



REVIEW ARTICLE

Stem cell therapy for preventing neonatal diseases in the 21st century: Current understanding and challenges

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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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INTRODUCTION

Infant mortality in the United States continues to be high (5.9/1000 live births in 2016)¹ compared to other developed 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 action, 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 then, similar cells have been isolated from adipose tissue (ADSC), and umbilical cord (UC) and umbilical cord blood (UCB) (Fig. 1).^{5,6} Since their initial description, these cells have received several names. As of today, the most accepted – yet still evolving – denomination of MSC, is that 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

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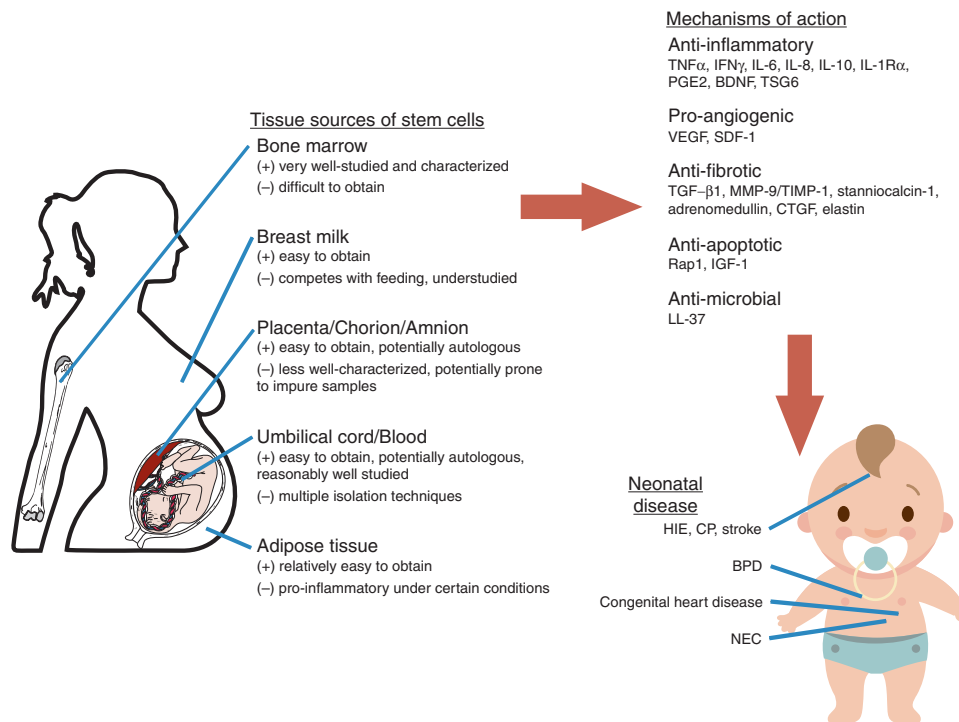


Fig. 1 Sources and potential mechanisms of action of stem cells for treating neonatal diseases. Stem cells from various sources have advantages (+) and disadvantages (-). Our understanding of mechanisms of action will inform applicability to neonatal diseases. BDNF brain-derived neurotrophic factor, 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 metalloproteinase inhibitor 1, TGF- β 1 transforming growth factor β 1, TSG6 tumor necrosis factor-inducible gene 6, VEGF vascular endothelial growth factor. Portion of figure made with resource from freepik.com

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-4¹⁷ 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-MS 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} (Fig. 1). These paracrine mechanisms²⁶ include beneficial modifications of the host niche/tissue environment with production of factors important in inflammation/immune signaling (e.g., IL-1R α ,²⁷ tumor necrosis factor- α -induced protein 6

