Published in final edited form as:

Clin Perinatol. 2015 September ; 42(3): 597–612. doi:10.1016/j.clp.2015.04.008.

STEM CELLS: POTENTIAL THERAPY FOR NEONATAL INJURY?

Momoko Yoshimoto, MD, PhD and

Assistant Research Professor, Pediatrics, Wells Center for Pediatric Research, Indiana University School of Medicine, 1044W Walnut Street R4-W116, Indianapolis, IN 46202, Tel: 317-278-0598

Joyce M Koenig, MD

Professor, Pediatrics, Molecular Microbiology & Immunology, E Doisy Research Center, Saint Louis University School of Medicine, 1100 S Grand Blvd, St Louis, MO 63106, Tel: (314) 977-9291

Momoko Yoshimoto: myoshimo@iu.edu; Joyce M Koenig: koenijm@slu.edu

Synopsis

Stem cell transplantation (SCT) is an established first-line or adjunctive therapy for a variety of neonatal and adult diseases. New evidence in preclinical models as well as a small number of human studies show the potential utility of SCT in neuroprotection and in the modulation of inflammatory injury in at risk-neonates. In this review we briefly summarize current understanding of human stem cell biology during ontogeny and present recent evidence supporting SCT as a viable approach for post-insult neonatal injury.

Keywords

stem cells; hematopoiesis; cord blood; transplantation; neonate; injury

Introduction

Stem cell transplantation (SCT) is an established first-line or adjunctive therapy for a variety of neonatal diseases, including those involving inborn errors of metabolism, types of primary immune deficiencies, certain neutrophil disorders, and hematologic malignancies such as neonatal leukemia. The utility of SCT in these and related conditions have been extensively discussed in the literature, and are beyond the scope of the present review^{1–6}. We here briefly summarize current understanding of human stem cell biology during ontogeny and present recent evidence of the potential role of SCT for the treatment of post-insult neonatal injury.

Correspondence to: Joyce M Koenig, koenijm@slu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosure Statement: The authors have no conflicts of interest relevant to the present review to disclose.

Stem Cell Biology: A Brief Review

Stem cell theory: Pluripotent stem cells and tissue specific somatic stem cells

Two main types of stem cells have been described: Pluripotent stem cells (PSCs) and somatic stem cells. The PSCs are multipotent stem cells that can differentiate into all cell types in the body and include *embryonic stem cells (ESCs)* and *inducible pluripotent stem cells (iPSCs)*. Tissue specific somatic stem cells give rise to organ-specific cell types. Embryonic stem cells (ESCs) are first established from the inner cell mass of the blastocysts in a fertilized egg⁷⁻⁹. *In vitro* studies have shown that cultured ESCs display self-renewal ability and have the capacity for multi-lineage differentiation. ESCs can differentiate into cell types that include all three germ layers, and *in vivo* studies have shown that ESCs can form teratomas when inoculated into immune deficient mice. Murine studies have shown that when a fertilized egg is injected with ESCs and implanted into a pseudo-pregnant dam, ESC-derived cells contribute to all embryonic cell types, forming a chimeric animal. The multi-lineage differentiation ability of ESCs both *in vivo* and *in vitro* highlights their potential utility for stem cell therapies (SCT). However, this therapeutic potential is accompanied by ethical problems since ESCs can be derived only from fertilized eggs.

More recently, embryonic stem (ES)-like pluripotent stem cells have been established from postnatal mouse testis and adult mouse/human somatic cells following the introduction of “stemness genes” such as *Oct4*, *c-myc*, *Sox2*, and *Klf4*¹⁰⁻¹². These ‘inducible pluripotent stem cells’ (iPSC) overcome the ethical problems associated with ESCs; thus, iPSC biology and its possibilities for clinical applications have been the focus of intensive research. However, although mESC/iPSC have been shown to differentiate into somatic stem cells *in vivo* in chimeric animals, the induction of tissue-specific somatic stem cells from iPS cells remains a challenging problem. One primary reason for this is the difficulty in maintaining iPS-derived stem cells in cell lineages that require rapid cell cycling of their progenitors to maintain cellular homeostasis (such as in the blood, skin, and skeletal muscles). Thus, the use of iPSC to produce functional progenitor cells, or even mature cells, may be most successful when cellular targets have relatively slow intrinsic cycling rates and thus do not require rapid somatic cellular replacement. Recent major advances in iPS-derived cell therapy have been reported in a non-human primate spinal injury model and in a clinical trial of iPS-derived retinal pigment epithelium replacement^{13;14}.

According to currently accepted stem cell theory, each tissue in the body is maintained by tissue-specific stem cells with the capacity for self-renewal and specific lineage differentiation. During embryonic organogenesis, stem cells differentiate into lineage cells that form specific tissues. These stem cells are maintained in the tissues even during adulthood: for example, cell types such as hair, skin, melanocytes, blood, muscle, intestinal epithelium, and sperm are continuously regenerated by tissue specific stem/progenitor cells. Although the healthy liver does not typically undergo tissue regeneration, if damaged the liver becomes a regenerative organ. Stem/progenitor cells, which have been identified in every tissue/organ, reside in a special microenvironment, called a *niche*, which facilitates the maintenance of self-renewal capacity. Although the brain and nervous system were not previously considered to have regenerative abilities, recent studies have identified stem/

progenitor cells and their niches even in adult animals^{15–17}. These cells may play a role in the maintenance of tissue homeostasis and can acquire the ability to produce lineage-specific cells for tissue regeneration following injury.

Recent technological advances have facilitated the identification, isolation, and purification of human somatic stem cells. The hematopoietic stem cell (HSC), the first stem cell to be experimentally proven in humans, resides in the bone marrow and is thus readily accessible for clinical purposes. Blood stem/progenitor cells have a homing ability that enables their migration to and engraftment in the bone marrow of the recipient when administered intravenously, without the need for surgical “implanting” procedures. HSC transplantation has been successfully performed to treat a wide spectrum of hematopoietic disorders and malignancies.

