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Stem Cell Therapy for Preventing Neonatal Diseases in the 21st Century: Current Understanding and Challenges

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Abstract

Diseases of the preterm newborn such as bronchopulmonary dysplasia, necrotizing enterocolitis, cerebral palsy, and hypoxic-ischemic encephalopathy continue to be major causes of infant mortality and long-term morbidity. Effective therapies for the prevention or treatment for these conditions are still lacking as recent clinical trials have shown modest or no benefit. Stem cell therapy is rapidly emerging as a novel therapeutic tool for several neonatal diseases with encouraging pre-clinical results that hold promise for clinical translation. However, there are a number of unanswered questions and facets to the development of stem cell therapy as a clinical intervention. There is much work to be done to fully elucidate the mechanisms by which stem cell therapy is effective (e.g., anti-inflammatory versus pro-angiogenic), identifying important paracrine mediators, and determining the timing and type of therapy (e.g., cellular versus secretomes), as well as patient characteristics that are ideal. Importantly, the interaction between stem cell therapy and current, standard-of-care interventions is nearly completely unknown. In this review, we will focus predominantly on the use of mesenchymal stromal cells for neonatal diseases, highlighting the promises and challenges in clinical translation towards preventing neonatal diseases in the 21st century.

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Keywords

mesenchymal stem cell; neonatal; bronchopulmonary dysplasia; necrotizing enterocolitis

Introduction

Infant mortality in the United States continues to be high (5.9/1000 live births in 2016 (1) compared to other civilized nations. Diseases affecting extremely premature infants such as bronchopulmonary dysplasia (BPD) and necrotizing enterocolitis (NEC), remain major contributors to infant mortality and morbidity. Continued efforts to prevent these diseases have met with only modest success in the last decade, mandating new therapeutic strategies to improve disease outcomes in the 21st century. Stem cell (SC) and SC-derived therapies have emerged as promising options with over 900 registered trials on clinicaltrials.gov. Given the plasticity and regenerative potential of developing organs, SC use represents an exciting therapeutic strategy. However, fundamental questions regarding mechanisms of action and optimal treatment strategies remain unanswered, restricting their broad clinical applicability (2). Focusing primarily on mesenchymal stromal cells (MSC), we will discuss aspects of SC therapy relevant to neonatal diseases including mechanisms of actions, sources, preclinical studies, and clinical trials herein. We highlight the promises and challenges of this novel therapy and provide a blueprint for successful clinical translation to prevent neonatal diseases in the 21st century.

MSC

SC-based therapies have received major attention over the past 20 years after the initial discovery that bone marrow-derived cells can regenerate infarcted myocardium (3). This observation ignited a new field of investigation into the capacity of various types of “stem” cells to repair damaged organs including the brain, heart, gut and lung. One cell type, first described in the 1960s as colony forming fibroblasts, has received particular attention (4). These cells had clonogenic capability, and were able to differentiate into chondrocytes, osteocytes and adipocytes, and therefore qualified as “stem” cells. These bone marrow-derived cells, rapidly identified as important niche cells for the hematopoietic SC, were progressively investigated for their apparent repair functions. Since, similar cells have been isolated from adipose tissue (ADSC), and umbilical cord (UC) and umbilical cord blood (UCB) (Figure 1) (5,6). Since their initial description, these cells have received several names. As of today, the most accepted – yet still evolving – denomination is that of MSC proposed by the International Society for Cellular Therapy (ISCT) (7). MSC characterization is based on expression of cell surface markers, plastic-adherent growth, and differentiation potential into osteocytes, adipocytes, and chondrocytes (7). Although some features unique to the MSC secretome are known (8,9), tissue-specific definitions are lacking. MSC are attractive due to their wide therapeutic potential, low immunogenicity due to lack of MHC class II receptors, ease of isolation, self-renewing capacity, and rapid and extensive *ex vivo* expansion capacity (10). They exhibit multi-lineage differentiation and their secretome, consisting of a broad array of growth factors, cytokines, chemokines and extracellular vesicles, exhibits pluripotent effects (11).

Other SC therapies

Some non-MSC stem cells can be similar, but are not identical to MSC; for example, amniotic fluid stem cells (AFSC) have the same cell surface markers and tripotent differentiation potential but also express stem cell embryonic antigen-4 (SSEA-4) (12), CD29 (13,14), CD49e, OCT-4 (13), and may be weakly MHC class II positive (15). Other SCs, such as decidual stromal cells (DSCs), express many of the MSC surface markers, but differentiate poorly into osteocytes, adipocytes, and chondrocytes (16). Similarly, cardiac progenitor cells (CPCs) can be isolated from myocardium during surgical palliation of congenital heart defects, but unlike MSC, express cardiac-specific transcription factors like GATA-4 (17) and critically, can be positive for the classical hematopoietic stem cell marker CD34 (18). Finally, umbilical cord blood mononuclear cells (UCB-MNC) refers to the cell fraction obtained by centrifugation which contain UCB-MSC in a small proportion and are mostly CD133 positive (19). Researchers have also administered minimally processed umbilical cord blood (UCB) without immunophenotyping or *ex vivo* expansion, to rapidly provide SC therapy (19–22).

Postulated mechanisms of action

It was initially believed that cell replacement at the site of injury by engraftment and differentiation was the key mechanism of MSC action (23). However, extremely low rates of engraftment (typically <1–5%), and recent evidence indicate that MSC exert their therapeutic benefits via cell-to-cell communication and the secretion of bioactive molecules capable of modulating reparative processes (24,25) (Figure 1). These paracrine mechanisms (26) include beneficial modifications of the host niche/tissue environment with production of factors important in inflammation/immune signaling [*e.g.*, IL-1Rα (27), tumor necrosis factor-α-induced protein 6 (TSG6) (28), prostaglandin E2 (29), and IL-10 (30)), angiogenesis (*e.g.*, vascular endothelial growth factor), fibrosis (*e.g.*, stanniocalcin-1 (31) and adrenomedullin (32)], and cell death/repair [*e.g.*, hepatocyte, insulin, and keratinocyte growth factors (25,33–35)].

The MSC secretome also includes extracellular vesicles such as exosomes, which are nanoparticle-sized, lipid-bilayer-enclosed vesicles that mediate the therapeutic benefit of MSC. Exosomes carry nucleic acids, including microRNAs, and proteins that, upon secretion into the extracellular space fuse with the cell membranes of host cells, effecting transcriptional and post-translational modifications (36,37). This discovery opens new exciting avenues towards cell-free therapy as *in vitro* (38) and *in vivo* (34,39–41) experiments demonstrate that the cell-free conditioned media or exosomes obtained from MSC exert the same therapeutic benefit as whole SC therapy. Administering the secretome/exosomes confers advantages related to SC manufacturing, storage, and ability to provide “off-the-shelf” pharmaceuticals (42), while avoiding potential ethical, legal, and scientific challenges, including a concern for tumorigenicity (43,44). Their potential to modulate inflammation/immune signaling, angiogenesis, fibrosis and cell death/repair make them ideal candidates for therapeutic exploration in diseases affecting preterm infants.

Homing & Engraftment

MSC (45,46) and AFSC (12,15) home to sites of injury including the lung in animal models of BPD and the intestinal villi in models of NEC. Administered SC are often undetectable after a few days from the site of implantation (38,40), but may home to remote sites such as the spleen and liver (16,47). Some animal studies suggest that up to 21% of administered UC-MSC differentiate into neurons in the 35 days after transplant (48) while others do not (49). Even without engraftment, the effect of SC appears to be long-lasting for up to 14 months (50). This may be related to route of administration, as those given intra-arterially (21) and intra-nasally (51) engraft, and intravenously (49) administered SC do not, or related to SC type, as SC contained in intraperitoneally (19) or intravenously (20) administered UCB also fail to engraft. Therefore, while engraftment is not necessary for function, and the fate of transfused SC remain unclear, consideration of SC type, and administration near the site of action is important.

Inflammation

MSC were initially noted to be anti-inflammatory and more recent findings have confirmed their immunomodulatory ability. MSC have several anti-inflammatory effects, inducing a shift from pro-inflammatory cytokines such as IL-1β (52), IL-6 (52,53), TNFα (52–54), IFNγ (54), IL-1Rα (27), and prostaglandin E2 (29) to anti-inflammatory cytokines like IL-10 (30,54,55) and TNFα-induced protein 6 (TSG-6) (28). Increases in regulatory T-cells (54) and a switch from M1 to M2 macrophage polarization (51,56) also contribute to the anti-inflammatory signature.

Angiogenesis

MSC have pro-angiogenic effects, exerted primarily through the vascular endothelial growth factor (VEGF) family of pro-angiogenic growth factors essential for normal vascular development (57). MSC from placenta (53) and UCB (34), and AFSCs (12) secrete VEGF and induce endogenous VEGF secretion, improving lung vascular density in BPD models, and silencing MSC VEGF abolishes this effect (34). *In vitro*, UCB-MSC conditioned media induces endothelial cell proliferation and tubule formation similar to that induced by direct VEGF application (58).

Fibrosis

Fibrosis is a common feature of chronic diseases such as BPD where parenchyma is replaced with scar tissue putatively via TGF-β1/SMAD2/SMAD3 signaling (59). MSC often reduce fibrosis (53,60), MMP-9/TIMP-1 expression (60), connective tissue growth factor (53), elastin (61), and myofibroblast formation (61). They have also been reported to increase fibrosis (62) and TGF-β1 (46), and to decrease MMP-9 (63) in BPD. In cardiac disease, BM-MSC (64) and CPCs (17) reduce fibrosis and collagen I, perhaps via adrenomedullin (32). Overall, the anti-fibrotic impact of MSC is less well-established, but still a likely mechanism of action.

Future Mechanistic Studies

While inflammation and regeneration are mechanisms relevant to all perinatal diseases, other MSC-functions may not be equally applicable to all diseases. Therefore, it is imperative that we further understand MSC function from a mechanistic perspective. Further, as disease processes are complex with pathogenic mechanisms that vary with stage of disease, consideration of best type of SC therapy and pre-conditioning is important for developing precision SC approaches to prevent or rehabilitate neonatal disease.

Disease outcomes

BPD and pulmonary hypertension

BPD, a chronic lung disease that develops in premature infants, remains a major cause of morbidity and mortality (65). BPD is a phenotype of disrupted lung growth arising from exposure of neonatal lung to chorioamnionitis and nosocomial infection, malnutrition, hyperoxia and positive pressure ventilation (66–70). The multifactorial nature of the disease has challenged development of novel therapies (71). The putative ability of MSC to sense their microenvironment and to modulate the repair response accordingly via pleiotropic secreted factors, makes them appealing for the treatment of BPD (24,72,73).