(TSG6),²⁸ prostaglandin E2,²⁹ and IL-10³⁰), angiogenesis (e.g., vascular endothelial growth factor), fibrosis (e.g., stanniocalcin-1³¹ and adrenomedullin³²), 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 vitro³⁸ 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-MS differentiate into neurons in the 35 days after transplant⁴⁸ 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-arterially²¹ and intra-nasally⁵¹ engraft, and intravenously⁴⁹ administered SC do not, or related to SC type, as SC contained in intraperitoneally¹⁹ or intravenously²⁰ 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-1 β ,⁵² IL-6,^{52,53} TNF α ,⁵²⁻⁵⁴ IFN γ ,⁵⁴ IL-1R α ,²⁷ and prostaglandin E2²⁹ to anti-inflammatory cytokines like IL-10^{30,54,55} and TNF α -induced protein 6 (TSG-6).²⁸ Increases in regulatory T-cells⁵⁴ 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 placenta⁵³ and UCB,³⁴ and AFSCs¹² 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- β 1/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 fibrosis⁶² and TGF- β 1,⁴⁶ and to decrease MMP-9⁶³ in BPD. In cardiac disease, BM-MSC⁶⁴ and CPCs¹⁷ 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 intravenous³⁹ or intra-tracheal injection³⁸ 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, 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 (Fig. 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 and 14 days of age⁷⁸ (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 (NCT02381366) and phase I trial in Spain (NCT02443961) 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 <28 weeks gestation still requiring mechanical ventilation at 14 days. Finally, a phase II double-blinded, multicenter, randomized controlled trial administering 10^7 MSC/kg is ongoing (NCT01828957) with a planned long-term follow-up (NCT01897987).

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

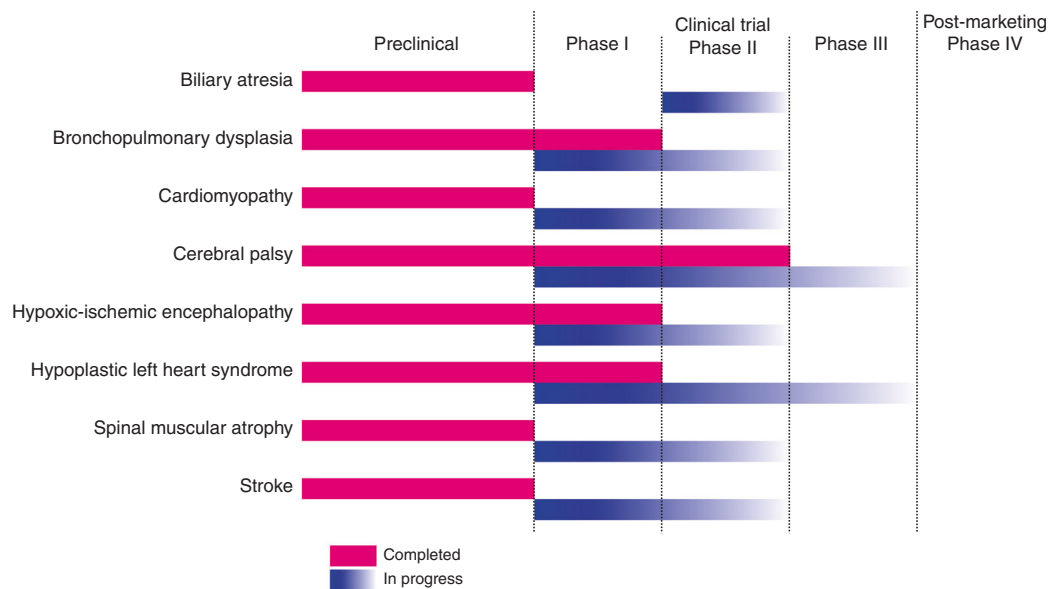


Fig. 2 Current stage of clinical trial development for neonatal diseases. There is accumulating pre-clinical evidence of stem cell efficacy for neonatal diseases, driving initiation of phase I–III clinical trials. No completed phase III or post-marketing phase IV trials have yet been completed for neonatal diseases

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 IFN γ appear to be important mediators of this effect as BDNF knockdown eliminates the beneficial effects of MSC⁹⁴ and the secretome of IFN γ -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 responses¹⁰⁷ are associated with NEC pathogenesis. Survivors of NEC frequently have long term sequelae including short gut syndrome and neurodevelopmental delays.¹⁰² SC have 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 h, to tissues with a high level of inflammation and injury in experimental NEC, decreasing ascites¹¹⁴ 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 (Fig. 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-MSC⁶⁴ and CPC¹⁷ reduce RV dilation and RV strain and reduce cardiomyocyte apoptosis in mice undergoing left anterior

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	NCT01297205	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-theal	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-theal	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-theal	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-theal	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	NCT01528436	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	NCT01273857	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" O: improvement in right ventricle ejection fraction, fewer unplanned catheter interventions, higher weight-for-age z score	119
HIE	I	NCT00593242	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