The hematopoietic system is the best *in vivo* example of the somatic stem cell theory. Post-transplantation bioassays have defined HSCs and confirmed their self-renewal and multi-lineage blood cell differentiation capacities. Long term (LT-) HSCs exhibit prolonged engraftment in recipient BM (more than 4 months in mice, 4–8 months in humans) and are secondary transplantable. LT-HSCs sit at the apex of the hematopoietic hierarchy system (Figure) and give rise to short term (ST)- HSCs, or multipotent progenitor cells (MPP), that engraft recipient BM for less than 4 months. ST-HSCs/MPPs differentiate into lymphoid-primed progenitor cells (LMPP) and common myeloid progenitor cells (CMP) in mice, or multilymphoid progenitors (MLPs) and CMP in humans. Murine LMPPs and human MLPs are primarily lymphoid progenitors that produce B and T cells but still retain myeloid potential. The more lineage-specific CMPs give rise to erythrocyte, megakaryocytes, granulocytes, monocytes/macrophages, and dendritic cells. Thus, HSCs produce a wide variety of blood cells and are maintained by self-renewal mechanisms within the hematopoietic BM niche.

Since the discovery of HSCs in umbilical cord blood (CB) and the first successful CB transplantation in a patient with Fanconi anemia in 1989, CB has been widely used for HSC transplantation in addition to BM or mobilized peripheral blood (PB) HSCs^{18–20}. In this chapter, we will describe the development of HSCs in the human embryo/fetus and other stem/progenitor cells found in CB. We will also discuss ongoing and possible clinical applications utilizing CB and related stem cells.

Hematopoietic Stem Cells

Human Embryonic HSC Development in Mice and Humans

The first HSCs are produced during embryogenesis, but are also found in the adult BM niche at steady state. Human cord blood contains proportionately greater numbers of circulating CD34+ HSCs than does the peripheral blood of adults²¹.

Developmental hematopoiesis has been well described in mice, an ideal model given a short (19-day) gestation.²² The first site of hematopoiesis, the extra-embryonic *yolk sac (YS)* at embryonic day (E) 7.5, consists of mainly large erythroid cells, called *primitive erythrocytes*²³. Primitive erythrocytes express embryonic-type hemoglobin molecules and

have a large nucleus²⁴. Erythroid progenitor cells that express adult type hemoglobin molecules are called *definitive* erythroid progenitor cells and are detected from E8.0 YS together with myeloid progenitors²⁵. The progenitors of definitive erythroid cells and myeloid cells are called *erythro-myeloid progenitors* (EMPs) and are produced mainly in the YS during E8 to 10²⁶. The first murine HSCs that can reconstitute lethally irradiated adult marrow by transplantation assay are detectable at E11 in the *aorta-gonado-mesonephros* (AGM) region^{27;28}. These EMPs and HSCs are all derived from endothelial cells, called *hemogenic endothelial cells* (HECs)^{29–32}. The transition from HECs to hematopoietic cells occurs between E7.5 to E11.5³³. HSCs produced by the HECs in the aortic area migrate into the fetal liver and placenta, where they subsequently undergo a massive expansion^{34–36}. HSCs migrate into the spleen and bone marrow at the end of gestation, just before birth, and HSCs ultimately reside in the bone marrow niche to maintain hematopoiesis throughout the life.

Similarly, in humans, hematopoiesis is first observed in the YS as early as embryonic day 18 (presomite stage)³⁷. Primitive and definitive hematopoiesis are observed in the YS up to 7 weeks of gestation and are gradually replaced by fetal liver hematopoiesis, which has been initiated by gestational day 30^{38;39}. Hematopoietic clusters expressing CD34 and CD45 have been observed in the ventral wall of the dorsal aorta beginning on day 27^{40–42}. These clusters rapidly increase in size and can attain several hundreds of cells by day 36. The intra-aortic CD34+ cells express important hematopoietic transcriptional factors including *tal*/*SCL*, *c-myb*, and *GATA2*, as well as CD143 (angiotensin-converting enzyme), which is known to enrich HSC activity in CB CD34+ cells^{43;44}. Studies have shown that NOD/SCID/IL2R γ c^{null} (NSG) mice can be reconstituted with human HSC⁴⁵, and active human colony forming activities are detectable in the recipient mouse bone marrow. The first human HSCs shown to reconstitute NSG mice for up to 8 months have been identified in the day 32 AGM region⁴⁶. These AGM-derived HSC are detectable in the peripheral blood of recipient mice 3 months post transplantation. Their numbers gradually increase over time, reaching up to 90% of chimerism by 8 months following transplantation. Human AGM-derived cells also reconstitute secondary recipient mice and display self-renewal capacity (a key measure of stem cell function), generating at least 300 daughter HSCs⁴⁶. HSC in the AGM region express CD34, CD45, VE-cadherin, c-kit, thy-1, and endoglin, but not CD38⁴⁷. Since VE-cadherin is an endothelial specific marker, these observations suggest that human HSCs are derived from HECs as has been observed in murine HSCs. Fetal liver HSCs are first detected on gestational day 42 and express CD34, CD45. VE-cadherin is also initially expressed on these HSCs, although this is lost after 10 weeks of gestation⁴⁸.

Circulating CD34+ HSCs are found in both full term and preterm CB^{49;50}. The proportions of CD34+ cells in preterm CB are generally higher than in full term CB, although the absolute numbers of CD34+ cells reported has been variable. While preterm CB CD34+ cells exhibit higher colony forming ability than full term CB CD34+ cells⁴⁹, preterm CD34+ cells may have lower repopulating ability⁴⁸. The ability of transplanted CD34+ cells to repopulate the bone marrow in patients with severe combined immune deficiency (SCID) has been shown to correlate with the expression level of CXCR4, a surface receptor involved in cellular migration, or homing, to the bone marrow³⁹. Thus functional differences

between preterm and term CD34+ cells may be linked to a relative developmental deficiency of CXCR4 in preterm CD34+ cells⁴⁹.