Proof-of-concept experiments suggested a single intravenous (39) or intra-tracheal injection (38) of BM-MSC was lung-protective in neonatal rodents exposed to hyperoxia, leading to improvements in survival, lung inflammation, pulmonary hypertension and alveolar structure. Similarly, extensive studies have shown that a single intra-tracheal administration of human UC-/UCB-MSC prevents and rescues neonatal rats from hyperoxia-induced lung injury (74). They also have long-term efficacy and safety as exemplified by persistent improvement in lung structure and exercise capacity, with no evidence of tumor formation (40). Similar benefits on lung structure and inflammation in BPD models have been reported using human UCB-MSC (75). Cell-free therapy has considerable promise in BPD, as MSC-derived exosomes administered intravenously, intraperitoneally, or intratracheally prevent oxygen-induced lung injury in neonatal rodents via the modulation of macrophage activity and secretion of miRNA and TSG-6 (56,76).

The extensive pre-clinical evidence regarding MSC therapy in experimental neonatal lung injury was recently confirmed in a systematic review including 25 studies (26). MSC significantly improved alveolarization irrespective of timing of treatment, source, dose, or route of administration, except for one study using the intra-nasal approach. MSC also significantly ameliorated secondary endpoints including pulmonary hypertension, lung inflammation, fibrosis, angiogenesis, and apoptosis. Notably, numerous risks of bias were identified, highlighting the need for more rigorous experimental design and reporting of pre-clinical studies as set forth by the ARRIVE guidelines for animal studies (77). Furthermore, all 25 studies were performed in the neonatal hyperoxia-induced rodent model of BPD and models of sepsis-induced remodeling in BPD (66), indicating the need for studies in large animal models that allow the study of more complex disease pathology and in-depth physiologic assessments. Despite these shortcomings, first-in-human trials with MSC have been initiated (Figure 2).

The first phase I trial used a single intra-tracheal injection of allogeneic UCB-MSC in nine preterm infants born between 23 and 29 weeks gestation requiring mechanical ventilation between 5 to 14 days of age (78) (Table 1). This dose escalation study testing 10^7 or 2×10^7 SC/kg suggested that the procedure was feasible and well tolerated with no serious adverse events reported (78). The follow-up study at 2 years of age indicates no adverse growth, respiratory or neurodevelopmental outcomes (79). Similarly, a phase I/II trial at Rush University Medical Centre () and phase I trial in Spain () are pending (Table 2), the latter of which will test the safety and feasibility of up to 3 doses of intravenous UC-MSC in infants born at less than 28 weeks gestation still requiring mechanical ventilation at 14 days. Finally, a phase II double-blinded, multicenter, randomized controlled trial administering 107 MSC/kg is ongoing () with a planned long-term follow-up ().

Hypoxic-ischemic encephalopathy

Hypoxic-ischemic encephalopathy (HIE) is caused by an acute reduction in cerebral blood flow and ischemia with necrosis, followed by inflammatory reperfusion injury (80). SC can reduce these phases of injury through anti-inflammatory, pro-angiogenic, anti-oxidant, and anti-apoptotic mechanisms. Pre-clinical studies indicate that when given within hours to days of hypoxia-ischemia, BM-MSC, UC-MSC, and UCB-MSC improve behavioral and motor outcomes (81–83), BM-MSC decrease the size of injured brain (84), and adipose-derived MSC and placenta-derived MSC reduce inflammation (52,54). Similar effects are found with administration of minimally processed UCB (85,86), endothelial colony forming cells (85), and neural stem cells (87), potentially making it difficult to investigate mechanisms of action.

Results of one clinical trial suggests that volume- and erythrocyte-reduced umbilical cord blood is safe and benefits neurodevelopmental outcome, although significant differences in gender, severity and attrition at follow-up between the intervention and control groups could have biased results in favor of SC therapy (88). The future of SC therapy for HIE lies in its past and present: identifying the target group of patients (mild, moderate, or severe and term or preterm) and the appropriate timing of intervention (within a certain time-frame), and conducting well-designed clinical trials to answer these fundamental questions.

Cerebral palsy

Cerebral palsy (CP) is a non-progressive motor disorder suffered by both preterm and full-term infants, associated with intellectual disability, impaired mobility, and epilepsy (89). MSC may modulate resident host progenitor cells to enhance plasticity, survival, and differentiation (90,91). Administering MSC in animal models of intraventricular hemorrhage improves behavioral outcomes, fosters growth of oligodendrocytes, and reduces inflammation (92,93). Brain-derived neurotrophic factor (BDNF) and IFNγ appear to be important mediators of this effect as BDNF knockdown eliminates the beneficial effects of MSC (94) and the secretome of IFNγ-treated MSC, but not untreated MSC, restores myelination defects (95).

In human studies of CP, administration of minimally processed UCB into the cerebrospinal fluid has been generally safe (96), as adverse effects reported relate to lumbar puncture (e.g.,

headache, vomiting) or mild immunologic reaction (e.g., fever). UCB administration improves motor symptoms (97–99), with UCB-MSC also showing benefit to gross and fine motor function for up to two years after administration (100). There may be a genetic basis of response, as twins are more likely to respond or not respond as a pair (101), and not all participants improve, suggesting that unrecognized variables that impact SC therapy efficacy exist.

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is a major cause of morbidity and mortality among premature infants with mortality remaining between 20–30% in the last two decades (102). Although the exact pathogenesis of the disease is unknown, infants who develop NEC typically are born prematurely and have low birth weight (103). Additionally, alterations in the intestinal microbiome (104), genetic factors (105,106), and exaggerated inflammatory responses (107) are associated with NEC pathogenesis. Survivors of NEC frequently have long term sequelae including short gut syndrome and neurodevelopmental delays (102). SC therapy has been investigated as a possible treatment for NEC due to their ability to reduce inflammation, differentiate, and self-replicate, and they therefore have the potential to improve tissue health, function, and regeneration (108–110).

Recent studies have investigated the ability of several types of SC and their secreted products to protect the intestines against experimental NEC. Bone marrow-derived MSC (BM-MSC), amniotic fluid-derived MSC (AF-MSC), amniotic fluid-derived neural stem cells (AF-NSC), and enteric neural stem cells (E-NSC) have similar effects on NEC in animal models (111). However, AF-NSC and E-NSC, compared to MSC, are challenging to isolate and culture (15,111), potentially limiting their clinical utility. AFSC administered intraperitoneally significantly reduce the incidence and severity of NEC in animal models (15), significantly decreasing histologic intestinal injury and improving gut barrier function (111,112). Furthermore, exosomes derived from MSC and NSC are just as effective in reducing the incidence and severity of experimental NEC as the SC from which they were derived (13). After intraperitoneal injection, AFSC migrate to bowel, liver, and spleen in healthy animals (113), and within 48–72 hours, to tissues with a high level of inflammation and injury in experimental NEC, decreasing ascites (114) and improving survival, intestinal function, and inflammation in a COX-2 dependent manner (15). AFSC in NEC-afflicted animals primarily localize in damaged tissue (15,113,115). Preliminary studies also suggest that extracellular vesicles from bovine milk-derived SC may be protective in NEC (116), preventing ileal injury and reduction in goblet cells via enhanced expression of the endoplasmic reticulum chaperone protein glucose-regulated protein 94. Although there have not yet been any clinical trials of SC in NEC, SC or their secreted products could be a promising, novel therapy for NEC and such trials may become a reality in the future (Figure 3).

Heart disease

Congenital heart defects are the most common birth defects and are often repaired in a staged manner, allowing harvesting of autologous material (e.g., cardiac progenitor/stem cells) for *ex vivo* manipulation prior to direct myocardial administration (18,22) during

subsequent surgical repair. Pre-clinical studies indicate that BM-MSC (64) and CPC (17) reduce RV dilation and RV strain and reduce cardiomyocyte apoptosis in mice undergoing left anterior descending artery ligation. A novel prenatal administration of CPC rescued mouse pups from heart failure and increased live births five-fold (117). Cell-free therapy may also be applicable as MSC-derived exosomes significantly alter CPC miRNA which promotes survival, long-term cardiac function, and reduced fibrosis in rats (118).

The first human clinical trial for infants with hypoplastic left heart syndrome (HLHS) has been performed and 36 month follow-up data are available. The TICAP (Transcoronary Infusion of CPC in Patients with Single-Ventricle Physiology) pilot trial administered CPC directly into the coronary arteries of fourteen children under six years or age before stage 2 or 3 repair. At 36 months post-infusion, right ventricular function and somatic growth improved more in those who received CPC. Interestingly, responses were more favorable in infants with lower ejection fractions and those who were treated earlier (119). The stage I/II ELPIS trial (Allogeneic Human MSC Injection in Patients With HLHS) (120) follows up on this study but will administer BM-MSC rather than CPC. ELPIS is enrolling up to thirty patients with HLHS who will receive intramyocardial allogeneic BM-MSC, 2.5×10^5 MSC/kg, at the time of stage 2 repair, with the primary outcome being need for emergent serious adverse event in the first month after infusion in the first ten patients, and the change in right ventricular ejection fraction in the next twenty patients.

Other diseases

Early studies of SC for congenital diaphragmatic hernia (CDH), retinopathy of prematurity (ROP), neonatal stroke, and sepsis show encouraging results. For example, MSC administration in rabbit CDH models improves pulmonary hypoplasia (121), and AFSC administration decreases pulmonary hypertension (14). Similarly, intra-vitreal administration of BM-MSC reduces neovascularization in a mouse model of ROP (122). As these disorders often co-occur with BPD, IVH, and NEC, the first clinical studies may in fact be from coincident findings in studies in which MSC therapy are further developed. Neonatal stroke treatment with MSC reduces infarct size, improves neurodevelopmental outcomes, promotes angiogenesis, and reduces inflammation (123,124). Interestingly, BDNF-overexpressing MSC appear to be more effective than non-transfected SC in reducing injury size and motor deficits in the short-term (125,126), again highlighting the need for understanding mechanism of action in addition to simply observing clinical outcomes. Inflammation from infections and sepsis can be targeted by MSC, improving survival and lung inflammation, though pre-conditioning with IFNγ does not improve efficacy in a model of neonatal sepsis in rats (127).

Moving MSC to the bedside – Need for a tiered, evidence-based, pragmatic approach

Due to enthusiasm for novel SC therapeutics, and relative safety, at least of MSC, early phase clinical trials are already underway (Figure 2). While these will provide some degree of information about the safety and feasibility of this approach, more needs to be learned about the mechanisms of action of MSC in order to harness their full therapeutic potential

(Figure 3). Translation of adult SC studies directly to children and babies without consideration of neonatal physiology and pathogenesis is likely to limit success. Side-by-side comparisons of MSC and their secretome (i.e., exosomes, microvesicles, or conditioned media) are especially important to determine if cell-free therapies are an effective and potentially safer option. Although it is tempting to consider MSC as a universal therapy for any and all patients and diseases, several technical and fundamental aspects must be addressed. Relating MSC therapy to traditional, single-chemical pharmacologic therapies offers a useful framework for considering translation into clinical practice. The core pharmacokinetic principles can be extended, relating Absorption to route, Distribution to homing, and Metabolism to dose and co-treatment interactions, recipient factors and timing. Pharmacodynamically, receptor agonism/antagonism and drug potency relates to MSC pre-conditioning and secretome manipulation. The concepts of additive, antagonistic, or synergistic interactions are important when considering MSC as just one of many therapies an infant may be receiving. Until these factors are better understood, moving forward into large-scale, advanced phase clinical trials requiring years of long-term follow-up may be premature.