– 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	NCT03468699	Autologous BM-MNC	–	–	1–15y	20
BPD	I	NCT01207869	UC-MSC	–	intra-tracheal	up to 6m	10
BPD	I	NCT01297205	Allogeneic UCB-MSC	10 or 20 × 10 ⁶ cells/kg	intra-tracheal	up to 14d	9
BPD	I	NCT01632475	Allogeneic UCB-MSC	1 or 2 × 10 ⁷ cells/kg (2 cohorts)	intra-tracheal	4–48m	9
BPD	I	NCT02023788	Allogeneic UCB-MSC	1 or 2 × 10 ⁷ cells/kg (2 cohorts)	intra-tracheal	45–63m	8
BPD	I	NCT02443961	MSC (not further specified)	5 × 10 ⁶ cells × 3 doses	–	1m–28wk	10
BPD	I	NCT03378063	Allogeneic UCB-MSC	–	–	1–3m	100
BPD	I	NCT03631420	UC-MSC	3, 10, or 30 × 10 ⁶ cells/kg (3 cohorts)	–	36–38wk	9
BPD	I	NCT03683953	MSC (not further specified)	25 × 10 ⁶ cells/kg	intra-tracheal	28–37wk	200
BPD	I	NCT03857841	BM-MSC extracellular vesicles	20, 60, 200 pmol phospholipid/kg	intravenous	up to 14d	18
BPD	I	NCT03873506	UC-MSC	1 or 5 × 10 ⁶ cells/kg	intravenous	1m–5y	30
BPD	I–II	NCT02381366	Allogeneic UCB-MSC	1 or 2 × 10 ⁷ cells/kg	–	up to 14d	12
BPD	I–II	NCT03558334	UCB-MSC	1 or 5 × 10 ⁶ cells/kg	intravenous	–	30
BPD	I–II	NCT03645525	UC-MSC	2 × 10 ⁷ cells/kg	intra-tracheal	up to 3wk	180
BPD	I–II	NCT03774537	UC-MSC	1 or 5 × 10 ⁶ cells/kg	intravenous	up to 14d	20
BPD	II	NCT01828957	Allogeneic UCB-MSC	1 × 10 ⁷ cells/kg	intra-tracheal	up to 14d	70
BPD	II	NCT01897987	Allogeneic UCB-MSC	1 × 10 ⁷ cells/kg	intra-tracheal	7m	70
BPD	II	NCT03392467	Allogeneic UCB-MSC	–	–	up to 13d	60
BPD	II	NCT03601416	UC-MSC	1 or 5 × 10 ⁶ cells/kg	intravenous	up to 1y	57
Cardiomyopathy	–	NCT02479776	Autologous BM stem cells	–	–	1–16y	10
Cardiomyopathy	I	NCT03129568	CPC	3 × 10 ⁵ cells/kg	intra-coronary	up to 17y	31
Cardiomyopathy	I	NCT01504594	Autologous CD34+ stem cells	–	intra-coronary	1–16y	10
Cardiomyopathy	I–II	NCT01219452	UC-MSC	multiple (not further specified)	intra-muscular	1–14y	30
Cardiomyopathy	I–II	NCT02256501	Autologous BM-MNC	–	intra-coronary	1–16y	30
CP	–	NCT01193660	Allogeneic UCB	>3e7 nucleated cells/kg	intravenous	10m–10y	105
CP	–	NCT03005249	Neural stem cells	–	–	1–12y	20
CP	–	NCT03414697	UC-MSC	≥1 × 10 ⁷ cells/kg	intravenous vs intra-thecal vs intra-nasal	2–18y	44
CP	I	NCT01404663	CD133+ stem cells	–	intra-thecal	4–12y	12
CP	I	NCT01978821	Autologous BM-MNC	–	–	17m–22y	40
CP	I	NCT02241395	Autologous BM-MNC	–	–	6m–35y	500
CP	I	NCT03087110	12/12 HLA-matched sibling cord blood cells	≥1 × 10 ⁷ cells/kg	intravenous	1–16y	12
CP	I–II	NCT01072370	MNC-enriched cord blood	–	intravenous	1–12y	40
CP	I–II	NCT01763255	BM CD133+ stem cells	–	intra-thecal	4–12y	8
CP	I–II	NCT03078621	Autologous BM-MSC	–	intra-thecal	2–12y	50
CP	I–II	NCT03130816	UCB	≥2 × 10 ⁷ cells/kg	intravenous	10m–20y	90
CP	I–II	NCT03473301	Allogeneic UCB or UC-MSC	1 × 10 ⁸ UCB or 2 × 10 ⁶ MSC/kg	intravenous	2–5y	90
CP	II	NCT01988584	Autologous UCB or BM-MNC	–	–	2–10y	20
CP	II	NCT02231242	Autologous BM nucleated cells	10 mL	intra-thecal	7–9y	60
CP	II	NCT02569775	Autologous BM-MNC	–	intra-thecal	1–15y	40
CP	II	NCT03123562	Autologous BM-MNC	–	intra-thecal	1–15y	25

