Bone Marrow Transplantation (2013) **48,** 890–900

www.nature.com/bmt

REVIEW

Rescuing the neonatal brain from hypoxic injury with autologous cord blood

Y Liao[1], M Cotten[2], S Tan[3], J Kurtzberg[2] and MS Cairo[1,4,5,6,7]

Brain injury resulting from perinatal hypoxic-ischemic encephalopathy (HIE) is a major cause of acute mortality in infants and chronic neurologic disability in surviving children. Recent multicenter clinical trials demonstrated the effectiveness of hypothermia initiated within the first 6 postnatal hours to reduce the risk of death or major neurological disabilities among neonates with HIE. However, in these trials, approximately 40% of cooled infants died or survived with significant impairments. Therefore, adjunct therapies are required to improve the outcome in neonates with HIE. Cord blood (CB) is a rich source of stem cells. Administration of human CB cells in animal models of HIE has generally resulted in improved outcomes and multiple mechanisms have been suggested including anti-inflammation, release of neurotrophic factors and stimulation of endogenous neurogenesis. Investigators at Duke are conducting studies of autologous CB infusion in neonates with HIE and in children with cerebral palsy. These pilot studies indicate no added risk from the regimens used, but results of ongoing placebo-controlled trials are needed to assess efficacy. Meanwhile, further investigations are warranted to determine the best strategies, that is, timing, dosing, route of delivery, choice of stem cells and *ex vivo* modulations, to attain long-term benefits of CB stem cell therapy.

Bone Marrow Transplantation (2013) **48,** 890–900; doi:10.1038/bmt.2012.169; published online 10 September 2012

Keywords: cord blood; stem cell therapy; HIE; hypothermia

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) is defined as brain injury in newborn infants diagnosed in the immediate postnatal period. HIE is induced by a combination of inadequate blood flow and oxygen delivery to the neonatal brain. HIE occurs in an estimated 2.5 (1.2–7.7) of every 1000 term births in high income countries.[1] Neonates with HIE have low Apgar scores at 5 min, exhibit abnormal neurologic examination, metabolic acidosis and often have seizures, and require respiratory support within the first postnatal hours of life.[2]

Neonatal brain injuries are graded in general by Sarnat scores, based on serial neurobehavioral evaluations in the first postnatal days.[3] Neonates with persistent severe encephalopathy (Sarnat III) have a risk of death or significant disability that approaches 100%. Close to half of neonates with moderate encephalopathy (Sarnat II) die or will have significant neuro-behavioral deficits.[4,5] Cerebral palsy (CP) is a major form of significant morbidity associated with HIE.[1] Children born at term who develop CP following HIE are twice as likely to have a severe composite disability score and three times more likely to die in the first 5 postnatal years than infants with CP attributed to etiologies other than HIE.[6]

Hypothermia has been demonstrated to reduce the percentage of deaths or major sensorineural disabilities among the neonates with HIE, and is becoming the standard approach to newborns with HIE worldwide.[7,8] However, in the clinical trials demonstrating benefit, a significant number of the HIE infants still died or survived with neurologic and functional impairment.[9]

Stem cell regenerative therapies offer great promise in a variety of degenerative diseases including neurological disorders. Stem cells from different sources, such as neural stem/progenitor cells derived from either fetal tissue or embryonic stem-induced pluripotent stem cells, MSCs, human umbilical cord blood (HUCB)-derived stem cells or HUCB mononuclear cells, have been utilized in several animal models of neurological diseases.[10–14] Cord blood (CB) has been clinically proven to be an effective stem cell source to prevent neurological deterioration in patients with inborn metabolic disorders, that is, Krabbe's disease and Hurler's syndrome.[15,16] A significant advantage of HUCB cells over other sources of stem cells in tissue regeneration is the ready availability of HUCB. This is particularly important for diseases such as HIE, where at least one of the proven windows of opportunity for rescue therapy (hypothermia) is within a few hours after the insult. CB collected shortly after delivery is a rich source for stem and progenitor cells, and requires minimal *ex vivo* manipulation and no immunosuppression for autologous transplantation, as is the case for neonates with HIE (Table 1).[17] CB is widely collected for public and private banking, with well-established standard operating procedures. Properly collected CB cells remain viable at room temperature for 48–72 h. When dosed in neonates, red blood cell depletion and volume reduction is necessary to avoid volume overload or polycythemia in newborns infused with autologous cells.[18] The Carolinas Cord Blood Bank has worked with Biosafe to develop specialized bags for short-term storage of red-cell and volume-reduced CB, which allows aliquots of cells to be used prior