Mesenchymal stem cells (MSCs)

Original observations showed that implantation of BM cells (without bone) into non-hematopoietic tissues (such as under skin or peritoneal cavity) induced the development of reticular tissue followed by bone formation that could sustain ectopic hematopoiesis^{51–53}. This ectopic bone formation is derived from a non-hematopoietic cell population that forms a clonal fibroblastic colony *in vitro*, the CFU-F⁵⁴. The progeny of CFU-F contribute to bone formation following transplantation and can differentiate into multiple skeletal tissues including bone, cartilage, adipose tissue, and fibroblastic tissue *in vivo*. This rare BM population was first named an *osteogenic stem cell* or a BM stromal stem cell^{55;56}. The discovery of this cell type was important in that it identified the presence of a second stem cell population in the BM in addition to HSCs. The currently used term, *mesenchymal stem cell (MSC)*, was first proposed in 1991 based on the capacity to differentiate into cells of the mesenchymal lineage (bone, cartilage, tendon, ligament, BM stroma, adipocytes, dermis, muscle, and connective tissue)⁵⁷. However, the “multipotency” of MSCs remains controversial^{56;58}. Furthermore, while MSCs are often referred to as “stem cells”, they do not meet strict criteria regarding self-renewal capacity and multilineage potential at the single cell level⁵⁵. A more conservative definition of MSCs (*mesenchymal stromal cells*⁵⁹ or skeletal stem/progenitors⁶⁰) include the following: 1) They possess CRU-F that can form fibroblastic colonies *in vitro*; 2) They have the capacity to differentiate into adipocytes, osteocytes, and chondrocytes *in vivo* and *in vitro*; and 3) They support hematopoietic development.

MSCs can be derived from embryonic limbs, postnatal BM, umbilical cord blood and other tissues in mouse and man^{51;57;61–63}, including the perivascular area or BM sinusoids⁶⁴. Their developmental origins are primarily from mesoderm but they also originate in part from neuroepithelium⁶⁵. MSCs express unique surface antigens including Stro-1, CD271, CD146, but lack hematopoietic and endothelial markers (CD45, CD14, CD11b, CD79, CD19, HLA-DR, CD34, and CD31).

MSC exhibit poor engraftment capacity following intravenous injection, and the small number that do engraft survive for only a short time in the recipient animals⁶⁶. Despite this shortcoming, MSCs may be useful for: 1) Direct cell replacement for tissue regeneration; and 2) Indirect effects on damaged tissue related to MSC secretion of immunosuppressive factors. The therapeutic benefit of MSCs appears to be greatest for the treatment of inflammatory diseases through the release of anti-inflammatory cytokines.

Endothelial progenitors: endothelial colony forming cells (ECFC)

The first putative endothelial progenitors were isolated from human adult PB in 1997 by Asahara *et al.*⁶⁷. The major discovery that endothelial progenitor cells (EPCs) circulate in the peripheral blood led to the concept that these “EPCs” contribute to the repair of vascular injuries. However, this nomenclature was unfortunate, as it has created long-standing confusion regarding the true definition of “EPCs”: the cells originally defined as EPCs have

since been designated as hematopoietic cells, not endothelial cells⁶⁸. The term that now most reliably defines circulating endothelial progenitors obtained from human PB or CB is *endothelial colony-forming cell (ECFC)*⁶⁹. ECFCs are strictly defined as endothelial progenitors and are differentiated from “EPCs” based on specific criteria. These include: 1) The *in vitro* ability of cloned cells to form large colonies with high proliferative potential that can be re-cultured to form secondary colonies; 2) The *in vivo* capacity to form capillaries that anastomose with host vessels; 3) The *in vitro* capacity to form tubes with lumens when plated in collagen; 4) Absence of hematopoietic surface markers, but surface expression of other unique markers including CD31, CD146, CD144, CD105, CD34 (partially), KDR, vWF, and lectins; and 5) Lack of phagocytic function. ECFCs or endothelial cells do not have phagocytic function, whereas “EPCs” or macrophages easily phagocytize *E. coli* fragments.

ECFCs are quite rare in CB (2 out of 10⁸ CB MNCs); however, their numbers are relatively enriched in CB compared to adult PB. CB-ECFCs have long telomeres and their HPP is also much higher than adult PB-ECFC⁶⁹. While the cells currently designated as EPCs are not true endothelial cells, they share similarities to the recently reported pro-angiogenic AC133⁺CD34⁺CD45^{dim}lin⁻ hematopoietic stem/progenitor cells and may contribute to neovascularization in cardiovascular disease or tumor progression^{70–72}.

iPS and iPS-derived ECFC

Human iPS cells have been established in various human cells. Although CB does not contain truly pluripotent stem cells, CB can be induced into iPS cells when cultured in the presence of defined factors^{73;74}. Cord blood iPS cells are hardy: a recent report showed that CB MNCs cryopreserved for over 20 years could still be utilized for HSC transplantation, ECFC isolation and the induction of iPS cells⁷⁵. In addition, a novel culture system to produce ECFCs from human iPS has been established⁷⁶. Human iPS derived-ECFCs display high clonal proliferative ability and have the capacity to form human vessels in mice. Importantly, these pluripotent cells can repair ischemic regions in mouse retina and limb injury models without inducing secondary teratoma formation. Thus, CB is useful not only for its stem cell populations but also for the induction of iPS cells that can be stored for future clinical use.

Stem Cells for the Treatment of Post-Insult Injury in Neonates

Neonates are highly susceptible to inflammatory and/or ischemic insults, particularly to critical organs such as the brain and lungs; this risk is considerably higher in the most immature, preterm infants^{77–79}. The damaging and often permanent effects related to these newborn insults are associated with high economic and societal costs, while the personal suffering of the afflicted and their families is incalculable^{80;81}. Thus, the successful prevention or treatment of insult-related injury is of utmost and immediate importance.

Neonatal injury and its inherent complications have been intensively studied for many years. Investigations in relevant animal models and human studies have advanced our understanding of the underlying mechanisms; however, treatments to prevent or to arrest injury as a result of these insults in many cases have been disappointing, until recently. A

growing body of evidence and exciting new discoveries indicate the potential (and in some cases, actual) regenerative role of SCT in the treatment of neonatal injury, as briefly reviewed below.

Brain injury and SCT

Hypoxic-ischemic encephalopathy—Studies in animal models have been critical to defining the mechanisms underlying post-insult brain injury. These models have been based on approaches that focus on either cellular/molecular mechanisms or that recapitulate the physiologic events that produce injury; these have been recently elegantly reviewed⁸². However, existing evidence has been distilled from studies using a spectrum of animal models and SCT approaches, primarily involving human amnion epithelial-derived cells (hAECs), MSC, whole cord blood and neural stem cells. Thus, the reparative benefits of SCT for post-insult brain injury and the specific cells involved remain unclearly defined (reviewed in⁸³).

