal rats. *Experimental Neurology* 2006;**201**(2):470–8. [PUBMED: 16884714]

Oki 2012

Oki K, Tatarishvili J, Wood J, Koch P, Wattananit S, Mine Y, et al. Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. *Stem Cells (Dayton, Ohio)* 2012;**30**(6):1120–33. [PUBMED: 22495829]

Owji 2017

Owji ZP, Gilbert G, Saint-Martin C, Wintermark P. Brain temperature is increased during the first days of life in asphyxiated newborns: developing brain injury despite hypothermia treatment. *AJNR. American Journal of Neuroradiology* 2017;**38**(11):2180–6. [PUBMED: 28860214]

Ozyener 2012

Ozyener F, Cetinkaya M, Alkan T, Goren B, Kafa IM, Kurt MA, et al. Neuroprotective effects of melatonin administered alone or in combination with topiramate in neonatal hypoxic-ischemic rat model. *Restorative Neurology and Neuroscience* 2012;**30**(5):435–44. [PUBMED: 22751353]

Palmer 1993

Palmer C, Towfighi J, Roberts RL, Heitjan DF. Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. *Pediatric Research* 1993;**33**(4 Pt 1):405–11. [PUBMED: 8479823]

Park 2016

Park WS, Sung SI, Ahn SY, Sung DK, Im GH, Yoo HS, et al. Optimal timing of mesenchymal stem cell therapy for neonatal intraventricular hemorrhage. *Cell Transplantation* 2016;**25**(6):1131–44. DOI: 10.3727/096368915X689640; PUBMED: 26440762

Parodi 2015

Parodi A, Morana G, Severino MS, Malova M, Natalizia AR, Sannia A, et al. Low-grade intraventricular hemorrhage: is ultrasound good enough?. *Journal of Maternal-fetal & Neonatal Medicine* 2015;**28**(Suppl 1):2261–4. DOI: 10.3109/14767058.2013.796162; PUBMED: 23968243

Parody 2013

Parody R, Caballero D, Marquez-Malaver FJ, Vazquez L, Saldana R, Madrigal MD, et al. To freeze or not to freeze peripheral blood stem cells prior to allogeneic transplantation from matched related donors. *European Journal of Haematology* 2013;**91**(5):448–55. [PUBMED: 23710624]

Parolini 2008

Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring HJ, Evangelista M, et al. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international workshop on placenta derived stem cells. *Stem Cells* 2008;**26**(2):300–11.

Parolini 2014

Parolini O, De D, Rodrigues M, Caruso M. Placental stem/progenitor cells: isolation and characterization. *Perinatal Stem Cells*. New York (NY): Springer, 2014:141–57. DOI: 10.1007/978-1-4939-1118-9_13

Peeters-Scholte 2003

Peeters-Scholte C, Braun K, Koster J, Kops N, Blomgren K, Buonocore G, et al. Effects of allopurinol and deferoxamine on reperfusion injury of the brain in newborn piglets after neonatal hypoxia-ischemia. *Pediatric Research* 2003;**54**(4):516–22. [PUBMED: 12815112]

Penrice 1997

Penrice J, Amess PN, Punwani S, Wylezinska M, Tyszczuk L, D'Souza P, et al. Magnesium sulfate after transient hypoxia-ischemia fails to prevent delayed cerebral energy failure in the newborn piglet. *Pediatric Research* 1997;**41**(3):443–7. [PUBMED: 9078550]

Pfister 2010

Pfister RH, Soll RF. Hypothermia for the treatment of infants with hypoxic-ischemic encephalopathy. *Journal of Perinatology* 2010;**30 Suppl**:S82–7. [PUBMED: 20877413]

Pierro 2017
Pierro M, Thebaud B, Soll R. Mesenchymal stem cells for the prevention and treatment of bronchopulmonary dysplasia in preterm infants. *Cochrane Database of Systematic Reviews* 2017, Issue 11. DOI: 10.1002/14651858.CD011932.pub2; PUBMED: 29125893