Table 2 continued

Disease	Phase	NCT number	Cell type	Dose	Route	Age	Target enrollment
CP	II	NCT02574923	Autologous BM-MNC	–	intra-theal	2–15y	30
CP	II	NCT03795974	Allogeneic BUC-MNC or UC-MSc	–	intra-theal	4–14y	108
CP	II	NCT03826498	Allogeneic UCB-MNC	2–5 × 10 ⁷ cells/kg	–	1–10y	40
CP	II	NCT01832454	Autologous MNC	–	intra-theal	3–15y	100
CP	III	NCT01929434	Stem cells (not further specified)	–	intra-theal	1–14y	300
HIE	–	NCT01506258	Autologous CD34+ stem cells	–	intravenous	37–42wk	20
HIE	–	NCT02854579	Fetal neural progenitor cells	4 × 10 ⁶ cells/kg × 3 doses	intra-theal	up to 14d	120
HIE	–	NCT01506258	Autologous CB stem cells	–	intravenous	37–42wk	20
HIE	–	NCT01019733	Autologous BM CD34+ stem cells	–	intra-theal	1–8y	18
HIE	–	NCT01284673	Autologous cord blood	–	–	up to 20 min	10
HIE	I	NCT01962233	UCB-MSc	1–8 × 10 ⁸ cells	intravenous	–	10
HIE	I	NCT03696745	Autologous UCB-SC	5 × 10 ⁷ cells/kg up to 4 times	–	28–37wk	200
HIE	I–II	NCT02881970	Autologous UCB-SC	–	–	up to 3d	20
HIE	II	NCT02434965	Autologous cord blood or placental stem cells	–	–	up to 6h	20
HLHS	–	NCT01856049	UCB harvest	not applicable	not applicable	pregnant women	100
HLHS	I	NCT01273857	Autologous CPC	3 × 10 ⁵ cells/kg	intra-coronary	up to 6y	14
HLHS	I	NCT01883076	Autologous UCB-MNC	3 × 10 ⁶ cells/kg	intra-myocardial	up to 18m	10
HLHS	I	NCT02398604	Allogeneic MSCs	25 × 10 ⁴ cells/kg	intra-myocardial	up to 30d	30
HLHS	I	NCT03406884	Autologous c-kit+ cells	–	intra-coronary	up to 27d	30
HLHS	I	NCT03431480	Autologous UCB-MNC	–	intra-coronary	up to 4d	12
HLHS	I–II	NCT03525418	Allogeneic BM-MSc	25 × 10 ⁴ cells/kg	intra-myocardial	up to 1y	30
HLHS	I–II	NCT03079401	Allogeneic mesenchymal precursor cells	2 × 10 ⁷ cells/kg	intra-myocardial	up to 5y	24
HLHS	II	NCT01829750	CPC	3 × 10 ⁵ cells/kg	intra-coronary	up to 20y	34
HLHS	II	NCT03779711	Autologous UCB-MNC	1–3 × 10 ⁵ cells/kg	intra-myocardial	up to 8m	100
HLHS	III	NCT02781922	Autologous CSC	3 × 10 ⁵ cells/kg	intra-coronary	up to 6y	40
IVH	I	NCT02274428	Allogeneic UCB-MSc	–	–	23–34wk	9
IVH	I	NCT02673788	Allogeneic UCB-MSc	–	–	6m–2y	9
IVH	II	NCT02890953	Allogeneic UCB-MSc	–	intra-ventricular	up to 28d	22
SMA	I–II	NCT02855112	A-MSc	1 × 10 ⁶ cells/kg × 3 doses	intra-theal	5–12m	10
Stroke	I–II	NCT03356821	Allogeneic BM-MSc	5 × 10 ⁷ cells/kg	intra-nasal	up to 10d	10
Urea Cycle Disorders	I–II	NCT01765283	Heterologous adult liver-derived progenitor cells	12.5, 50, or 200 × 10 ⁵ cells/kg (3 cohorts)	–	up to 17y	20
Urea Cycle Disorders	II	NCT02489292	Heterologous adult liver-derived progenitor cells	5 × 10 ⁷ cells/kg	–	up to 12y	20