Umbilical cord blood is a repository of a plethora of stem cell types, including those of hematopoietic, endothelial and mesenchymal origin, as was discussed in the first part of this review. Rodent models of neonatal hypoxic-ischemic brain injury have exhibited structural and behavioral improvements following transplantation with human cord blood cells, including measurable improvements in cognition{de Paula S., 2012 19297 /id;Wasielewski, 2012 20076 /id;Geissler, 2011 20087 /id}. However, the specific stem cell type(s) or cell combinations in cord blood that confer therapeutic properties have not been well defined. The underlying mechanisms are also unclear; evidence of poor engraftment in animals that respond positively to SCT suggest that the ameliorative effects are not due to the regenerative capabilities of the administered stem cells⁸⁷. A relationship has been established between neuroprotection and indirect (paracrine and trophic) modulatory effects of administered stem cells, including MSCs and hAECs, on inflammatory and excitotoxic neural responses^{88;89}. Administration of hAECs to sheep fetuses was also shown to confer protection from inflammation-induced brain injury⁹⁰.

Studies of cord blood SCT in humans, while sparse, are increasing. In a recently reported prospective trial, 23 infants with hypoxic-ischemic encephalopathy received both head cooling and autologous cord blood transfusion within the first 72 hours of life⁹¹. The authors concluded that this form of SCT was both safe and feasible even in outborn infants transferred to a tertiary care hospital. However, no firm conclusions could be made regarding hospital and developmental outcomes or 1-year survival. In a feasibility pilot study, the transfusion of autologous cord blood from private cord blood banks to young children with neurologic disorders was found to be safe⁹². In a Korean study of 96 infants and children with cerebral palsy, subjects who received matched allogeneic cord blood cells with erythropoietin fared better developmentally six months later than did their counterparts given erythropoietin only or placebo⁹³. Importantly, this study highlights the potential therapeutic utility of banked allogeneic donor cord blood cells in SCT of brain injury.

Neonatal stroke—Stroke is a primarily ischemic event that occurs in the fetal or neonatal periods and involves ischemia of cerebral arteries or periventricular venous infarction⁹⁴.

Strokes in the perinatal period commonly (60% of cases) result in neurologic deficits, with hemiplegic cerebral palsy being a frequent complication. A variety of preclinical studies have shown potential therapeutic value of stem cell populations derived from human umbilical CB in limiting injury and in promoting functional recovery. In rodent stroke models, the administration of neural stem cells lead to glial and neuronal differentiation at sites of injury, while transplanted MSCs were indirectly beneficial by inducing the release of neuroprotective trophic factors that dampened inflammation^{95;96}. Human CB-derived AC133+ EPCs limited infarct size and shortened the resolution period in a rat stroke model⁹⁷. Stem cell types in addition to those derived from CB may also be of value. The administration of amniotic fluid-derived stem cells to adult rats with ischemic stroke also resulted in significant functional recovery⁹⁸. However, the most beneficial stem cell type for the treatment of stroke remains unclear. Transplantation with the broader array of stem cells contained in human CD34+ cells bestowed a limited benefit in one rodent stroke model, while another study showed greater therapeutic effect and less inflammation in animals receiving CB mononuclear cells compared to CB MSCs^{99;100}. While the potential therapeutic utility of SCT in neonates with stroke is supported by preclinical studies, however its actual utility in human neonates, while promising, remains to be defined¹⁰¹.

Lung injury and SCT

Extremely preterm infants are at high risk for numerous long-term complications, of which bronchopulmonary dysplasia (BPD) is the most common¹⁰². The “new” BPD is associated with lung growth arrest as well as abnormal vascularization and fibrosis, and it can result in disabling lung abnormalities that persist into adulthood⁷⁹. Studies in neonatal animal models of BPD have shown that transplantation with MSC or soluble MSC-derived proteins (the MSC “secretome”) can modulate BPD by dampening inflammatory processes^{96;103–105}. In a recently reported phase I dose-escalation trial, a small group of extremely preterm infants who received intra-tracheal autologous MSC had diminished BPD severity and lowered inflammatory cytokine levels in tracheal aspirates compared to historical controls¹⁰⁶. Importantly, MSC treatment was clinically well tolerated in these tiny babies and was not associated with discernable short-term safety issues. The potential benefits of cord blood MSCs have stimulated numerous ongoing clinical trials addressing their use for the treatment of diverse disorders in adults and several in preterm infants.

Intestinal injury and SCT

Necrotizing enterocolitis (NEC), an intestinal inflammatory disorder with infectious components, is a common and potentially devastating complication of prematurity¹⁰⁷. The etiology of NEC is multifactorial and includes ischemia-reperfusion injury, disturbances of the intestinal microbiome and prior inflammatory exposure. The potential role of SCT in the treatment of neonatal NEC has only recently been addressed. In an *in vivo* neonatal rat model of NEC, intraperitoneal administration of bone-marrow derived MSCs was associated with attenuated histologic intestinal injury, improved weight gain, and decreased clinical illness scores¹⁰⁸. MSC homing to intestinal tissue was enhanced in the pups with NEC compared to normal controls, suggesting a potentiating role for inflammation in the engraftment of MSC. In another study, neonatal rats with NEC that were treated with amniotic fluid stem cells showed improved gut structure, survival and reduced inflammatory

mechanisms mediated via COX-2¹⁰⁹. Despite these encouraging preclinical data, a role for SCT in the treatment of NEC in human neonates has not been reported to date.

Utility of Neonatal SCT: General Considerations

A number of critical questions remain to be answered through well-designed pre-clinical large animal models and human clinical trials before the use of umbilical CB or specific stem cells afford reasonable therapeutic options. One important issue is that of the optimal timing of SCT administration in the context of the injury. Studies in infants with brain injury have shown a secondary post-injury period during which inflammatory and excitatory mechanisms become pronounced, thus the time between the initial insult and this later phase may be the ideal window to institute SCT as a therapeutic measure¹¹⁰. Conversely, a Korean study showed benefit of SCT in children with cerebral palsy, even in those who were 10 years of age¹¹¹, which suggests a possible benefit despite long-standing neurologic injury.

Another key issue to be addressed is the identification of the ideal stem cell source for a specific type of tissue injury. As detailed earlier in this review, umbilical cord blood contains numerous stem cell types, including those of hematopoietic, endothelial, and mesenchymal origins. However, it remains unclear if and how the perinatal factors contributing to neonatal hypoxic-ischemic encephalopathy or other injury could potentially influence the functionality and intrinsic properties of the cord blood stem cells themselves¹¹².