Donor

The first step to translation is to identify appropriate donors. Donor age impacts the SC phenotype, with neonatal MSC having greater anti-inflammatory capacity (128) and exosomes from preterm UC-MSC being better able to repair ischemic injury compared to exosomes from term UC-MSC (129). Donor sex may impact the MSC secretome as discussed above (55), but has typically been understudied because many studies use male donors and female hosts to identify engraftment. Also to be considered is that early studies show that the health status of donors can impact MSC phenotype and function (130), but studies of this type are in their infancy.

SC type

Numerous tissues sources have been investigated (Figure 1) with BM-MSC the most well-studied, but collection requires invasive procedures, making them difficult to obtain. Similarly, ADSC are typically obtained from liposuction aspirates (131). MSC from fetal membrane tissues (UC and UCB, placenta and amnion/chorion) and AFSC are especially appealing in the neonatal setting due to accessibility. Efficacy of autologous versus allogeneic SC is unknown, but the former are more likely to be accepted by families (132), and the latter have the advantage of being an “off-the-shelf” product readily available on-demand.

Culture methods

Once the appropriate SC type is identified, the optimal methods of isolation and culture are not yet known (9,133). In addition, traditional SC culture requires fetal bovine serum, which is undesirable for human administration and can change SC phenotype due to batch-to-batch variation (134). Xenobiotic-free culture methods utilizing human plasma or recombinant growth factors exist, but differences between products can alter MSC immunomodulatory capacity (135) and cytokine production (136).

Quality control and long-term follow up

A lack of *in vitro* potency assays to predict *in vivo* efficacy (137) is a major challenge to improving the manufacturing of a clinical-grade cellular therapies. Indeed, the "product" is the process" in SC therapy, *i.e.*, the process determines SC phenotype and function. Microarrays and genome sequencing may also be helpful once genetic profiles of various SC types and phenotypes are established. Safety is a critical factor, particularly the concern of carcinogenic transformation, as observed in induced pluripotent stem cells (138). Long-term cultures from higher-passage UC-MSC (139) can acquire chromosomal aberrations and proliferative advantage. Therefore, long-term follow-up is required and will rely on phase IV and post-marketing surveillance, but registries of MSC recipients may also foster such monitoring. MSC represent a radical new type of therapy, especially for fragile neonates, so recipients will likely need to be followed into adulthood.

Pre-conditioning

There are a wide variety of chemical agents that could optimize MSC efficacy (140), but the mechanisms of action and improvements in efficacy are incompletely understood. Preliminary studies of pro-inflammatory stimulation with factors like IFNγ (95,127) have found this promotes regeneration and anti-inflammatory effects, but pre-conditioning can also decrease efficacy (141). Oxidative stress may also be important, as the usual environment of MSC is relatively hypoxic (142) and some neonatal diseases are caused excessive (e.g., BPD) oxygen. For example, hyperoxia pre-conditioned enhances MSC efficacy in preventing pulmonary hypertension and alveolar simplification (41). These experiments also provide insight into how the MSC may respond when placed into the complex *in vivo* environment. Translating such findings to the bedside will require confirmation of these phenotypic changes with quality control assays as above and consideration of the technical and logistical challenges of pre-conditioning.

Co-treatments

Neonates in the intensive care unit receive many pharmaceuticals (143), as well as many non-pharmacologic treatments such as phototherapy and hypothermia, making it unlikely that MSC will be administered as a single agent. Some treatment-SC therapy interactions will probably be discovered, but studies of such interactions have thus far been limited. For example, inhaled nitric oxide and erythropoietin are synergistic with MSC therapy (60,63), enhancing pro-angiogenic and anti-fibrotic effects in models of BPD. Studies for HIE are more varied, showing therapeutic hypothermia and MSC can be synergistic (82) or antagonistic, producing increased brain inflammation (144). These unexpected findings indicate the need for caution as MSC treatment moves from the controlled laboratory setting to the complex and highly variable clinical setting.

Clinical trial design

Clinical trials require definition of appropriate clinical and surrogate endpoints, and should aim to clarify optimal timing, dose, and route of administration; this is especially important for diseases such as HIE which exhibit "critical windows" of susceptibility. Additionally, current therapeutic modalities must be incorporated into study protocols which may be

considered by appropriate target patient population or statistical analyses to control for patient heterogeneity. Dose is a potentially limiting factor because of the challenges in manufacturing sufficient quantities of MSC from limited donor sources. Generally, higher doses of MSC are more effective, as observed in models of stroke (49) and sepsis (145). The therapeutic ceiling of MSC is not yet defined and the dose in pre-clinical experiments can vary by several orders of magnitude. Route of administration affects dosing, as lower doses given directly into the site of injury are as efficacious as higher doses given intravenously by as much as five-fold (146). However, providing higher doses in a less invasive manner may be more acceptable to clinicians and families. Finally, the timing of administration must be investigated. One unique feature of SC-based therapy is its ability to affect initiation, propagation, and repair of disease, whereas conventional single agent therapies typically target one aspect of each phase. It is unclear whether SC should be used in a preventative or therapeutic manner, but efficacy may be diminished with later treatment in models of BPD (40,75) and HIE (86,147,148). Timing may also affect the ability to use autologous SC, as UC-MSC take up to three weeks to get to first passage (149).

Conclusions

Neonates with acute and chronic illnesses represent a unique clinical challenge, as these complex diseases encompass dynamic physiologic processes in immature developing organs. Current treatment strategies, including agents targeting single pathways, have resulted in small and only incremental improvements, since multi-organ, multi-pathway pathophysiology underpins these complex diseases. MSC and other SC may represent a paradigm shift in the treatment of these diseases as promising pre-clinical studies have led to early clinical trials. However, many challenges remain; including precise characterization of MSC and SC phenotypically, defining mechanisms of action, standardization across preparations and quality control, optimizing treatment protocols with due consideration of disease pathogenesis, and rigorous clinical trials. A systematic and coordinated approach by several teams looking at various aspects of SC therapy ranging from elucidation of mechanisms to clinical trial design, will likely deliver the promise of preventing neonatal disease in the 21st century using SC therapy.

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References

1. Kochanek KD, Murphy SL, Xu J, Arias E. Mortality in the United States, 2016. NCHS Data Brief, no 293. [Internet] Hyattsville, MD: 2017 Available from: https://www.cdc.gov/nchs/products/databriefs/db293.htm
2. Nitkin CR, Bonfield TL. Concise Review: Mesenchymal Stem Cell Therapy for Pediatric Disease: Perspectives on Success and Potential Improvements. Stem Cells Transl Med [Internet] 2016 [cited 2016 Sep 20];Available from: http://www.ncbi.nlm.nih.gov/pubmed/27625040
3. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature [Internet] 2001;410:701–5. Available from: http://www.nature.com/articles/35070587

4. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol [Internet] 1976;4:267–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/976387
5. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells [Internet] 2006;24:1294–301. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16410387
6. Miao Z, Jin J, Chen L, et al. Isolation of mesenchymal stem cells from human placenta: comparison with human bone marrow mesenchymal stem cells. Cell Biol Int [Internet] 2006;30:681–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16870478
7. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy [Internet] 2006 [cited 2014 Jul 9];8:315–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16923606
8. Wegmeyer H, Bröske A-M, Leddin M, et al. Mesenchymal stromal cell characteristics vary depending on their origin. Stem Cells Dev [Internet] 2013 [cited 2016 Feb 4];22:2606–18. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3780294&tool=pmcentrez&rendertype=abstract
9. Hoffmann A, Floerkemeier T, Melzer C, Hass R. Comparison of in vitro-cultivation of human mesenchymal stroma/stem cells derived from bone marrow and umbilical cord [Internet]. J. Tissue Eng. Regen. Med 2017 [cited 2018 Apr 20];11:2565–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27125777 [PubMed: 27125777]
10. Bernardo ME, Pagliara D, Locatelli F. Mesenchymal stromal cell therapy: a revolution in Regenerative Medicine? Bone Marrow Transplant [Internet] 2012;47:164–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21478914
11. Cunningham CJ, Redondo-Castro E, Allan SM. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. J Cereb Blood Flow Metab [Internet] 2018;38:1276–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29768965
12. Grisafi D, Pozzobon M, Dedja A, et al. Human amniotic fluid stem cells protect rat lungs exposed to moderate hyperoxia. Pediatr Pulmonol [Internet] 2013 [cited 2014 Feb 21];48:1070–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23533160
13. McCulloh CJ, Olson JK, Wang Y, et al. Treatment of experimental necrotizing enterocolitis with stem cell-derived exosomes. J Pediatr Surg [Internet] 2018 [cited 2018 Apr 19];53:1215–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29661576
14. Dekoninck P, Toelen J, Roubliova X, et al. The use of human amniotic fluid stem cells as an adjunct to promote pulmonary development in a rabbit model for congenital diaphragmatic hernia. Prenat Diagn [Internet] 2015 [cited 2018 Aug 31];35:833–40. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25976324
15. Zani A, Cananzi M, Fascetti-Leon F, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut [Internet] 2014 [cited 2018 Apr 26];63:300–9. Available from: http://gut.bmj.com/lookup/doi/10.1136/gutjnl-2012-303735
16. Erkers T, Kaipe H, Nava S, et al. Treatment of severe chronic graft-versus-host disease with decidual stromal cells and tracing with (111)indium radiolabeling. Stem Cells Dev [Internet] 2015;24:253–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4291217&tool=pmcentrez&rendertype=abstract
17. Wehman B, Pietris N, Bigham G, et al. Cardiac Progenitor Cells Enhance Neonatal Right Ventricular Function After Pulmonary Artery Banding. Ann Thorac Surg [Internet] 2017 [cited 2018 Apr 19];104:2045–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28760475
18. Avolio E, Rodriguez-Arabaolaza I, Spencer HL, et al. Expansion and characterization of neonatal cardiac pericytes provides a novel cellular option for tissue engineering in congenital heart disease. J Am Heart Assoc 2015;4:e002043. [PubMed: 26080813]
19. Nakanishi K, Sato Y, Mizutani Y, Ito M, Hirakawa A, Higashi Y. Rat umbilical cord blood cells attenuate hypoxic–ischemic brain injury in neonatal rats. Sci Rep [Internet] 2017 [cited 2018 Apr 19];7:44111 Available from: http://www.ncbi.nlm.nih.gov/pubmed/28281676