Pimentel-Coelho 2012
Pimentel-Coelho PM, Rosado-de-Castro PH, da Fonseca LM, Mendez-Otero R. Umbilical cord blood mononuclear cell transplantation for neonatal hypoxic-ischemic encephalopathy. *Pediatric Research* 2012;**71**(4 Pt 2):464–73. [PUBMED: 22430382]

Pluchino 2013
Pluchino S, Peruzzotti-Jametti L. Rewiring the ischaemic brain with human-induced pluripotent stem cell-derived cortical neurons. *Brain* 2013;**136**(Pt 12):3525–7. [PUBMED: 24335051]

Podesta 2015
Podesta M, Bruschettini M, Cossu C, Sabatini F, Dagnino M, Romantsik O, et al. Preterm cord blood contains a higher proportion of immature hematopoietic progenitors compared to term samples. *PloS One* 2015;**10**(9):e0138680. DOI: 10.1371/journal.pone.0138680; PUBMED: 26417990

Polderman 2008
Polderman KH. Induced hypothermia and fever control for prevention and treatment of neurological injuries. *Lancet* 2008;**371**(9628):1955–69. [PUBMED: 18539227]

Qin 2015
Qin J, Ma X, Qi H, Song B, Wang Y, Wen X, et al. Transplantation of induced pluripotent stem cells alleviates cerebral inflammation and neural damage in hemorrhagic stroke. *PloS One* 2015;**10**(6):e0129881. [PUBMED: 26086994]

Qu 2007
Qu R, Li Y, Gao Q, Shen L, Zhang J, Liu Z, et al. Neurotrophic and growth factor gene expression profiling of mouse bone marrow stromal cells induced by ischemic brain extracts. *Neuropathology* 2007;**27**(4):355–63. [PUBMED: 17899689]

Review Manager 2014 [Computer program]
Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager 5 (RevMan 5). Version 5.3. Copenhagen: Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Rogers 2014
Rogers EE, Bonifacio SL, Glass HC, Juul SE, Chang T, Mayock DE, et al. Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. *Pediatric Neurology* 2014;**51**(5):657–62. [PUBMED: 25439577]

Rothwell 1995
Rothwell NJ, Strijbos PJ. Cytokines in neurodegeneration and repair. *International Journal of Developmental Neuroscience* 1995;**13**(3-4):179–85. [PUBMED: 7572274]

Rowe 2010
Rowe DD, Leonardo CC, Hall AA, Shahaduzzaman MD, Collier LA, Willing AE, et al. Cord blood administration induces oligodendrocyte survival through alterations in gene expression. *Brain Research* 2010;**1366**:172–88. [PUBMED: 20883670]

Rutherford 2010
Rutherford MA, Supramaniam V, Ederies A, Chew A, Bassi L, Groppo M, et al. Magnetic resonance imaging of white matter diseases of prematurity. *Neuroradiology* 2010;**52**(6):505–21. DOI: 10.1007/s00234-010-0700-y; PUBMED: 20422407

Sanberg 2014
Sanberg PR, Eve DJ, Borlongan CV. Umbilical cord blood cells in the repair of central nervous system diseases. In: Atala A, Murphy S editor(s). *Perinatal Stem Cells*. New York (NY): Springer, 2014:269–87. DOI: 10.1007/978-1-4939-1118-9_25

Sarnat 1976
Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Archives of Neurology* 1976;**33**(10):696–705. [PUBMED: 987769]

Schwarting 2008
Schwarting S, Litwak S, Hao W, Bahr M, Weise J, Neumann H. Hematopoietic stem cells reduce postischemic inflammation and ameliorate ischemic brain injury. *Stroke* 2008;**39**(10):2867–75. [PUBMED: 18658037]

Schünemann 2013
Schünemann H, Broż ek J, Guyatt G, Oxman A, editor (s). Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach (updated October 2013). GRADE Working Group, 2013. Available from gdt.guidelinedevelopment.org/app/handbook/handbook.html.