The role of specific stem cell types in various target organs also remains incompletely defined. Mesenchymal stem cells (MSC) derived from developing humans exhibit a high degree of mesodermal pluripotency (the capacity to differentiate into multiple cell types); in addition, these MSC are associated with a low induction of immune responses in the recipient and exhibit anti-inflammatory properties^{96;113}. Another type of cell with pluripotent properties is the hAEC¹¹⁴. Like MSCs, hAECs are anti-inflammatory and appear to be immunologically well-tolerated^{115;116}. Studies in fetal sheep showed that AEDC administration was protective against brain inflammation, including periventricular white matter injury⁹⁰. As also observed with MSCs, the beneficial effect of hAECs may be primarily related to the release of soluble protective factors⁸⁷.

In addition to the optimal stem cell type and timing of administration necessary for therapeutic efficacy, other questions that remain to be answered include: the most ideal stem cell sources (autologous *versus* allogeneic, cord blood *versus* isolated stem cells, primary cells *versus* derived or genetically modified cells), host tolerance, and safety, among others^{110;117}. While short-term safety may be acceptable, as suggested by the very small number of recent human studies, long-term effects remain unknown. This latter point may be of particular importance to very preterm infants who are still undergoing developmental maturation, including of the immune and hematopoietic systems. Another critical safety feature to be defined involves the potential tumorigenicity or cancerous conversion of the transplanted stem cells, a question that remains enigmatic.

Summary

True stem cells or cells with pluripotent properties are found in a variety of tissues and organs. Umbilical cord blood can provide a spectrum of stem or pluripotent progenitor cells, many of which have a greater potential for proliferation (although not necessarily engraftment) compared to similar cells mobilized from the bone marrow into the peripheral blood of adults. Preclinical studies have shown a therapeutic benefit of SCT in perinatal and neonatal injuries, including those involving the brain, lungs and intestines. The very few human studies to date suggest short-term safety of MSCs or cord blood (both autologous and allogeneic) SCT and have variably shown measurable improvements in functional or inflammatory parameters. However, the therapeutic mechanisms remain incompletely defined, and the optimal usage of SCT for a particular type of injury is also unclear. Thus, despite its exciting potential for currently untreatable neonatal disorders, extensive studies in both preclinical models and in humans will be necessary before SCT becomes an accepted form of therapy. Although still quite limited relative to studies in adults, an increasing number of human trials now address these issues in neonates (<https://ClinicalTrials.gov>).

REFERENCES

1. Boelens JJ, Orchard PJ, Wynn RF. Transplantation in inborn errors of metabolism: current considerations and future perspectives. *Br J Haematol*. 2014; 167:293–303. [PubMed: 25074667]
2. Westgren M, Ringden O, Bartmann P, et al. Prenatal T-cell reconstitution after in utero transplantation with fetal liver cells in a patient with X-linked severe combined immunodeficiency. *Am J Obstet Gynecol*. 2002; 187:475–482. [PubMed: 12193946]
3. van d V, van den Berg TK, Kuijpers TW. Leukocyte adhesion deficiencies. *Hematol Oncol Clin North Am*. 2013; 27:101–116. [PubMed: 23351991]
4. Moyer MW. Cell banks: life blood. *Nature*. 2013; 498:S16. [PubMed: 23803945]
5. Sison EA, Brown P. Does hematopoietic stem cell transplantation benefit infants with acute leukemia? *Hematology Am Soc Hematol Educ Program*. 2013; 2013:601–604. [PubMed: 24319238]
6. Peffault de LR, Porcher R, Dalle JH, et al. Allogeneic hematopoietic stem cell transplantation in Fanconi anemia: the European Group for Blood and Marrow Transplantation experience. *Blood*. 2013; 122:4279–4286. [PubMed: 24144640]
7. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981; 292:154–156. [PubMed: 7242681]
8. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A*. 1981; 78:7634–7638. [PubMed: 6950406]
9. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145–1147. [PubMed: 9804556]
10. Kanatsu-Shinohara M, Inoue K, Lee J, et al. Generation of pluripotent stem cells from neonatal mouse testis. *Cell*. 2004; 119:1001–1012. [PubMed: 15620358]
11. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
12. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131:861–872. [PubMed: 18035408]
13. Kobayashi Y, Okada Y, Itakura G, et al. Pre-evaluated safe human iPSC-derived neural stem cells promote functional recovery after spinal cord injury in common marmoset without tumorigenicity. *PLoS One*. 2012

14. Kamao H, Mandai M, Okamoto S, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Reports*. 2014; 2:205–218. [PubMed: 24527394]
15. Song HJ, Stevens CF, Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat Neurosci*. 2002; 5:438–445. [PubMed: 11953752]
16. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 1999; 97:703–716. [PubMed: 10380923]
17. Tavazoie M, Van der Veken L, Silva-Vargas V, et al. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell*. 2008; 3:279–288. [PubMed: 18786415]
18. Nakahata T, Ogawa M. Hemopoietic colony-forming cells in umbilical cord blood with extensive capability to generate mono- and multipotential hemopoietic progenitors. *J Clin Invest*. 1982; 70:1324–1328. [PubMed: 7174797]
19. Broxmeyer HE, Douglas GW, Hangoc G, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci U S A*. 1989; 86:3828–3832. [PubMed: 2566997]
20. Gluckman E, Broxmeyer HA, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med*. 1989; 321:1174–1178. [PubMed: 2571931]
21. Bender JG, Unverzagt K, Walker DE, et al. Phenotypic analysis and characterization of CD34+ cells from normal human bone marrow, cord blood, peripheral blood, and mobilized peripheral blood from patients undergoing autologous stem cell transplantation. *Clin Immunol Immunopathol*. 1994; 70:10–18. [PubMed: 7505211]
22. Lin Y, Yoder MC, Yoshimoto M. Lymphoid progenitor emergence in the murine embryo and yolk sac precedes stem cell detection. *Stem Cells Dev*. 2014; 23:1168–1177. [PubMed: 24417306]
23. Moore MA, Metcalf D. Ontogeny of the haemopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. *Br J Haematol*. 1970:279–296. [PubMed: 5491581]
24. Barker JE. Development of the mouse hematopoietic system. I. Types of hemoglobin produced in embryonic yolk sac and liver. *Dev Biol*. 1968; 18:14–29. [PubMed: 5669501]
25. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development*. 1999; 126:5073–5084. [PubMed: 10529424]
26. Lux CT, Yoshimoto M, McGrath K, Conway SJ, Palis J, Yoder MC. All primitive and definitive hematopoietic progenitor cells emerging before E10 in the mouse embryo are products of the yolk sac. *Blood*. 2008; 111:3435–3438. [PubMed: 17932251]
27. Muller AM, Medvinsky A, Strouboulis J, Grosveld F, Dzierzak E. Development of hematopoietic stem cell activity in the mouse embryo. *Immunity*. 1994; 1:291–301. [PubMed: 7889417]
28. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell*. 1996; 86:897–906. [PubMed: 8808625]
29. Nishikawa SI, Nishikawa S, Kawamoto H, et al. In vitro generation of lymphohematopoietic cells from endothelial cells purified from murine embryos. *Immunity*. 1998; 8:761–769. [PubMed: 9655490]
30. de Bruijn MF, Ma X, Robin C, Ottersbach K, Sanchez MJ, Dzierzak E. Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. *Immunity*. 2002; 16:673–683. [PubMed: 12049719]
31. Chen MJ, Yokomizo T, Zeigler BM, Dzierzak E, Speck NA. Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature*. 2009; 457:887–891. [PubMed: 19129762]
32. Chen MJ, Li Y, De Obaldia ME, et al. Erythroid/myeloid progenitors and hematopoietic stem cells originate from distinct populations of endothelial cells. *Cell Stem Cell*. 2011; 9:541–552. [PubMed: 22136929]