20. Drobyshevsky A, Cotten CM, Shi Z, et al. Human Umbilical Cord Blood Cells Ameliorate Motor Deficits in Rabbits in a Cerebral Palsy Model. Dev Neurosci 2015;37:349–62. [PubMed: 25791742]
21. Greggio S, De Paula S, Azevedo PN, Venturin GT, Dacosta JC. Intra-arterial transplantation of human umbilical cord blood mononuclear cells in neonatal hypoxic-ischemic rats. Life Sci [Internet] 2014 [cited 2018 Apr 26];96:33–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24177600
22. Brizard CP, Looi JYJ, Smolich JJ, et al. Safety of intracoronary human cord blood stem cells in a lamb model of infant cardiopulmonary bypass. Ann Thorac Surg [Internet] 2015 [cited 2018 Apr 20];100:1021–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26209485
23. Prockop DJ, Kota DJ, Bazhanov N, Reger RL. Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). J Cell Mol Med [Internet] 2010;14:2190–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20716123
24. Fung ME, Thébaud B. Stem cell-based therapy for neonatal lung disease: it is in the juice. Pediatr Res [Internet] 2014 [cited 2015 Aug 25];75:2–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3940470&tool=pmcentrez&rendertype=abstract
25. Lee JW, Fang X, Krasnodembskaya A, Howard JP, Matthay MA. Concise review: Mesenchymal stem cells for acute lung injury: role of paracrine soluble factors. Stem Cells [Internet] 2011;29:913–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21506195
26. Augustine S, Avey MT, Harrison B, et al. Mesenchymal Stromal Cell Therapy in Bronchopulmonary Dysplasia: Systematic Review and Meta-Analysis of Preclinical Studies. Stem Cells Transl Med [Internet] 2017 [cited 2018 Apr 19];6:2079–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29045045
27. Ortiz LA, Dutreil M, Fattman C, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci U S A [Internet] 2007;104:11002–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17569781
28. Chaubey S, Thueson S, Ponnalagu D, et al. Early gestational mesenchymal stem cell secretome attenuates experimental bronchopulmonary dysplasia in part via exosome-associated factor TSG-6. Stem Cell Res Ther [Internet] 2018 [cited 2018 Aug 13];9:173 Available from: https://stemcellres.biomedcentral.com/articles/10.1186/s13287-018-0903-4
29. Bartosh TJ, Ylöstalo JH, Mohammadipoor A, et al. Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc Natl Acad Sci U S A [Internet] 2010;107:13724–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20643923
30. Németh K, Leelahavanichkul A, Yuen PST, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med [Internet] 2009;15:42–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19098906
31. Ono M, Ohkouchi S, Kanehira M, et al. Mesenchymal stem cells correct inappropriate epithelial-mesenchyme relation in pulmonary fibrosis using stanniocalcin-1. Mol Ther [Internet] 2015;23:549–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25373521
32. Li LL, Peng C, Zhang M, et al. Mesenchymal stem cells overexpressing adrenomedullin improve heart function through antifibrotic action in rats experiencing heart failure. Mol Med Rep [Internet] 2018;17:1437–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29138835
33. Xu Y-X, Chen L, Wang R, et al. Mesenchymal stem cell therapy for diabetes through paracrine mechanisms. Med Hypotheses [Internet] 2008;71:390–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18538944
34. Chang YS, Ahn SY, Jeon HB, et al. Critical Role of Vascular Endothelial Growth Factor Secreted by Mesenchymal Stem Cells in Hyperoxic Lung Injury. Am J Respir Cell Mol Biol [Internet] 2014;51:391–9. Available from: http://www.atsjournals.org/doi/abs/10.1165/rcmb.2013-0385OC
35. Lee JW, Fang X, Matthay MA, Serikov V, Gupta N. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci 2009;

36. van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: A Common Pathway for a Specialized Function. J Biochem [Internet] 2006;140:13–21. Available from: http://academic.oup.com/jb/article/140/1/13/2182139/Exosomes-A-Common-Pathway-for-a-Specialized
37. Stoorvogel W Functional transfer of microRNA by exosomes. Blood [Internet] 2012;119:646–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22262739
38. van Haaften T, Byrne R, Bonnet S, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. Am J Respir Crit Care Med [Internet] 2009 [cited 2014 Feb 5];180:1131–42. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3269236&tool=pmcentrez&rendertype=abstract
39. Aslam M, Baveja R, Liang OD, et al. Bone Marrow Stromal Cells Attenuate Lung Injury in a Murine Model of Neonatal Chronic Lung Disease. Am J Respir Crit Care Med [Internet] 2009 [cited 2014 Feb 19];180:1122–30. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2784417&tool=pmcentrez&rendertype=abstract
40. Pierro M, Ionescu L, Montemurro T, et al. Short-term, long-term and paracrine effect of human umbilical cord-derived stem cells in lung injury prevention and repair in experimental bronchopulmonary dysplasia. Thorax [Internet] 2013 [cited 2014 Feb 19];68:475–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23212278
41. Waszak P, Alphonse R, Vadivel A, Ionescu L, Eaton F, Thébaud B. Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats. Stem Cells Dev [Internet] 2012 [cited 2015 Aug 25];21:2789–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22533467
42. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. Int J Mol Sci [Internet] 2017;18 Available from: http://www.ncbi.nlm.nih.gov/pubmed/28841158
43. Park S-J, Kim H-J, Kim W, et al. Tumorigenicity Evaluation of Umbilical Cord Blood-derived Mesenchymal Stem Cells. Toxicol Res [Internet] 2016;32:251–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27437093
44. Barkholt L, Flory E, Jekerle V, et al. Risk of tumorigenicity in mesenchymal stromal cell-based therapies - Bridging scientific observations and regulatory viewpoints. Cytotherapy 2013;15:753–9. [PubMed: 23602595]
45. Jahromi S Hamidian, Estrada C, Li Y, Cheng E, Davies JE. Human Umbilical Cord Perivascular Cells and Human Bone Marrow Mesenchymal Stromal Cells Transplanted Intramuscularly Respond to a Distant Source of Inflammation. Stem Cells Dev [Internet] 2018;27:scd.2017.0248 Available from: http://online.liebertpub.com/doi/10.1089/scd.2017.0248
46. Luan Y, Ding W, Ju Z, Zhang Z, Zhang X, Kong F. Bone marrow-derived mesenchymal stem cells protect against lung injury in a mouse model of bronchopulmonary dysplasia. Mol Med Rep [Internet] 2015 [cited 2018 Apr 20];11:1945–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25406024
47. Gholamrezanezhad A, Mirpour S, Bagheri M, et al. In vivo tracking of 111In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl Med Biol [Internet] 2011;38:961–7. Available from: 10.1016/j.nucmedbio.2011.03.008
48. Zhang X, Zhang Q, Li W, et al. Therapeutic effect of human umbilical cord mesenchymal stem cells on neonatal rat hypoxic-ischemic encephalopathy. J Neurosci Res [Internet] 2014 [cited 2018 Apr 26];92:35–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24265136
49. Tanaka E, Ogawa Y, Mukai T, et al. Dose-dependent effect of intravenous administration of human umbilical cord-derived mesenchymal stem cells in neonatal stroke mice. Front Neurol [Internet] 2018 [cited 2018 Apr 19];9:133 Available from: http://www.ncbi.nlm.nih.gov/pubmed/29568282
50. Donega V, Nijboer CH, van Velthoven CTJ, et al. Assessment of long-term safety and efficacy of intranasal mesenchymal stem cell treatment for neonatal brain injury in the mouse. Pediatr Res [Internet] 2015 [cited 2018 Apr 20];78:520–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26270577
51. Donega V, Nijboer CH, van Tilborg G, Dijkhuizen RM, Kavelaars A, Heijnen CJ. Intranasally administered mesenchymal stem cells promote a regenerative niche for repair of neonatal ischemic brain injury. Exp Neurol [Internet] 2014 [cited 2018 Apr 26];261:53–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24945601

52. Park D, Lee SH, Bae DK, et al. Transplantation of Human Adipose Tissue-Derived Mesenchymal Stem Cells Restores the Neurobehavioral Disorders of Rats with Neonatal Hypoxic-Ischemic Encephalopathy. Cell Med [Internet] 2013 [cited 2018 Apr 26];5:17–28. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26858861

53. Chou HC, Li YT, Chen CM. Human mesenchymal stem cells attenuate experimental bronchopulmonary dysplasia induced by perinatal inflammation and hyperoxia. Am J Transl Res 2016;8:342–53. [PubMed: 27158330]

54. Ding H, Zhang H, Ding H, et al. Transplantation of placenta-derived mesenchymal stem cells reduces hypoxic-ischemic brain damage in rats by ameliorating the inflammatory response. Cell Mol Immunol [Internet] 2017;14:693–701. Available from: 10.1038/cmi.2015.99

55. Sammour I, Somashekar S, Huang J, et al. The Effect of Gender on Mesenchymal Stem Cell (MSC) Efficacy in Neonatal Hyperoxia-Induced Lung Injury. PLoS One [Internet] 2016 [cited 2018 Apr 19];11:e0164269 Available from: http://www.ncbi.nlm.nih.gov/pubmed/27711256

56. Willis GR, Fernandez-Gonzalez A, Anastas J, et al. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. Am J Respir Crit Care Med [Internet] 2018 [cited 2018 Apr 19];197:104–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28853608

57. Woik N, Kroll J. Regulation of lung development and regeneration by the vascular system. Cell Mol Life Sci [Internet] 2015;72:2709–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25894695

58. Montemurro T, Viganò M, Ragni E, et al. Angiogenic and anti-inflammatory properties of mesenchymal stem cells from cord blood: soluble factors and extracellular vesicles for cell regeneration. Eur J Cell Biol [Internet] 2016 [cited 2018 Apr 20];95:228–38. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27139721

59. Wen Y-C, Du M-K, Li M-W, Hsuan YC-Y, Su Y-C, Lin W. EphA2-positive human umbilical cord-derived mesenchymal stem cells exert anti-fibrosis and immunomodulatory activities via secretion of prostaglandin E2. Taiwan J Obstet Gynecol [Internet] 2018;57:722–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30342659

60. Luan Y, Zhang L, Chao S, et al. Mesenchymal stem cells in combination with erythropoietin repair hyperoxia-induced alveoli dysplasia injury in neonatal mice via inhibition of TGF-β1 signaling. Oncotarget [Internet] 2016 [cited 2018 Apr 20];7:47082–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27191651

61. Hou C, Peng D, Gao L, et al. Human umbilical cord-derived mesenchymal stem cells protect from hyperoxic lung injury by ameliorating aberrant elastin remodeling in the lung of O2-exposed newborn rat. Biochem Biophys Res Commun [Internet] 2018 [cited 2018 Apr 19];495:1972–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29242152

62. Liu L, Mao Q, Chu S, et al. Intranasal versus Intraperitoneal Delivery of Human Umbilical Cord Tissue–Derived Cultured Mesenchymal Stromal Cells in a Murine Model of Neonatal Lung Injury. Am J Pathol [Internet] 2014 [cited 2018 Apr 20];184:3344–58. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25455688

63. Zhang Z-H, Pan Y-Y, Jing R-S, et al. Protective effects of BMSCs in combination with erythropoietin in bronchopulmonary dysplasia-induced lung injury. Mol Med Rep [Internet] 2016 [cited 2018 Apr 20];14:1302–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27279073

64. Wehman B, Sharma S, Pietris N, et al. Mesenchymal stem cells preserve neonatal right ventricular function in a porcine model of pressure overload. Am J Physiol - Hear Circ Physiol [Internet] 2016;310:H1816–26. Available from: http://ajpheart.physiology.org/lookup/doi/10.1152/ajpheart.00955.2015