Sfaello 2005
Sfaello I, Baud O, Arzimanoglou A, Gressens P. Topiramate prevents excitotoxic damage in the newborn rodent brain. *Neurobiology of Disease* 2005;**20**(3):837–48. [PUBMED: 16009561]

Silverstein 1997
Silverstein FS, Barks JD, Hagan P, Liu XH, Ivacko J, Szaflarski J. Cytokines and perinatal brain injury. *Neurochemistry International* 1997;**30**(4-5):375–83. [PUBMED: 9106251]

Sirimanne 1996
Sirimanne ES, Blumberg RM, Bossano D, Gunning M, Edwards AD, Gluckman PD, et al. The effect of prolonged modification of cerebral temperature on outcome after hypoxic-ischemic brain injury in the infant rat. *Pediatric Research* 1996;**39**(4 Pt 1):591–7. [PUBMED: 8848330]

Taghizadeh 2014
Taghizadeh RR, Holzer PW, Marino T, Cetrulo KJ, Cetrulo CL, Cetrulo CL. Towards clinical applications of umbilical cord derived mesenchymal stem cells. In: Atala A, Murphy

S editor(s). *Perinatal Stem Cells*. New York (NY): Springer, 2014:347–59. DOI: 10.1007/978-1-4939-1118-9_31

Tagin 2013
Tagin M, Shah PS, Lee KS. Magnesium for newborns with hypoxic-ischemic encephalopathy: a systematic review and meta-analysis. *Journal of Perinatology* 2013;**33**(9):663–9. [PUBMED: 23743671]

Tan 1996
Tan WK, Williams CE, During MJ, Mallard CE, Gunning MI, Gunn AJ, et al. Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischemic injury in late gestation fetal sheep. *Pediatric Research* 1996; **39**(5):791–7. [PUBMED: 8726230]

Thoresen 1995
Thoresen M, Penrice J, Lorek A, Cady EB, Wylezinska M, Kirkbride V, et al. Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatric Research* 1995;**37**(5): 667–70. [PUBMED: 7603788]

Thoresen 2009
Thoresen M, Hobbs CE, Wood T, Chakkarapani E, Dingley J. Cooling combined with immediate or delayed xenon inhalation provides equivalent long-term neuroprotection after neonatal hypoxia-ischemia. *Journal of Cerebral Blood Flow and Metabolism* 2009;**29**(4):707–14. [PUBMED: 19142190]

Tornero 2013
Tornero D, Wattananit S, Gronning Madsen M, Koch P, Wood J, Tatarishvili J, et al. Human induced pluripotent stem cell-derived cortical neurons integrate in stroke-injured cortex and improve functional recovery. *Brain* 2013;**136**(Pt 12):3561–77. [PUBMED: 24148272]

Trounson 2015
Trounson A, Mcdonald C. Stem cell therapies in clinical trials: progress and challenges. *Stem Cell* 2015;**17**:11–22.

Tsuji 2014
Tsuji M, Taguchi A, Ohshima M, Kasahara Y, Sato Y, Tsuda H, et al. Effects of intravenous administration of umbilical cord blood CD34(+) cells in a mouse model of neonatal stroke. *Neuroscience* 2014;**263**:148–58. [PUBMED: 24444827]

van Velthoven 2009
van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Regeneration of the ischemic brain by engineered stem cells: fuelling endogenous repair processes. *Brain Research Reviews* 2009;**61**(1):1–13. [10.1016/j.brainresrev.2009.03.003; PUBMED: 19348860]

van Velthoven 2011
van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Mesenchymal stem cell transplantation changes the gene expression profile of the neonatal ischemic brain. *Brain Behavioral Immunology* 2011;**25**(7):1342–8. DOI: 10.1016/j.bbi.2011.03.021; PUBMED: 21473911

van Velthoven 2012
van Velthoven CT, Kavelaars A, Heijnen CJ. Mesenchymal stem cells as a treatment for neonatal ischemic brain damage. *Pediatric Research* 2012;**71**(4 Pt 2):474–81. DOI: 10.1038/pr.2011.64; PUBMED: 22430383