33. Tober J, Yzaguirre AD, Piwarzyk E, Speck NA. Distinct temporal requirements for Runx1 in hematopoietic progenitors and stem cells. *Development*. 2013; 140:3765–3776. [PubMed: 23924635]
34. Ema H, Nakauchi H. Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. *Blood*. 2000; 95:2284–2288. [PubMed: 10733497]
35. Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK. The placenta is a niche for hematopoietic stem cells. *Dev Cell*. 2005; 8:365–375. [PubMed: 15737932]
36. Ottersbach K, Dzierzak E. The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. *Dev Cell*. 2005; 8:377–387. [PubMed: 15737933]
37. Bloom W, Bartelmez GW. Hematopoiesis in young human embryos. *Am J Anat*. 1940; 67:21–53.
38. Huyhn A, Dommergues M, Izac B, et al. Characterization of hematopoietic progenitors from human yolk sacs and embryos. *Blood*. 1995; 86:4474–4485. [PubMed: 8541536]
39. Migliaccio G, Migliaccio AR, Petti S, et al. Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac---liver transition. *J Clin Invest*. 1986; 78:51–60. [PubMed: 3722384]
40. Tavian M, Hallais MF, Peault B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. *Development*. 1999; 126:793–803. [PubMed: 9895326]
41. Tavian M, Coulombel L, Luton D, Clemente HS, Dieterlen-Lievre F, Peault B. Aorta-associated CD34+ hematopoietic cells in the early human embryo. *Blood*. 1996; 87:67–72. [PubMed: 8547678]
42. Tavian M, Robin C, Coulombel L, Peault B. The human embryo, but not its yolk sac, generates lympho-myeloid stem cells: mapping multipotent hematopoietic cell fate in intraembryonic mesoderm. *Immunity*. 2001; 15:487–495. [PubMed: 11567638]
43. Jokubaitis VJ, Sinka L, Driessen R, et al. Angiotensin-converting enzyme (CD143) marks hematopoietic stem cells in human embryonic, fetal, and adult hematopoietic tissues. *Blood*. 2008; 111:4055–4063. [PubMed: 17993616]
44. Labastie MC, Cortes F, Romeo PH, Dulac C, Peault B. Molecular identity of hematopoietic precursor cells emerging in the human embryo. *Blood*. 1998; 92:3624–3635. [PubMed: 9808556]
45. Ito M, Hiramatsu H, Kobayashi K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood*. 2002; 100:3175–3182. [PubMed: 12384415]
46. Ivanovs A, Rybtsov S, Welch L, Anderson RA, Turner ML, Medvinsky A. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. *J Exp Med*. 2011; 208:2417–2427. [PubMed: 22042975]
47. Ivanovs A, Rybtsov S, Anderson RA, Turner ML, Medvinsky A. Identification of the niche and phenotype of the first human hematopoietic stem cells. *Stem Cell Reports*. 2014; 2:449–456. [PubMed: 24749070]
48. Oberlin E, Fleury M, Clay D, et al. VE-cadherin expression allows identification of a new class of hematopoietic stem cells within human embryonic liver. *Blood*. 2010; 116:4444–4455. [PubMed: 20693433]
49. Nakajima M, Ueda T, Migita M, et al. Hematopoietic capacity of preterm cord blood hematopoietic stem/progenitor cells. *Biochem Biophys Res Comm*. 2009; 389:290–294. [PubMed: 19720051]
50. Wisgrill L, Schuller S, Bammer M, et al. Hematopoietic stem cells in neonates: any differences between very preterm and term neonates? *PloS One*. 2014
51. Friedenstein AJ, Piatetzky S II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol*. 1966; 16:381–390. [PubMed: 5336210]
52. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation*. 1974; 17:331–340. [PubMed: 4150881]
53. Tavassoli M, Crosby WH. Transplantation of marrow to extramedullary sites. 20105. *Science*. 1968; 161:54–56. [PubMed: 4871792]
54. Friedenstein AJ, Chailakhyan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet*. 1970; 3:393–403. [PubMed: 5523063]