65. Dumpa V, Bhandari V. Surfactant, steroids and non-invasive ventilation in the prevention of BPD. Semin Perinatol [Internet] 2018;42:444–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30343941

66. Menden HL, Xia S, Mabry SM, Navarro A, Nyp MF, Sampath V. Nicotinamide Adenine Dinucleotide Phosphate Oxidase 2 Regulates LPS-Induced Inflammation and Alveolar Remodeling in the Developing Lung. Am J Respir Cell Mol Biol [Internet] 2016 [cited 2016 Sep 13];55:767–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27438994

67. Morty RE. Recent advances in the pathogenesis of BPD. Semin Perinatol 2018;42:404–12. [PubMed: 30384986]
68. Bhandari A, Bhandari V. Pathogenesis, pathology and pathophysiology of pulmonary sequelae of bronchopulmonary dysplasia in premature infants. Front Biosci 2003;8:e370–80. [PubMed: 12700058]
69. Baker CD, Alvira CM. Disrupted lung development and bronchopulmonary dysplasia. Curr Opin Pediatr 2014;26:306–14. [PubMed: 24739494]
70. Álvarez-Fuente M, Moreno L, Mitchell JA, et al. Preventing bronchopulmonary dysplasia: new tools for an old challenge. Pediatr Res 2019;85:432–41. [PubMed: 30464331]
71. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med [Internet] 2001;163:1723–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11401896
72. Caplan AI. Mesenchymal Stem Cells: Time to Change the Name! Stem Cells Transl Med [Internet] 2017 [cited 2017 May 2];6:1445–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28452204
73. Fischbach MA, Bluestone JA, Lim WA. Cell-based therapeutics: The next pillar of medicine. Sci. Transl. Med 2013;
74. Pierro M, Thébaud B, Soll R. Mesenchymal stem cells for the prevention and treatment of bronchopulmonary dysplasia in preterm infants. Cochrane Database Syst Rev [Internet] 2017 [cited 2018 Apr 19];11:CD011932 Available from: http://doi.wiley.com/10.1002/14651858.CD011932
75. Chang YS, Choi SJ, Ahn SY, et al. Timing of Umbilical Cord Blood Derived Mesenchymal Stem Cells Transplantation Determines Therapeutic Efficacy in the Neonatal Hyperoxic Lung Injury. PLoS One [Internet] 2013 [cited 2015 Jul 14];8:e52419 Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3549907&tool=pmcentrez&rendertype=abstract
76. Porzionato A, Zaramella P, Dedja A, et al. Intratracheal administration of clinical-grade mesenchymal stem cell-derived extracellular vesicles reduces lung injury in a rat model of bronchopulmonary dysplasia. Am J Physiol Cell Mol Physiol [Internet] 2019;316:L6–19. Available from: https://www.physiology.org/doi/10.1152/ajplung.00109.2018
77. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol [Internet] 2010;8:e1000412 Available from: https://dx.plos.org/10.1371/journal.pbio.1000412
78. Chang YS, Ahn SY, Yoo HS, et al. Mesenchymal Stem Cells for Bronchopulmonary Dysplasia: Phase 1 Dose-Escalation Clinical Trial. J Pediatr [Internet] 2014 [cited 2014 Feb 20];164:966–972.e6 Available from: http://www.ncbi.nlm.nih.gov/pubmed/24508444
79. Ahn SY, Chang YS, Kim JH, Sung SI, Park WS. Two-Year Follow-Up Outcomes of Premature Infants Enrolled in the Phase I Trial of Mesenchymal Stem Cells Transplantation for Bronchopulmonary Dysplasia. J Pediatr [Internet] 2017 [cited 2018 Apr 19];185:49–54.e2 Available from: http://www.ncbi.nlm.nih.gov/pubmed/28341525
80. Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. JAMA Pediatr [Internet] 2015;169:397–403. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25685948
81. Gu Y, Zhang Y, Bi Y, et al. Mesenchymal stem cells suppress neuronal apoptosis and decrease IL-10 release via the TLR2/NFκB pathway in rats with hypoxic-ischemic brain damage. Mol Brain [Internet] 2015 [cited 2018 Apr 20];8:65 Available from: http://www.ncbi.nlm.nih.gov/pubmed/26475712
82. Park WS, Sung SI, Ahn SY, et al. Hypothermia augments neuroprotective activity of mesenchymal stem cells for neonatal hypoxic-ischemic encephalopathy. PLoS One [Internet] 2015 [cited 2018 Apr 20];10:e0120893 Available from: http://www.ncbi.nlm.nih.gov/pubmed/25816095
83. Mueller M, Oppliger B, Joerger-Messerli M, et al. Wharton's Jelly Mesenchymal Stem Cells Protect the Immature Brain in Rats and Modulate Cell Fate. Stem Cells Dev [Internet] 2017 [cited 2018 Apr 19];26:239–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27842457
84. Donega V, Nijboer CH, Braccioli L, et al. Intranasal administration of human MSC for ischemic brain injury in the mouse: In vitro and in vivo neuroregenerative functions. PLoS One [Internet]

2014 [cited 2018 Apr 20];9:e112339 Available from: http://www.ncbi.nlm.nih.gov/pubmed/25396420

85. Grandvuillemin I, Garrigue P, Ramdani A, 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 Transl Med [Internet] 2017 [cited 2018 Apr 19]; 6:1987–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28980775
86. Li J, Yawno T, Sutherland A, et al. Preterm white matter brain injury is prevented by early administration of umbilical cord blood cells. Exp Neurol [Internet] 2016 [cited 2018 Apr 20]; 283:179–87. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27317990
87. Braccioli L, Heijnen CJ, Coffer PJ, Nijboer CH. Delayed administration of neural stem cells after hypoxia-ischemia reduces sensorimotor deficits, cerebral lesion size, and neuroinflammation in neonatal mice. Pediatr Res [Internet] 2017 [cited 2018 Apr 20];81:127–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27632779
88. Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. J Pediatr [Internet] 2014 [cited 2014 Jul 1];164:973–979.e1 Available from: http://www.ncbi.nlm.nih.gov/pubmed/24388332
89. Novak I, Walker K, Hunt RW, Wallace EM, Fahey M, Badawi N. Concise Review: Stem Cell Interventions for People With Cerebral Palsy: Systematic Review With Meta-Analysis. Stem Cells Transl Med [Internet] 2016 [cited 2018 Apr 20];5:1014–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27245364
90. Wang X, Cheng H, Hua R, et al. Effects of bone marrow mesenchymal stromal cells on gross motor function measure scores of children with cerebral palsy: a preliminary clinical study. Cytotherapy [Internet] 2013 [cited 2015 Jun 18];15:1549–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24100132
91. Finch-Edmondson M, Morgan C, Hunt RW, Novak I. Emergent Prophylactic, Reparative and Restorative Brain Interventions for Infants Born Preterm With Cerebral Palsy. Front Physiol [Internet] 2019;10:15 Available from: http://www.ncbi.nlm.nih.gov/pubmed/30745876
92. Ahn SY, Chang YS, Sung DK, et al. Mesenchymal stem cells prevent hydrocephalus after severe intraventricular hemorrhage. Stroke [Internet] 2013 [cited 2018 Sep 6];44:497–504. Available from: http://stroke.ahajournals.org/cgi/doi/10.1161/STROKEAHA.112.679092
93. Zhu LH, Bai X, Zhang N, Wang SY, Li W, Jiang L. Improvement of human umbilical cord mesenchymal stem cell transplantation on glial cell and behavioral function in a neonatal model of periventricular white matter damage. Brain Res [Internet] 2014 [cited 2018 Apr 26];1563:13–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24680746
94. Ahn SY, Chang YS, Sung DK, Sung SI, Ahn J-Y, Park WS. Pivotal Role of Brain-Derived Neurotrophic Factor Secreted by Mesenchymal Stem Cells in Severe Intraventricular Hemorrhage in Newborn Rats. Cell Transplant [Internet] 2017 [cited 2018 Apr 20];26:145–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27535166
95. Morioka C, Komaki M, Taki A, et al. Neuroprotective effects of human umbilical cord-derived mesenchymal stem cells on periventricular leukomalacia-like brain injury in neonatal rats. Inflamm Regen [Internet] 2017 [cited 2018 Apr 19];37:1 Available from: http://www.ncbi.nlm.nih.gov/pubmed/29259700
96. Feng M, Lu A, Gao H, et al. Safety of Allogeneic Umbilical Cord Blood Stem Cells Therapy in Patients with Severe Cerebral Palsy: A Retrospective Study. Stem Cells Int [Internet] 2015 [cited 2015 Aug 5];2015:325652 Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4510256&tool=pmcentrez&rendertype=abstract
97. Liu X, Fu X, Dai G, et al. Comparative analysis of curative effect of bone marrow mesenchymal stem cell and bone marrow mononuclear cell transplantation for spastic cerebral palsy. J Transl Med [Internet] 2017 [cited 2018 Apr 19];15:48 Available from: http://www.ncbi.nlm.nih.gov/pubmed/28235424
98. Kang M, Min K, Jang J, et al. Involvement of Immune Responses in the Efficacy of Cord Blood Cell Therapy for Cerebral Palsy. Stem Cells Dev [Internet] 2015 [cited 2015 Sep 8];Available from: http://www.ncbi.nlm.nih.gov/pubmed/25977995
99. Romanov YA, Tarakanov OP, Radaev SM, et al. Human allogeneic AB0/Rh-identical umbilical cord blood cells in the treatment of juvenile patients with cerebral palsy. Cytotherapy [Internet]

2015 [cited 2015 Sep 8];17:969–78. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25791070