Verina 2013
Verina T, Fatemi A, Johnston MV, Comi AM. Pluripotent possibilities: human umbilical cord blood cell treatment after neonatal brain injury. *Pediatric Neurology* 2013;**48**(5): 346–54. [PUBMED: 23583051]

Villa 2003
Villa P, Bigini P, Mennini T, Agnello D, Laragione T, Cagnotto A, et al. Erythropoietin selectively attenuates cytokine production and inflammation in cerebral ischemia by targeting neuronal apoptosis. *Journal of Experimental Medicine* 2003;**198**(6):971–5. [PUBMED: 12975460]

Wagner 2008
Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PloS One* 2008;**3**(5):e2213. DOI: 10.1371/journal.pone.0002213; PUBMED: 18493317

Wang 2004
Wang L, Zhang Z, Wang Y, Zhang R, Chopp M. Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke* 2004;**35**(7):1732–7. [PUBMED: 15178821]

Wang 2013
Wang XL, Zhao YS, Hu MY, Sun YQ, Chen YX, Bi XH. Umbilical cord blood cells regulate endogenous neural stem cell proliferation via hedgehog signaling in hypoxic ischemic neonatal rats. *Brain Research* 2013;**1518**:26–35. [PUBMED: 23632377]

Wang 2016
Wang J, Chen Y, Yang Y, Xiao X, Chen S, Zhang C, et al. Endothelial progenitor cells and neural progenitor cells synergistically protect cerebral endothelial cells from Hypoxia/reoxygenation-induced injury via activating the PI3K/Akt pathway. *Molecular Brain* 2016;**9**:12. [PUBMED: 26842559]

Williams 1991
Williams CE, Gunn A, Gluckman PD. Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. *Stroke* 1991;**22**(4): 516–21. [PUBMED: 2024281]

Wu 2012
Wu YW, Bauer LA, Ballard RA, Ferriero DM, Glidden DV, Mayock DE, et al. Erythropoietin for neuroprotection in neonatal encephalopathy: safety and pharmacokinetics. *Pediatrics* 2012;**130**(4):683–91. [PUBMED: 23008465]

Wu 2013
Wu CC, Chen YC, Chang YC, Wang LW, Lin YC, Chiang YL, et al. Human umbilical vein endothelial cells protect against hypoxic-ischemic damage in neonatal brain via stromal cell-derived factor 1/C-X-C chemokine receptor type 4. *Stroke* 2013;**44**(5):1402–9. [PUBMED: 23449265]

Wyllie 2015
Wyllie J, Perlman JM, Kattwinkel J, Wyckoff MH, Aziz K, Guinsburg R, et al. Part 7: neonatal resuscitation: 2015 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. *Resuscitation* 2015;**95**:e169–201. [PUBMED: 26477424]

Xu 2015
Xu L, Ryu J, Hiel H, Menon A, Aggarwal A, Rha E, et al. Transplantation of human oligodendrocyte progenitor cells in an animal model of diffuse traumatic axonal injury: survival and differentiation. *Stem Cell Research & Therapy* 2015;**6**:93. [PUBMED: 25971252]

Yoon 2016
Yoon S, Yun A, Chang S, Park WS. Stem cells for neonatal brain disorders. *Neonatology* 2016;**109**:377–83.