55. Friedenstein AJ. Precursor cells of mechanocytes. *Int Rev Cytol.* 1976; 47:327–359. [PubMed: 11195]
56. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell.* 2008; 2:313–319. [PubMed: 18397751]
57. Caplan AI. Mesenchymal stem cells. *J Orthop Res.* 1991; 9:641–650. [PubMed: 1870029]
58. Kluth SM, Radke TF, Kogler G. Potential application of cord blood-derived stromal cells in cellular therapy and regenerative medicine. *J Blood Transfus.* 2012:365182. [PubMed: 24066257]
59. 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.* 2006; 8:315–317. [PubMed: 16923606]
60. Bianco P, Robey PG, Saggio I, Riminucci M. "Mesenchymal" stem cells in human bone marrow (skeletal stem cells): a critical discussion of their nature, identity, and significance in incurable skeletal disease. *Hum Gene Ther.* 2010; 21:1057–1066. [PubMed: 20649485]
61. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol.* 2000; 109:235–242. [PubMed: 10848804]
62. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell.* 2008; 3:301–313. [PubMed: 18786417]
63. Yamamoto N, Akamatsu H, Hasegawa S, et al. Isolation of multipotent stem cells from mouse adipose tissue. *J Dermatol Sci.* 2007; 48:43–52. [PubMed: 17644316]
64. Sacchetti B, Funari A, Michienzi S, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell.* 2007; 131:324–336. [PubMed: 17956733]
65. Takashima Y, Era T, Nakao K, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. *Cell.* 2007; 129:1377–1388. [PubMed: 17604725]
66. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol.* 2014; 15:1009–1016. [PubMed: 25329189]
67. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* 1997; 275:964–967. [PubMed: 9020076]
68. Yoder MC, Mead LE, Prater D, et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood.* 2007; 109:1801–1809. [PubMed: 17053059]
69. Ingram DA, Mead LE, Tanaka H, et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood.* 2004; 104:2752–2760. [PubMed: 15226175]
70. Estes ML, Mund JA, Mead LE, et al. Application of polychromatic flow cytometry to identify novel subsets of circulating cells with angiogenic potential. *Cytometry A.* 2010; 77:831–839. [PubMed: 20803735]
71. Pradhan KR, Mund JA, Johnson C, Vik TA, Ingram DA, Case J. Polychromatic flow cytometry identifies novel subsets of circulating cells with angiogenic potential in pediatric solid tumors. *Cytometry B Clin Cytom.* 2011; 80:335–338. [PubMed: 21567939]
72. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med.* 2003; 348:593–600. [PubMed: 12584367]
73. Ye Z, Zhan H, Mali P, et al. Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders. *Blood.* 2009; 114:5473–5480. [PubMed: 19797525]
74. Okita K, Yamakawa T, Matsumura Y, et al. An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells. *Stem Cells.* 2013; 31:458–466. [PubMed: 23193063]
75. Broxmeyer HE, Lee MR, Hangoc G, et al. Hematopoietic stem/progenitor cells, generation of induced pluripotent stem cells, and isolation of endothelial progenitors from 21- to 23.5-year cryopreserved cord blood. *Blood.* 2011; 117:4773–4777. [PubMed: 21393480]

76. Prasain N, Lee MR, Vemula S, et al. Differentiation of human pluripotent stem cells to cells similar to cord-blood endothelial colony-forming cells. *Nat Biotechnol.* 2014; 32:1151–1157. [PubMed: 25306246]
77. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum Dev.* 2010; 86:329–338. [PubMed: 20554402]
78. Malaeb S, Dammann O. Fetal inflammatory response and brain injury in the preterm newborn. *J Child Neurol.* 2009; 24:1119–1126. [PubMed: 19605775]
79. Jobe AH. The new bronchopulmonary dysplasia. *Curr Opin Pediatr.* 2011; 23:167–172. [PubMed: 21169836]
80. Johnston KM, Gooch K, Korol E, et al. The economic burden of prematurity in Canada. *BMC Pediatr.* 2014; 14:93. [PubMed: 24708755]
81. Flood K, Malone FD. Prevention of preterm birth. *Semin Fetal Neonatal Med.* 2012; 17:58–63. [PubMed: 21893439]
82. Fleiss B, Guillot PV, Titomanlio L, Baud O, Hagberg H, Gressens P. Stem cell therapy for neonatal brain injury. *Clin Perinatol.* 2014; 41:133–148. [PubMed: 24524451]
83. Castillo-Melendez M, Yawno T, Jenkin G, Miller SL. Stem cell therapy to protect and repair the developing brain: a review of mechanisms of action of cord blood and amnion epithelial derived cells. *Front Neurosci.* 2013; 7:194. [PubMed: 24167471]
84. de Paula S, Greggio S, Marinowicz DR, Machado DC, DaCosta JC. The dose-response effect of acute intravenous transplantation of human umbilical cord blood cells on brain damage and spatial memory deficits in neonatal hypoxia-ischemia. *Neuroscience.* 2012; 210:431–441.
85. Wasielewski B, Jensen A, Roth-Harer A, Dermietzel R, Meier C. Neuroglial activation and Cx43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury. *Brain Res.* 2012; 1487:39–53. [PubMed: 22796290]
86. Geissler M, Dinse HR, Neuhoﬀ S, Kreikemeier K, Meier C. Human umbilical cord blood cells restore brain damage induced changes in rat somatosensory cortex. *PLoS One.* 2011; 6:e20194. [PubMed: 21673795]
87. Tan JL, Chan ST, Wallace EM, Lim R. Human amnion epithelial cells mediate lung repair by directly modulating macrophage recruitment and polarization. *Cell Transplant.* 2014; 23:319–328. [PubMed: 23294809]
88. Meier C, Middelani J, Wasielewski B, et al. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr Res.* 2006; 59:244–249. [PubMed: 16439586]
89. Pimentel-Coelho PM, Magalhaes ES, Lopes LM, deAzevedo LC, Santiago MF, Mendez-Otero R. Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection in the striatum. *Stem Cells Dev.* 2010; 19:351–358. [PubMed: 19296724]
90. Yawno T, Schuilwerf J, Moss TJ, et al. Human amnion epithelial cells reduce fetal brain injury in response to intrauterine inflammation. *Dev Neurosci.* 2013; 35:272–282. [PubMed: 23571644]
91. Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr.* 2014; 164:973–979. [PubMed: 24388332]
92. Sun J, Allison J, McLaughlin C, et al. Differences in quality between privately and publicly banked umbilical cord blood units: a pilot study of autologous cord blood infusion in children with acquired neurologic disorders. *Transfusion.* 2010; 50:1980–1987. [PubMed: 20546200]
93. Min K, Song J, Kang JY, et al. Umbilical cord blood therapy potentiated with erythropoietin for children with cerebral palsy: a double-blind, randomized, placebo-controlled trial. *Stem Cells.* 2013; 31:581–591. [PubMed: 23281216]
94. Kirton A, Shroﬀ M, Pontigon AM, deVeber G. Risk factors and presentations of periventricular venous infarction vs arterial presumed perinatal ischemic stroke. *Arch Neurol.* 2010; 67:842–848. [PubMed: 20625091]
95. Kim ES, Ahn SY, Im GH, et al. Human umbilical cord blood-derived mesenchymal stem cell transplantation attenuates severe brain injury by permanent middle cerebral artery occlusion in newborn rats. *Pediatr Res.* 2012; 72:277–284. [PubMed: 22669296]