100. Huang L, Zhang C, Gu J, et al. A Randomized, Placebo-Controlled Trial of Human Umbilical Cord Blood Mesenchymal Stem Cell Infusion for Children With Cerebral Palsy. Cell Transplant [Internet] 2018;27:325–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29637820
101. Wang X, Hu H, Hua R, et al. Effect of umbilical cord mesenchymal stromal cells on motor functions of identical twins with cerebral palsy: pilot study on the correlation of efficacy and hereditary factors. Cytotherapy [Internet] 2015 [cited 2015 Sep 8];17:224–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25593078
102. Neu J, Walker WA. Necrotizing Enterocolitis. N Engl J Med [Internet] 2011;364:255–64. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra1005408
103. Herrmann K, Carroll K. An Exclusively Human Milk Diet Reduces Necrotizing Enterocolitis. Breastfeed Med [Internet] 2014;9:184–90. Available from: http://www.liebertpub.com/doi/10.1089/bfm.2013.0121
104. Warner BB, Deych E, Zhou Y, et al. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. Lancet [Internet] 2016;387:1928–36. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673616000817
105. Cuna A, George L, Sampath V. Genetic predisposition to necrotizing enterocolitis in premature infants: Current knowledge, challenges, and future directions. Semin Fetal Neonatal Med [Internet] 2018;23:387–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1744165X18300982
106. Sampath V, Menden H, Helbling D, et al. SIGIRR genetic variants in premature infants with necrotizing enterocolitis. Pediatrics [Internet] 2015;135:e1530–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25963006
107. Hackam DJ, Sodhi CP. Toll-Like Receptor-Mediated Intestinal Inflammatory Imbalance in the Pathogenesis of Necrotizing Enterocolitis. Cell Mol Gastroenterol Hepatol [Internet] 2018;6:229–238.e1 Available from: http://www.ncbi.nlm.nih.gov/pubmed/30105286
108. Galindo LT, Filippo TRM, Semedo P, et al. Mesenchymal Stem Cell Therapy Modulates the Inflammatory Response in Experimental Traumatic Brain Injury. Neurol Res Int [Internet] 2011;2011:564089 Available from: http://www.hindawi.com/journals/nri/2011/564089/
109. Rowart P, Erpicum P, Detry O, et al. Mesenchymal Stromal Cell Therapy in Ischemia/Reperfusion Injury. J Immunol Res [Internet] 2015;2015:1–8. Available from: http://www.hindawi.com/journals/jir/2015/602597/
110. Diaco N, Diamandis Z, Borlongan C. Amniotic fluid-derived stem cells as an effective cell source for transplantation therapy in stroke. Brain Circ [Internet] 2015;1:119 Available from: http://www.braincirculation.org/text.asp?2015/1/2/119/172881
111. McCulloh CJ, Olson JK, Zhou Y, Wang Y, Besner GE. Stem cells and necrotizing enterocolitis: A direct comparison of the efficacy of multiple types of stem cells. J Pediatr Surg [Internet] 2017 [cited 2018 Apr 19];52:999–1005. Available from: https://linkinghub.elsevier.com/retrieve/pii/S002234681730177X
112. McCulloh CJ, Olson JK, Wang Y, Vu J, Gartner S, Besner GE. Evaluating the efficacy of different types of stem cells in preserving gut barrier function in necrotizing enterocolitis. J Surg Res [Internet] 2017;214:278–85. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022480417301531
113. Ghionzoli M, Cananzi M, Zani A, et al. Amniotic fluid stem cell migration after intraperitoneal injection in pup rats: implication for therapy. Pediatr Surg Int [Internet] 2010;26:79–84. Available from: http://link.springer.com/10.1007/s00383-009-2504-x
114. Zani A, Cananzi M, Lauriti G, et al. Amniotic Fluid Stem Cells Prevent Development of Ascites in a Neonatal Rat Model of Necrotizing Enterocolitis. Eur J Pediatr Surg [Internet] 2013;24:057–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23852724
115. Drucker NA, McCulloh CJ, Li B, Pierro A, Besner GE, Markel TA. Stem cell therapy in necrotizing enterocolitis: Current state and future directions. Semin Pediatr Surg [Internet] 2018 [cited 2018 Apr 19];27:57–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1055858617301476

116. Li B, Hock A, Wu RY, et al. Bovine milk-derived exosomes enhance goblet cell activity and prevent the development of experimental necrotizing enterocolitis. PLoS One [Internet] 2019;14:e0211431 Available from: http://dx.plos.org/10.1371/journal.pone.0211431

117. Liu X, Hall SRR, Wang Z, et al. Rescue of neonatal cardiac dysfunction in mice by administration of cardiac progenitor cells in utero. Nat Commun [Internet] 2015;6:8825 Available from: http://www.nature.com/articles/ncomms9825

118. Zhang Z, Yang J, Yan W, Li Y, Shen Z, Asahara T. Pretreatment of cardiac stem cells with exosomes derived from mesenchymal stem cells enhances myocardial repair. J Am Heart Assoc 2016;5.

119. Tarui S, Ishigami S, Ousaka D, et al. Transcoronary infusion of cardiac progenitor cells in hypoplastic left heart syndrome: Three-year follow-up of the Transcoronary Infusion of Cardiac Progenitor Cells in Patients with Single-Ventricle Physiology (TICAP) trial. J Thorac Cardiovasc Surg [Internet] 2015 [cited 2018 Apr 19];150:1198–207. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26232942

120. Kaushal S, Wehman B, Pietris N, et al. Study design and rationale for ELPIS: A phase I/IIb randomized pilot study of allogeneic human mesenchymal stem cell injection in patients with hypoplastic left heart syndrome. Am Heart J [Internet] 2017 [cited 2018 Apr 19];192:48–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28938963

121. Yuniartha R, Alatas FS, Nagata K, et al. Therapeutic potential of mesenchymal stem cell transplantation in a nitrofen-induced congenital diaphragmatic hernia rat model. Pediatr Surg Int [Internet] 2014 [cited 2018 Aug 31];30:907–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25092488

122. Wang J-D, An Y, Zhang J-S, et al. Human bone marrow mesenchymal stem cells for retinal vascular injury. Acta Ophthalmol [Internet] 2017 [cited 2018 Apr 19];95:e453–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27807930

123. Wei ZZ, Gu X, Ferdinand A, et al. Intranasal Delivery of Bone Marrow Mesenchymal Stem Cells Improved Neurovascular Regeneration and Rescued Neuropsychiatric Deficits after Neonatal Stroke in Rats. Cell Transplant [Internet] 2015 [cited 2018 Apr 20];24:391–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25647744

124. van Velthoven CT, Dzietko M, Wendland MF, et al. Mesenchymal stem cells attenuate MRI-identifiable injury, protect white matter, and improve long-term functional outcomes after neonatal focal stroke in rats. J Neurosci Res [Internet] 2017 [cited 2018 Apr 19];95:1225–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27781299

125. Van Velthoven CTJ, Sheldon RA, Kavelaars A, et al. Mesenchymal stem cell transplantation attenuates brain injury after neonatal stroke. Stroke 2013;44:1426–32. [PubMed: 23539530]

126. Jeong CH, Kim SM, Lim JY, Ryu CH, Jun JA, Jeun SS. Mesenchymal stem cells expressing brain-derived neurotrophic factor enhance endogenous neurogenesis in an ischemic stroke model. Biomed Res Int [Internet] 2014 [cited 2018 Apr 26];2014:1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24672780

127. Zhu Y, Xu L, Collins JJP, et al. Human Umbilical Cord Mesenchymal Stromal Cells Improve Survival and Bacterial Clearance in Neonatal Sepsis in Rats. Stem Cells Dev [Internet] 2017 [cited 2018 Apr 19];26:1054–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28401804

128. Jahromi S Hamidian, Li Y, Davies JE. Effect of Tumor Necrosis Factor Alpha Dose and Exposure Time on Tumor Necrosis Factor Induced Gene-6 Activation by Neonatal and Adult Mesenchymal Stromal Cells. Stem Cells Dev [Internet] 2017 [cited 2018 Apr 19];27:scd.2017.0179 Available from: http://www.ncbi.nlm.nih.gov/pubmed/29121823

129. Panfoli I, Ravera S, Podesta M, et al. Exosomes from human mesenchymal stem cells conduct aerobic metabolism in term and preterm newborn infants. FASEB J [Internet] 2016 [cited 2018 Apr 20];30:1416–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26655706

130. Montanucci P, Pescara T, Pennoni I, et al. Functional Profiles of Human Umbilical Cord-Derived Adult Mesenchymal Stem Cells in Obese/Diabetic Versus Healthy Women. Curr Diabetes Rev [Internet] 2016 [cited 2018 Apr 20];12:1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27363411

131. Turner L US stem cell clinics, patient safety, and the FDA. Trends Mol Med [Internet] 2015;21:271–3. Available from: 10.1016/j.molmed.2015.02.008

132. Hodges RJ, Bardien N, Wallace E. Acceptability of stem cell therapy by pregnant women. Birth [Internet] 2012 [cited 2016 Mar 22];39:91–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23281856

133. Park WS, Ahn SY, Sung SI, Ahn J-Y, Chang YS. Strategies to enhance paracrine potency of transplanted mesenchymal stem cells in intractable neonatal disorders. Pediatr Res [Internet] 2018 [cited 2018 Apr 19];83:214–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28972960

134. Honn KV, Singley JA, Chavin W. Fetal Bovine Serum: A Multivariate Standard. Exp Biol Med [Internet] 1975;149:344–7. Available from: http://ebm.sagepub.com/lookup/doi/10.3181/00379727-149-38804

135. Oikonomopoulos A, van Deen WK, Manansala A-R, et al. Optimization of human mesenchymal stem cell manufacturing: the effects of animal/xeno-free media. Sci Rep [Internet] 2015 [cited 2016 Jul 10];5:16570 Available from: http://www.ncbi.nlm.nih.gov/pubmed/26564250

136. Swamynathan P, Venugopal P, Kannan S, et al. Are serum-free and xeno-free culture conditions ideal for large scale clinical grade expansion of Wharton's jelly derived mesenchymal stem cells? A comparative study. Stem Cell Res Ther [Internet] 2014 [cited 2016 Mar 22];5:88 Available from: http://stemcellres.com/content/5/4/88 [PubMed: 25069491]

137. Boregowda SV, Phinney DG. Quantifiable Metrics for Predicting MSC Therapeutic Efficacy. J Stem Cell Res Ther [Internet] 2016;6 Available from: https://www.omicsonline.org/open-access/quantifiable-metrics-for-predicting-msc-therapeutic-efficacy-2157-7633-1000365.php?aid=82018

138. Volarevic V, Markovic BS, Gazdic M, et al. Ethical and Safety Issues of Stem Cell-Based Therapy. Int J Med Sci [Internet] 2018;15:36–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29333086

139. Borghesi A, Avanzini MA, Novara F, et al. Genomic alterations in human umbilical cord-derived mesenchymal stromal cells call for stringent quality control before any possible therapeutic approach. Cytotherapy [Internet] 2013 [cited 2018 Apr 26];15:1362–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24094488

140. Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Gonçalves RM. Mesenchymal Stromal Cell Secretome: Influencing Therapeutic Potential by Cellular Pre-conditioning. Front Immunol [Internet] 2018;9:2837 Available from: http://www.ncbi.nlm.nih.gov/pubmed/30564236

141. Kilpinen L, Impola U, Sankkila L, et al. Extracellular membrane vesicles from umbilical cord blood-derived MSC protect against ischemic acute kidney injury, a feature that is lost after inflammatory conditioning. J Extracell vesicles [Internet] 2013;2 Available from: http://www.ncbi.nlm.nih.gov/pubmed/24349659

142. Hoch AI, Leach JK. Concise review: optimizing expansion of bone marrow mesenchymal stem/stromal cells for clinical applications. Stem Cells Transl Med [Internet] 2014 [cited 2016 May 23];3:643–52. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4006491&tool=pmcentrez&rendertype=abstract

143. Krzyżaniak N, Pawłowska I, Bajorek B. Review of drug utilization patterns in NICUs worldwide. J Clin Pharm Ther [Internet] 2016 [cited 2018 Oct 17];41:612–20. Available from: http://doi.wiley.com/10.1111/jcpt.12440

144. Herz J, Köster C, Reinboth BS, et al. Interaction between hypothermia and delayed mesenchymal stem cell therapy in neonatal hypoxic-ischemic brain injury. Brain Behav Immun [Internet] 2018 [cited 2018 Apr 19];70:118–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29454023

145. Perlee D, van Vught LA, Scicluna BP, et al. Intravenous Infusion of Human Adipose Mesenchymal Stem Cells Modifies the Host Response to Lipopolysaccharide in Humans: A Randomized, Single-Blind, Parallel Group, Placebo Controlled Trial. Stem Cells [Internet] 2018 [cited 2018 Aug 14];Available from: http://doi.wiley.com/10.1002/stem.2891

146. Ahn SY, Chang YS, Sung DK, et al. Optimal route for mesenchymal stem cells transplantation after severe intraventricular hemorrhage in newborn rats. PLoS One 2015;10:1–14.