Zhao 1996
Zhao W, Richardson JS, Mombourquette MJ, Weil JA, Ijaz S, Shuaib A. Neuroprotective effects of hypothermia and U-78517F in cerebral ischemia are due to reducing oxygen-based free radicals: an electron paramagnetic resonance study with gerbils. *Journal of Neuroscience Research* 1996;**45** (3):282–8. [PUBMED: 8841989]

Zhu 2009
Zhu C, Kang W, Xu F, Cheng X, Zhang Z, Jia L, et al. Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy. *Pediatrics* 2009;**124** (2):e218–26. [PUBMED: 19651565]

* *Indicates the major publication for the study*

ADDITIONAL TABLES

Table 1. Types of regenerative cells

Cell type	Source	Rationale	Mechanism of action	Preclinical/clinical results	References
MSC	Human umbilical cord tissue/ blood; rodent/human bone marrow	Safe and feasible in phase I RCT for bronchopulmonary dysplasia • Low immunogenicity (low MHC II), easily obtainable, rapid expansion • Autologous/ allogeneic administration • Paracrine release of trophic factors	• Paracrine release of IGF-1, EGF, VEGF, BDNF • Immunomodulatory: regulate T-cell, B-cell function, and production of inflammatory cytokines • Mitochondrial transfer	• Nerve fibre remyelination and axonal regeneration • Improve behavioural/motor tests • Enhance neural cell proliferation, survival, function • Decrease infarct size	Ahn 2016; Boshuizen 2018; Chopp 2002; Hsu 2016; Islam 2012; Liu 2010; Murphy 2013; Park 2016
MNC	Human umbilical cord blood	• Readily collected and large supply in cord blood with high plasticity • Safe and feasible in phase I RCT for hypoxic-ischaemic encephalopathy • Low immunogenicity (minimal HLA matching)	• Increase expression of BDNF, NGF, VEGF, GDNF • Activation of pro-survival Akt pathway • Decrease TNF-α and increase IL-10 gene expression • Reduce CD4+ T cell infiltration • Regulate	• Decrease neuronal apoptosis, astrogliosis, inflammation • Improve oligodendrocyte survival • Induce axonal growth • Improve neurobehavioral outcome	Aridas 2016; Cotten 2014; Fan 2005; McDonald 2018; Pimentel-Coelho 2012; Rowe 2010; Wang 2013

Table 1. Types of regenerative cells (*Continued*)

		• Paracrine release of trophic factors • Autologous/ allogeneic administration	hedgehog signalling		
OPC	Rodent/human embryonic stem cell; human NSC derivation	• Differentiate into oligodendrocytes (cells highly susceptible to hypoxic-ischaemic injury) • Remyelinate injured axons	• Diffuse biodistribution along white matter tracts and differentiate into myelin sheath-producing oligodendrocytes	• Promote myelin sheath formation, NSC proliferation, and inhibit apoptosis • Motor recovery following CNS injury	Chen 2015; Gopagondanahalli 2016; Kim 2018; Manley 2017; Niimi 2018; Xu 2015
NSC	Human fetal striatum; human ESC; human iPSC	• Differentiate into cells necessary for brain repair, including: neurons, astrocytes, and oligodendrocytes • Paracrine release of trophic factors • Low immunogenicity and tumorigenicity	• Immunomodulation • Paracrine secretion of BDNF, VEGF, and EGF • Attenuate NF-κB signalling • Upregulate glutamate transport	• Stimulate survival and migration of endogenous NSCs and neurons • Reduce inflammation and reactive oxygen species production • Improve axonal growth, motor function, decreased infarct size	Daadi 2016; Huang 2018; Ji 2015; Mine 2013
HSC	Umbilical cord blood	• Paracrine release of neurotrophic factors • Multipotent capacity and ability to transdifferentiate into neuronal cells • Autologous/ allogeneic administration	• Reduce microglial cells and T lymphocytes • Secrete VEGF, HGF, IGF-1	• Decrease infarct size and maintains cerebral blood flow • Enhance axonal growth • Ameliorate neuronal apoptosis and postischaemic inflammation	Schwarting 2008; Tsuji 2014; Verina 2013
EPC	Human umbilical vein; human umbilical cord blood; human adipose stem cell; human iPSC	• Umbilical cord-derived EPCs have higher regenerative potential than adult bone marrow-derived EPCs • Endothelial cell protection, repair, angiogenesis	• Anti-inflammatory effects: reduce $CD4^+$ infiltration to the brain • Activation of PI3/Akt pathway • Axonal growth: BDNF secretion	• Improve cognitive and motor function • Inhibit neuronal apoptosis • Stimulate blood vessel formation and reduce infarct size	Grandvuillemin 2017; Kidani 2016; McDonald 2018; Nabetani 2018; Wang 2016; Wu 2013