96. Cheng Q, Zhang Z, Zhang S, et al. Human umbilical cord mesenchymal stem cells protect against ischemic brain injury in mouse by regulating peripheral immunoinflammation. *Brain Res.* 2015; 1594:293–304. [PubMed: 25449888]
97. Iskander A, Knight RA, Zhang ZG, et al. Intravenous administration of human umbilical cord blood-derived AC133+ endothelial progenitor cells in rat stroke model reduces infarct volume: magnetic resonance imaging and histological findings. *Stem Cells Transl Med.* 2013; 2:703–714. [PubMed: 23934909]
98. Tajiri N, Acosta S, Glover LE, et al. Intravenous grafts of amniotic fluid-derived stem cells induce endogenous cell proliferation and attenuate behavioral deficits in ischemic stroke rats. *PLoS One.* 2012; 7:e43779. [PubMed: 22912905]
99. Karlupia N, Manley NC, Prasad K, Schafer R, Steinberg GK. Intraarterial transplantation of human umbilical cord blood mononuclear cells is more efficacious and safer compared with umbilical cord mesenchymal stromal cells in a rodent stroke model. *Stem Cell Res Ther.* 2014; 5:45. [PubMed: 24690461]
100. Tsuji M, Taguchi A, Ohshima M, et al. Effects of intravenous administration of umbilical cord blood CD34(+) cells in a mouse model of neonatal stroke. *Neuroscience.* 2014; 263:148–158. [PubMed: 24444827]
101. Basu AP. Early intervention after perinatal stroke: opportunities and challenges. *Dev Med Child Neurol.* 2014; 56:516–521. [PubMed: 24528276]
102. McEvoy CT, Jain L, Schmidt B, Abman S, Bancalari E, Aschner JL. Bronchopulmonary dysplasia: NHLBI Workshop on the Primary Prevention of Chronic Lung Diseases. *Ann Am Thorac Soc.* 2014; 11(Suppl 3):S146–S153. [PubMed: 24754823]
103. 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.* 2009; 180:1122–1130. [PubMed: 19713447]
104. 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.* 2009; 180:1131–1142. [PubMed: 19713449]
105. Abman SH, Matthay MA. Mesenchymal stem cells for the prevention of bronchopulmonary dysplasia: delivering the secretome. *Am J Respir Crit Care Med.* 2009; 180:1039–1041. [PubMed: 19923401]
106. Chang YS, Ahn SY, Yoo HS, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. *J Pediatr.* 2014; 164:966–972. [PubMed: 24508444]
107. Neu J. Necrotizing enterocolitis. *World Rev Nutr Diet.* 2014; 110:253–263. [PubMed: 24751635]
108. Tayman C, Uckan D, Kilic E, et al. Mesenchymal stem cell therapy in necrotizing enterocolitis: a rat study. *Pediatr Res.* 2011; 70:489–494. [PubMed: 21772224]
109. 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.* 2014; 63:300–309. [PubMed: 23525603]
110. Kourembanas S. Stem cell-based therapy for newborn lung and brain injury: feasible, safe, and the next therapeutic breakthrough? *J Pediatr.* 2014; 164:954–956. [PubMed: 24630358]
111. Lee YH, Choi KV, Moon JH, et al. Safety and feasibility of countering neurological impairment by intravenous administration of autologous cord blood in cerebral palsy. *J Transl Med.* 2012; 10:58. [PubMed: 22443810]
112. Wu CF, Huang FD, Sui RF, Sun JX. Preeclampsia serum upregulates CD40/CD40L expression and induces apoptosis in human umbilical cord endothelial cells. *Reprod Biol Endocrinol.* 2012; 10:28. [PubMed: 22510585]
113. English K, Wood KJ. Mesenchymal stromal cells in transplantation rejection and tolerance. *Cold Spring Harb Perspect Med.* 2013; 3:a015560. [PubMed: 23637312]
114. Parolini O, Alviano F, Bagnara GP, et al. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. *Stem Cells.* 2008; 26:300–311. [PubMed: 17975221]

115. Solomon A, Wajngarten M, Alviano F, et al. Suppression of inflammatory and fibrotic responses in allergic inflammation by the amniotic membrane stromal matrix. *Clin Exp Allergy*. 2005; 35:941–948. [PubMed: 16008682]
116. Solomon A, Espana EM, Tseng SC. Amniotic membrane transplantation for reconstruction of the conjunctival fornices. *Ophthalmology*. 2003; 110:93–100. [PubMed: 12511352]
117. Munoz J, Shah N, Rezvani K, et al. Concise review: umbilical cord blood transplantation: past, present, and future. *Stem Cells Transl Med*. 2014; 3:1435–1443. [PubMed: 25378655]

Key Points

- Umbilical cord blood contains a plethora of stem cells and multipotent progenitor cells.
- In preclinical animal models, transplantation with cord blood or specific stem-like cells can limit brain and lung injury and/or preserve or restore function in part through anti-inflammatory mechanisms.
- The small number of human studies to date suggests the short-term safety of cord blood-derived stem cells, however additional preclinical and human studies are needed to establish therapeutic efficacy and long-term safety.

Figure 1. Hematopoietic hierarchy system

Long-term hematopoietic stem cells (LT-HSCs) sit on the apex of the hematopoietic hierarchy system. LT-HSCs that reside in the bone marrow hematopoietic niche maintain self-renewal and multi-lineage differentiation capacity through asymmetric cell division. SH-HSC: short-term hematopoietic stem cell, MPP: multipotent progenitor cell, MLP: multilymphoid progenitor cell, CMP: common myeloid progenitor cell, ETP: earliest T lymphoid progenitor cell, GMP: granulocyte-macrophage progenitor cell, MEP: megakaryocyte-erythroid progenitor cell.