147. Cameron SH, Alwakeel AJ, Goddard L, et al. Delayed post-treatment with bone marrow-derived mesenchymal stem cells is neurorestorative of striatal medium-spiny projection neurons and improves motor function after neonatal rat hypoxia-ischemia. Mol Cell Neurosci [Internet] 2015 [cited 2018 Apr 20];68:56–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25828540

148. Park WS, Sung SI, Ahn SY, et al. Optimal Timing of Mesenchymal Stem Cell Therapy for Neonatal Intraventricular Hemorrhage. Cell Transplant [Internet] 2016 [cited 2018 Apr 20]; 25:1131–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26440762

149. Araújo AB, Furlan JM, Salton GD, et al. Isolation of human mesenchymal stem cells from amnion, chorion, placental decidua and umbilical cord: comparison of four enzymatic protocols. Biotechnol Lett [Internet] 2018 [cited 2018 Apr 19];40:989–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29619744

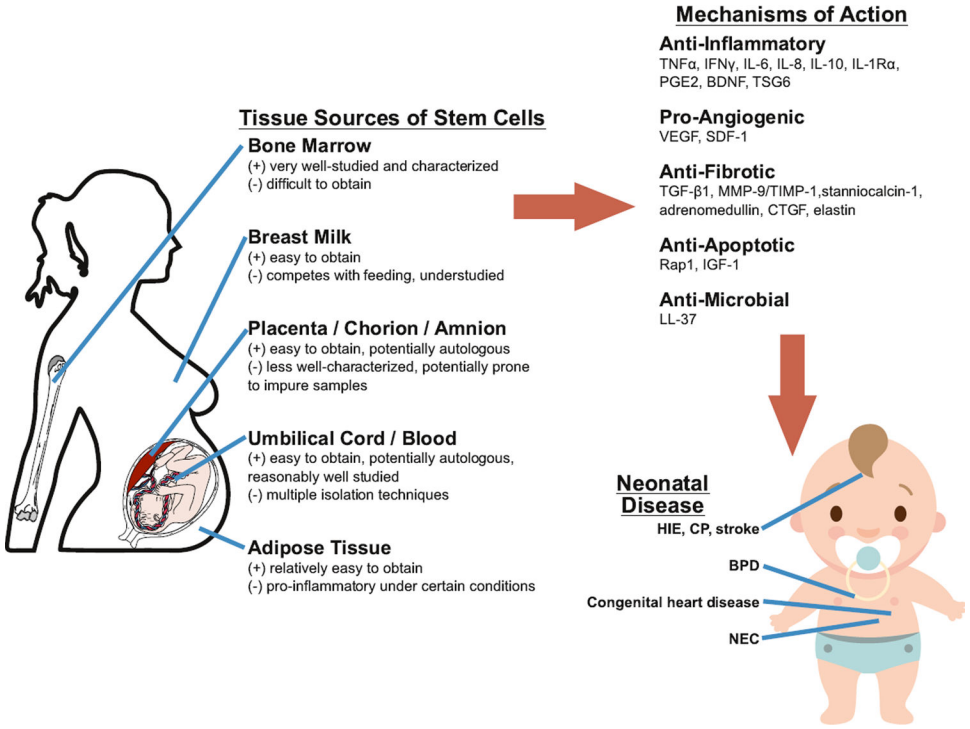

Figure 1:

Sources and potential mechanisms of action of stem cells for treating neonatal diseases. Stem cells from various sources have advantages (+) and disadvantages (−). Our understanding of mechanisms of action will inform applicability to neonatal diseases. *Abbreviations*: BDNF, brain-derived neurotrophic factor; BPD, bronchopulmonary dysplasia; CP, cerebral palsy; CTGF, connective tissue growth factor; HIE, hypoxic-ischemic encephalopathy; IFN, interferon; IGF-1, insulin-like growth factor 1; IL, interleukin; MMP-9, matrix metalloprotein-9; NEC, necrotizing enterocolitis; TNF, tumor necrosis factor; PGE2, prostaglandin E2; SDF-1, stromal cell-derived factor 1; TIMP-1, TIMP metallopeptidase inhibitor 1; TGF-β1, transforming growth factor β1; TSG6, tumor necrosis factor-inducible gene 6; VEGF, vascular endothelial growth factor. Portion of figure made with resource from freepik.com.

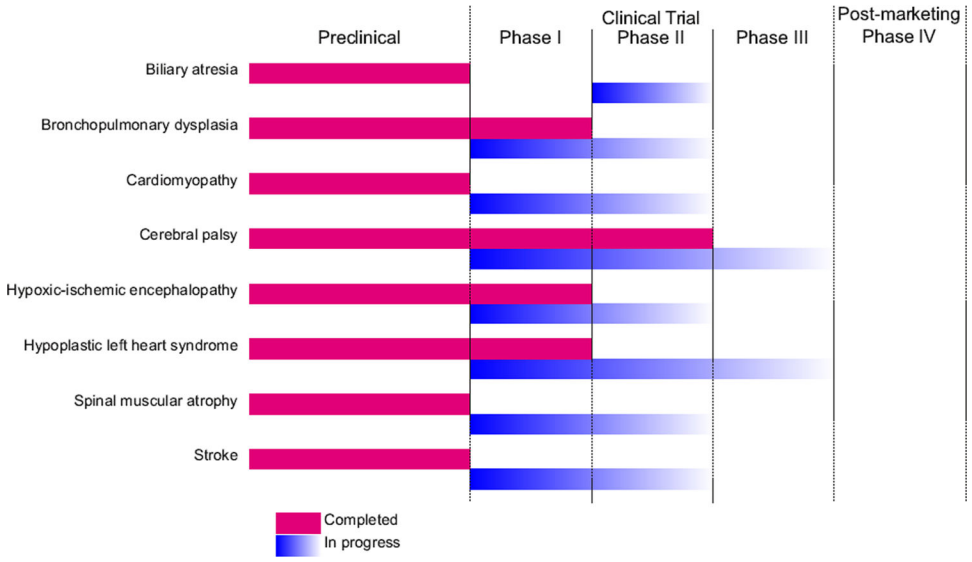

Figure 2:

Current stage of clinical trial development for neonatal diseases. There is accumulating pre-clinical evidence of stem cell efficacy for neonatal diseases, driving initiation of phase I-III clinical trials. No completed phase III or post-marketing phase IV trials have yet been completed for neonatal diseases.

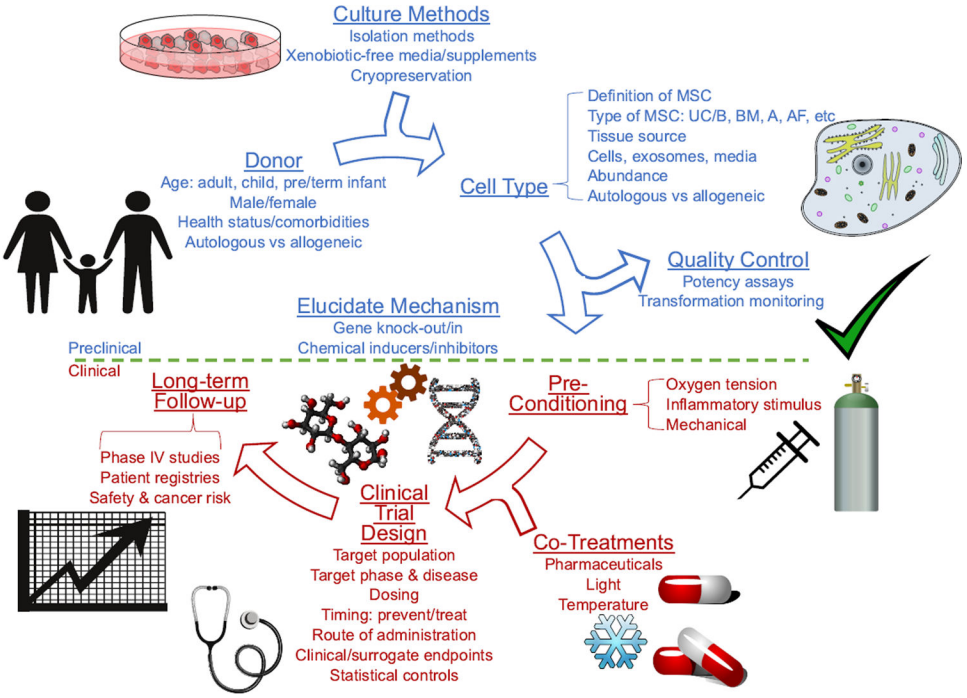

Figure 3:

Blueprint for developing stem cell therapy for the 21st century. There are a variety of factors, both pre-clinical and clinical, that may impact stem cell efficacy that require further investigation, such as donor, culture methods, stem cell type, quality control, stem cell pre-conditioning, co-treatments, clinical trial design, and long-term follow-up, all of which are centered around studies to elucidate the mechanisms of stem cell action.

Table 1:

Published clinical trials mesenchymal stromal cells and other cell therapies for neonatal and pediatric diseases.