Table 1. Types of regenerative cells (*Continued*)

		• Low immunogenicity • Paracrine release of regenerative factors • Autologous administration	• Angiogenesis: VEGF, IGF-1 secretion		
iPSC	Skin fibroblasts, umbilical cord tissue, amniotic tissue	• Autologous administration • Differentiation into multiple neural lineage cells • Low immunogenicity	• Differentiate into functional neural cells (electrophysiological properties) • Decrease infiltration of MPO+ neutrophils and CD11b+ microglia • VEGF expression and organelle transfer	• Improve survival and sensorimotor function • Establish axonal connections • Inhibit inflammation, neural apoptosis, and glial scar formation	Cai 2010; Hsu 2016; Oki 2012; Pluchino 2013; Qin 2015; Tornero 2013

BDNF: brain-derived neurotrophic factor; CNS: central nervous system; ESC: embryonic stem cell; EGF: epidermal growth factor; GDNF: glial cell-line derived neurotrophic factor; HGF: hepatocyte growth factor; HLA: human leukocyte antigen; HSC: hematopoietic stem cells; IGF-1: insulin-like growth factor 1; iPSC: inducible pluripotent stem cells; IL: interleukin; MHC: major histocompatibility complex; MSC: mesenchymal stem cell; MNC: mononuclear cells; NF-κB: nuclear factor kappa beta; NGF: nerve growth factor; NSC: neural stem cells; OPC: oligodendrocyte progenitor cells; RCT: randomized controlled trial; TNF: tumour necrosis factor; VEGF: vascular endothelial growth factor.

APPENDICES

Appendix 1. Search strategy

PubMed:
("Stem Cells"[Mesh] OR "Stem Cell Transplantation"[Mesh] OR "Stromal Cells"[Mesh] OR "stem cell"[tiab] OR "stem cells"[tiab] OR "mesenchymal cell" OR "mesenchymal cells" OR "mononuclear cell" OR "mononuclear cells" OR "progenitor cell" OR "progenitor cells" OR "cord blood cell" OR "cord blood cells" OR "regenerative cell" OR "regenerative cells" OR "stromal cell" OR "stromal cells") AND (brain injury OR neuro-protect* OR neuro-restorative OR neuroprotect* OR Asphyxia Neonatorum[MeSH] OR Hypoxia-Ischemia, Brain[MeSH] OR Asphyxia* OR Hypoxia OR Hypoxic OR Hypoxemia OR Hypoxaemia OR Ischemia OR Ischaemia OR Ischemic OR Ischaemic OR anoxia)
The PubMed search will be translated for the other databases detailed in the search methods, and combined with the following Cochrane Neonatal standard search strategies:
PubMed: ((infant, newborn[MeSH] OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW or infan* or neonat*) AND (randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab]) NOT (animals [mh] NOT humans [mh]))

Embase: ((exp infant) OR (infan* OR newborn or neonat* OR premature or very low birth weight or low birth weight or VLBW or LBW).mp AND (human not animal) AND (randomized controlled trial or controlled clinical trial or randomized or placebo or clinical trials as topic or randomly or trial or clinical trial).mp
CINAHL: (infan* OR newborn OR neonat* OR premature OR low birth weight OR VLBW OR LBW) AND (randomized controlled trial OR controlled clinical trial OR randomized OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial)
Cochrane Library: (infan* or newborn or neonat* or premature or preterm or very low birth weight or low birth weight or VLBW or LBW)

Appendix 2. Risk of bias tool

We will use the standard methods of Cochrane and Cochrane Neonatal to assess the methodological quality of the trials. For each trial, we will seek information regarding the method of randomization, blinding, and reporting of all outcomes of all the infants enrolled in the trial. We will assess each criterion as being at a low, high, or unclear risk of bias. Two review authors will separately assess each study. We will resolve any disagreements by discussion. We will add this information to the 'Characteristics of included studies' table. We will evaluate the following issues and enter the findings into the 'Risk of bias' table.

1. Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

For each included study, we will categorize the method used to generate the allocation sequence as:

- low risk (any truly random process, e.g. random number table; computer random number generator);
- high risk (any non-random process, e.g. odd or even date of birth; hospital or clinic record number); or
- unclear risk.

2. Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?

For each included study, we will categorize the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomization; consecutively numbered sealed opaque envelopes);
- high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
- unclear risk.

3. Blinding of participants and personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?

For each included study, we will categorize the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding will be assessed separately for different outcomes or class of outcomes. We will categorize the methods as:

- low risk, high risk, or unclear risk for participants; and
- low risk, high risk, or unclear risk for personnel.

4. Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?

For each included study, we will categorize the methods used to blind outcome assessment. Blinding will be assessed separately for different outcomes or class of outcomes. We will categorize the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

5. Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?

For each included study and for each outcome, we will describe the completeness of data including attrition and exclusions from the analysis. We will note whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomized participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information is reported or supplied by the trial authors, we will reinclude missing data in the analyses. We will categorize the methods used to deal with missing data as:

- low risk (less than 20% missing data);
- high risk (20% or greater missing data); or
- unclear risk.

6. Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?

For each included study, we will describe how we investigated the possibility of selective outcome reporting bias and what we found. We will search study protocols of the included trials in ClinicalTrials.gov; the World Health Organization's International Trials Registry and Platform, and the ISRCTN Registry. For studies in which study protocols were published in advance, we will compare prespecified outcomes versus outcomes eventually reported in the published results. If the study protocols were not published in advance, we will contact study authors to gain access to the study protocol. We will assess the likelihood of selective reporting bias as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review were reported);
- high risk (where not all the study's prespecified outcomes were reported; one or more reported primary outcomes were not prespecified outcomes of interest and were reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

7. Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?

For each included study, we will describe any important concerns we had about other possible sources of bias (e.g. whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We will assess whether each study was free of other problems that could put it at risk of bias as:

- low risk;
- high risk; or
- unclear risk

If needed, we will explore the impact of the level of bias through undertaking sensitivity analyses.

CONTRIBUTIONS OF AUTHORS

MB, OR, and AM reviewed the literature and wrote the protocol.

DL and BT commented on and reviewed the protocol.

DECLARATIONS OF INTEREST

OR: no known conflicts of interest.

MB: no known conflicts of interest.

AM: is supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant KL2 TR001118. AM also has submitted a provisional patent to the US Patent and Trademark Office that involves byproducts of stem cells ("cell-free therapies"). This patent does not conflict with this planned systematic review, as the review is only considering different types of cell-based interventions.

BT: work on stem cells is supported by the Canadian Institute for Health Research, the Canadian Stem Cell Network, the Canadian Thoracic Society, the Ottawa Hospital Research Institute and the Children's Hospital of Eastern Ontario Research Institute, and the Ontario Institute of Regenerative Medicine.

DL: no known conflicts of interest.

SOURCES OF SUPPORT

Internal sources

- Institute for Clinical Sciences, Lund University, Lund, Sweden.

MB, OR and DL are employed by this organization

- The University of Texas Health San Antonio School of Medicine Clinical Investigator Kickstart Pilot Grant, USA.

to AM

External sources

- Vermont Oxford Network, USA.

Cochrane Neonatal Reviews are produced with support from Vermont Oxford Network, a worldwide collaboration of health professionals dedicated to providing evidence-based care of the highest quality for newborn infants and their families.