– 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

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 6 years of 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 × 10⁵ MSC/kg, at the time of stage 2 repair,

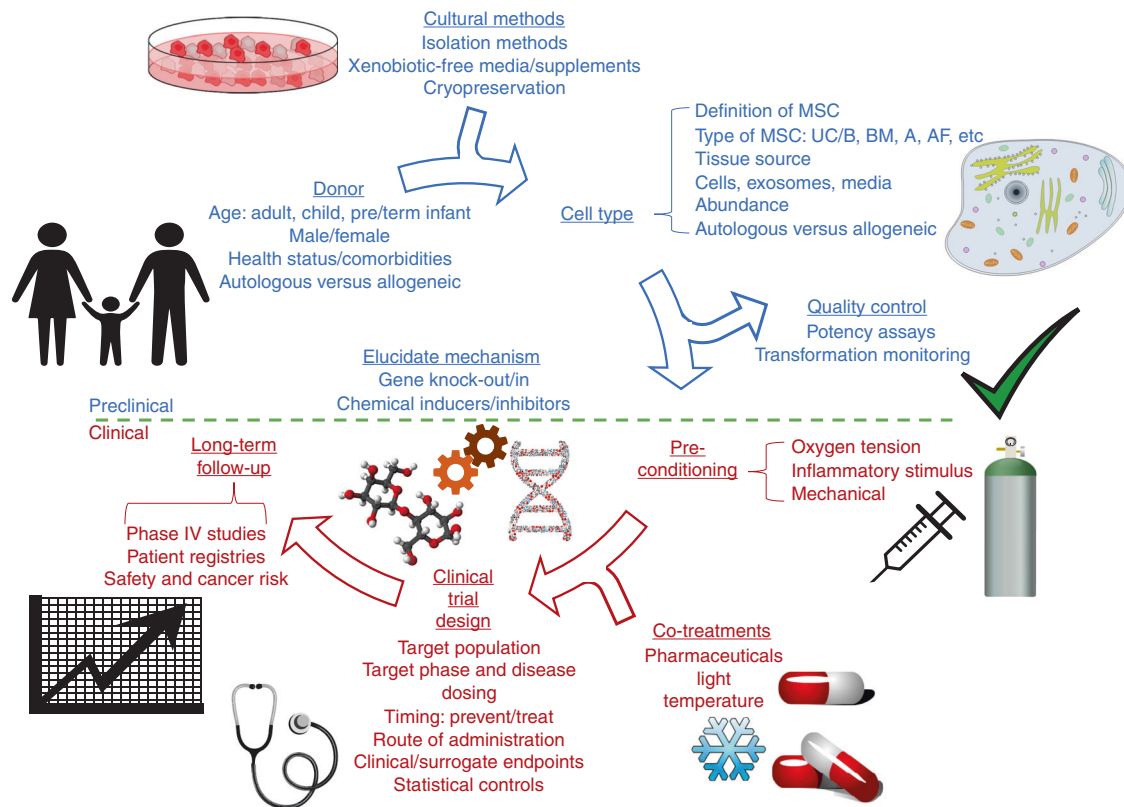


Fig. 3 Blueprint for developing stem cell therapy for the 21st century. There are a variety of factors, both pre-clinical and clinical, that may impact stem cell efficacy that require further investigation, such as donor, culture methods, stem cell type, quality control, stem cell pre-conditioning, co-treatments, clinical trial design, and long-term follow-up, all of which are centered around studies to elucidate the mechanisms of stem cell action

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 20 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-vitreous 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 IFN γ 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 for MSC, early phase clinical trials are already underway

(Fig. 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 (Fig. 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 capacity¹²⁸ 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 (Fig. 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 capacity¹³⁵ and cytokine production.¹³⁶

Quality control and long-term follow up

A lack of *in vitro* potency assays to predict *in vivo* efficacy¹³⁷ 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-MSC¹³⁹ 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 IFN γ ^{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 hypoxic¹⁴², and some neonatal diseases are caused by exposure to hyperoxia (e.g., BPD). For example, hyperoxia pre-conditioning 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 synergistic⁸² 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, considered by appropriate target patient population, and 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 stroke⁴⁹ 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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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, drafting the article, revising it critically for important intellectual content, and gave final approval of the version to be published.

ADDITIONAL INFORMATION

Competing interests: GB is a scientific founder and stock option holder in Scioto Biosciences. CN, RJ, BT, and VS have no financial or other conflicts.

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