Disease	Phase	Trial Registration	Cell Type	Dose	Route	Age	Subjects	Rationale (R) & Outcome (O)	Ref
BPD	I		Allogeneic UCB-MSCs	1–2×10^7 cells/kg	intra-tracheal	24–26.6wk GA, 7–14 days of age	9	R: MSCs in rat pups were protective, and safe in hyperoxia BPD model O: decreased BPD severity and need for postnatal steroids	78
CP	--	ChiCTR-TNRC-10000928	Autologous BM-MSCs	2×10^7 cells/dose for up to 4 doses	intra-thecal	6–180 months	46	R: Hypothesis that MSC's immunomodulatory and trophic factors could "enhance CNS plasticity, survival, and differentiation of host cells" O: improved GMFM scores and GMFCS classification 18 months after treatment	90
CP	--	--	Allogeneic UCB-MSCs	2–3×10^7 cells/ dose for up to 8 doses	intravenous and intra-thecal	1–29 years	47	R: UCB-MSC is safe with low immunogenicity and preliminary animal studies showed benefit for a model of hypoxic/ischemic brain injury O: minor infusion-related adverse events but no serious effect 6 months after treatment	96
CP	--	--	Allogeneic UCB-MSCs	1–1.5×10^7 cells/ dose for 4 doses	intra-thecal	3–12 years	16	R: Extend preclinical studies that "MSC can ameliorate motor dysfunction in central nervous system diseases" O: improvement in GMFM but not FMFM scores 6 months after treatment	101
CP	--	--	Allogeneic UC-MSCs	25×10^7 cells/dose (average) for 2 doses	intravenous	1–12 years	80	R: UCB-MSC produce "cytokines and immunomodulatory and neurotrophic factors that can modulate brain plasticity and contribute to functional brain repair" O: improved physical/mental scores of symptom severity	99
CP	--	--	Allogeneic UCB-MSCs	5×10^7 cells/dose for 6 doses	intravenous	3–12 years	54	R: Extension of reported case reports and preliminary clinical trials O: improvement in GMFM-88 and CFA 24 months after treatment, improved EEG background rhythms	100
CP	I-II	CHiCTR-TRC-12002568	Autologous BM-MNCs or BM-MSCs	1×10^6 cells/kg for 4 doses	intra-thecal	7–132 months	105	R: Extension of "previous clinical experiments" that BM-MSC "improve[s] spastic CP including motor function, language, and cognition" O: improvement in GMFM and FMFM scores 12 months after treatment in BM-MSC group only	97
CP	II		Allogeneic UCB	2.25–7.1×10^7 total nucleated cells	intravenous or intra-arterial	6 months-20 years	17	R: Extension of "previous clinical research" where UCB and erythropoietin "produced therapeutic benefit in children with CP" O: improved MMT and GMPM scores 6 months after treatment, decreased periventricular inflammation on PET scans	98
HLHS	I		Autologous cardiac progenitor cells	3×10^5 cells/kg	intra-coronary	up to 6 years	14	R: "Decline of cardiomyocyte replication might be associated with the absolute loss of intrinsic progenitor cells or reduced potential of preexisting mature myocyte proliferation during heart development" and CPC can produce "cytokine or specified molecular-targeted therapy"	119

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Disease	Phase	Trial Registration	Cell Type	Dose	Route	Age	Subjects	Rationale (R) & Outcome (O)	Ref
								O: improvement in right ventricle ejection fraction, fewer unplanned catheter interventions, higher weight-for-age z score	
HIE	I		Autologous UCB	1–5×10^7 nucleated cells/dose for up to 4 doses	intravenous	up to 14 days	23	R: Neonatal rodents with HIE treated with UCB “have improved anatomic and neurobehavioral outcomes” O: improvement in survival or Bayley III Scores of Infant Development >=85 (mild/moderate range impairment)	88

Abbreviations: --, not specified; BM, bone marrow; BPD, bronchopulmonary dysplasia; CFA, comprehensive functional assessment; CP, cerebral palsy; EEG, electroencephalogram; FMFM, fine motor function measure; GA, gestational age; GMFCS, gross motor functional classification system; GMFM, gross motor function measure; HIE, hypoxic ischemic encephalopathy; HLHS, hypoplastic left heart syndrome; MNC, mononuclear cell; MSC, mesenchymal stromal cell; PET, positron emission tomography; UC, umbilical cord; UCB, umbilical cord blood

Table 2:

Current clinical trials of cell-based therapy for neonatal diseases.

Disease	Phase	NCT Number	Cell Type	Dose	Route	Age	Target Enrollment
Biliary atresia	II		Autologous BM-MNC	--	--	1–15y	20
BPD	I		UC-MSC		intra-tracheal	up to 6m	10
BPD	I		Allogeneic UCB-MSC	10 or 20×10^6 cells/kg	intra-tracheal	up to 14d	9
BPD	I		Allogeneic UCB-MSC	1 or 2×10^7 cells/kg (2 cohorts)	intra-tracheal	4–48m	9
BPD	I		Allogeneic UCB-MSC	1 or 2×10^7 cells/kg (2 cohorts)	intra-tracheal	45–63m	8
BPD	I		MSC (not further specified)	5×10^6 cells x 3 doses	--	1m-28wk	10
BPD	I		Allogeneic UCB-MSC	--	--	1–3m	100
BPD	I		UC-MSC	3, 10, or 30×10^6 cells/kg (3 cohorts)	--	36–38wk	9
BPD	I		MSC (not further specified)	25×10^6 cells/kg	intra-tracheal	28–37wk	200
BPD	I		BM-MSC extracellular vesicles	20, 60, 200 pmol phosphlipid/kg	intravenous	up to 14d	18
BPD	I		UC-MSC	1 or 5×10^6 cells/kg	intravenous	1m-5y	30
BPD	I-II		Allogeneic UCB-MSC	1 or 2×10^7 cells/kg	--	up to 14d	12
BPD	I-II		UCB-MSC	1 or 5×10^6 cells/kg	intravenous	--	30
BPD	I-II		UC-MSC	2×10^7 cells/kg	intra-tracheal	up to 3wk	180
BPD	I-II		UC-MSC	1 or 5×10^6 cells/kg	intravenous	up to 14d	20
BPD	II		Allogeneic UCB-MSC	1×10^7 cells/kg	intra-tracheal	up to 14d	70
BPD	II		Allogeneic UCB-MSC	1×10^7 cells/kg	intra-tracheal	7m	70
BPD	II		Allogeneic UCB-MSC	--	--	up to 13d	60
BPD	II		UC-MSC	1 or 5×10^6 cells/kg	intravenous	up to 1y	57
Cardio-myopathy	--		Autologous BM stem cells	--	--	1–16y	10
Cardio-myopathy	I		CPC	3×10^5 cells/kg	intra-coronary	up to 17y	31
Cardio-myopathy	I		Autologous CD34+ stem cells	--	intra-coronary	1–16y	10
Cardio-myopathy	I-II		UC-MSC	multiple (not further specified)	intra-muscular	1–14y	30
Cardio-myopathy	I-II		Autologous BM-MNC	--	intra-coronary	1–16y	30
CP	--		Allogeneic UCB	>3e7 nucleated cells/kg	intravenous	10m-10y	105

Disease	Phase	NCT Number	Cell Type	Dose	Route	Age	Target Enrollment
CP	--		Neural stem cells	--	--	1–12y	20
CP	--		UC-MSC	≥1×10^7 cells/kg	intravenous vs intra-thecal vs intra-nasal	2–18y	44
CP	I		CD133+ stem cells	--	intra-thecal	4–12y	12
CP	I		Autologous BM-MNC	--	--	17m-22y	40
CP	I		Autologous BM-MNC	--	--	6m-35y	500
CP	I		12/12 HLA-matched sibling cord blood cells	≥1×10^7 cells/kg	intravenous	1–16y	12
CP	I-II		MNC-enriched cord blood	--	intravenous	1–12y	40
CP	I-II		BM CD133+ stem cells	--	intra-thecal	4–12y	8
CP	I-II		Autologous BM-MSC	--	intra-thecal	2–12y	50
CP	I-II		UCB	≥2×10^7 cells/kg	intravenous	10m-20y	90
CP	I-II		Allogeneic UCB or UC-MSC	1×10^8 UCB or 2×10^6 MSC/kg	intravenous	2–5y	90
CP	II		Autologous UCB or BM-MNC	--	--	2–10y	20
CP	II		Autologous BM nucleated cells	10mL	intra-thecal	7–9y	60
CP	II		Autologous BM-MNC	--	intra-thecal	1–15y	40
CP	II		Autologous BM-MNC	--	intra-thecal	1–15y	25
CP	II		Autologous BM-MNC	--	intra-thecal	2–15y	30
CP	II		Allogeneic BUC-MNC or UC-MSC	--	intra-thecal	4–14y	108
CP	II		Allogeneic UCB-MNC	2–5×10^7 cells/kg	--	1–10y	40
CP	II		Autologous MNC	--	intra-thecal	3–15y	100
CP	III		Stem cells (not further specified)	--	intra-thecal	1–14y	300
HIE	--		Autologous CD34+ stem cells	--	intravenous	37–42wk	20
HIE	--		Fetal neural progenitor cells	4×10^6 cells/kg x 3 doses	intra-thecal	up to 14d	120
HIE	--		Autologous CB stem cells	--	intravenous	37–42wk	20
HIE	--		Autologous BM CD34+ stem cells	--	intra-thecal	1–8y	18
HIE	--		Autologous cord blood	--	--	up to 20 minutes	10
HIE	I		UCB-MSC	1–8×10^8 cells	intravenous	--	10
HIE	I		Autologous UCB-SC	5×10^7 cells/kg up to 4 times	--	28–37wk	200

Disease	Phase	NCT Number	Cell Type	Dose	Route	Age	Target Enrollment
HIE	I-II		Autologous UCB-SC	--	--	up to 3d	20
HIE	II		Autologous cord blood or placental stem cells	--	--	up to 6h	20
HLHS	--		UCB harvest	not applicable	not applicable	pregnant women	100
HLHS	I		Autologous CPC	3×10^5 cells/kg	intra-coronary	up to 6y	14
HLHS	I		Autologous UCB-MNC	3×10^6 cells/kg	intra-myocardial	up to 18m	10
HLHS	I		Allogeneic MSCs	25×10^4 cells/kg	intra-myocardial	up to 30d	30
HLHS	I		Autologous c-kit+ cells	--	intra-coronary	up to 27d	30
HLHS	I		Autologous UCB-MNC	--	intra-coronary	up to 4d	12
HLHS	I-II		Allogeneic BM-MSC	25×10^4 cells/kg	intra-myocardial	up to 1y	30
HLHS	I-II		Allogeneic mesenchymal precursor cells	2×10^7 cells/kg	intra-myocardial	up to 5y	24
HLHS	II		CPC	3×10^5 cells/kg	intra-coronary	up to 20y	34
HLHS	II		Autologous UCB-MNC	$1–3\times10^6$ cells/kg	intra-myocardial	up to 8m	100
HLHS	III		Autologous CSC	3×10^5 cells/kg	intra-coronary	up to 6y	40
IVH	I		Allogeneic UCB-MSC	--	--	23–34wk	9
IVH	I		Allogeneic UCB-MSC	--	--	6m-2y	9
IVH	II		Allogeneic UCB-MSC	--	intra-ventricular	up to 28d	22
SMA	I-II		A-MSC	1×10^6 cells/kg x 3 doses	intra-thecal	5–12m	10
Stroke	I-II		Allogeneic BM-MSC	5×10^7 cells/kg	intra-nasal	up to 10d	10
Urea Cycle Disorders	I-II		Heterologous adult liver-derived progenitor cells	12.5, 50, or 200×10^5cells/kg (3 cohorts)	--	up to 17y	20
Urea Cycle Disorders	II		Heterologous adult liver-derived progenitor cells	5×10^7 cells/kg	--	up to 12y	20

Abbreviations: --, not specified; A-MSC, adipose mesenchymal stromal cell; BM, bone marrow; BPD, bronchopulmonary dysplasia; CP, cerebral palsy; CPC, cardiac progenitor cell; CSC, cardiac stem cell; HIE, hypoxic ischemic encephalopathy; HLHS, hypoplastic left heart syndrome; MNC, mononuclear cell; MSC, mesenchymal stromal cell; IVH, intraventricular hemorrhage; SMA, spinal muscular atrophy; UC, umbilical cord; UCB, umbilical cord blood