[1]Department of Pediatrics, New York Medical College, Valhalla, NY, USA; [2]Department of Pediatrics, Duke University School of Medicine, Durham, NC, USA; [3]Department of Pediatrics, Evanston Northwestern Healthcare and Northwestern University, Evanston, IL, USA; [4]Department of Medicine, New York Medical College, Valhalla, NY, USA; [5]Department of Pathology, New York Medical College, Valhalla, NY, USA; [6]Department of Immunology and Microbiology, New York Medical College, Valhalla, NY, USA and [7]Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY, USA. Correspondence: Dr MS Cairo, Department of Pediatrics, Maria Fareri Children's Hospital, Westchester Medical Center, New York Medical College, Munger Pavilion, Room 110, Valhalla, NY 10595, USA.
E-mail: Mitchell_Cairo@nymc.edu
Received 1 June 2012; revised 2 August 2012; accepted 3 August 2012; published online 10 September 2012

to freezing (Figure 1a) and allowing residual cells to be frozen for long-term storage, and thawed and infused after the first few postnatal days of life (Figures 1b–d).

PATHOPHYSIOLOGY OF HIE

HIE in term infants can be briefly defined into two phases of pathologic events, the primary and secondary energy failure with a latency period in between (Figure 2).[19–21] The primary energy failure occurs within minutes after initial cerebral ischemia. This phase is associated with acidosis and a rapid depletion of glucose and high-energy phosphates (ATP) in the brain, resulting in acute intracellular derangements, including membrane depolarization, release and blocked re-uptake of excitatory neurotransmitter, defective osmoregulation and inhibition of protein synthesis, ultimately leading to cell death and necrosis.[19–21]

Resolution of hypoxia–ischemia (HI) and resuscitation reverses the primary energy failure and promotes recycling of neurotransmitters. The duration of time for HI to be successfully reversed is likely influenced by maturation, preconditioning events, substrate availability, body temperature, simultaneous disease processes and possibly individual genetic variations.[22]

Table 1. Advantages and disadvantages of CB stem cell infusions for the treatment of neonatal HIE

CB stem cell infusions for the treatment of neonatal HIE	
Advantages	*Disadvantages*
Easy to obtain	Limited number of cells
Autologous and allogeneic are available	May not be universal donor
Rich in primitive stem cells	Not as pluripotent as embryonic stem cells
Long telomere length	One time source
Easily inducible and highly proliferative	Regenerative mechanisms still not elucidated
High efficiency of gene transduction	Potentially transmit infections
Available during first few days of the life. Can be cryopreserved for $\geqslant$30 years	Potentially transmit genetic diseases

Depending on the extent of the primary energy failure, delayed secondary energy failure may occur after a variable period of time following the initial insult. Secondary energy failure also leads to altered growth factor and protein synthesis, which ultimately adds the ongoing insult of altered brain development to the evolving HI injury without acidosis.[23–28] More extensive secondary energy failure and disruption of oxidative metabolism worsens the outcome.[29] Much of the later injury after secondary energy failure occur secondary to the process of apoptosis.[30–32] Although acute cellular necrosis occurs, apoptotic cell death appears to be a more significant contributor to abnormal outcome for the developing brain of the term infant.[33,34] After the secondary phase of injury, there is another chronic phase of injury where there is further loss of brain cells.[35]

Emerging evidence in animal models of neonatal brain injury and evidence from advanced neuroimaging techniques in infants with HIE, suggest that areas of the brain that are connected to areas of initial injury suffer evolving pathology. Functional neuronal development and connectivity deteriorate after acute brain injury in neonates.[36,37] These more distal and chronic pathologies may be more heavily influenced by inflammation and alteration of growth factors secondary to early neuronal loss.

LIMITED ENDOGENOUS NEUROGENESIS FOLLOWING HYPOXIC ISCHEMIC INSULTS

Endogenous neural stem cells (NSCs) reside in germinal zones in the embryonic and adult central nervous system, including the subventricular zone, the external germinal layer of the cerebellum and the subgranular zone of the dentate gyrus.[38–41] Accumulating evidence indicates that endogenous neurogenesis and gliogenesis occur in response to experimental models of ischemia, traumatic brain injury and seizures.[42–44] However, in contrast to adult brains after ischemic injury, where newly generated neurons are predominantly medium-spiny projection neurons, the regenerated neurons in the neonatal striatum, following the asphyxia, are restricted to only calretinin-expressing interneurons.[44] As calretinin interneurons represent only 0.5% of all striatal neurons in the adult rat brain, this study implies a limited regenerative capacity of endogenous neurogensis after neonatal HI brain injuries. Therefore, therapeutic

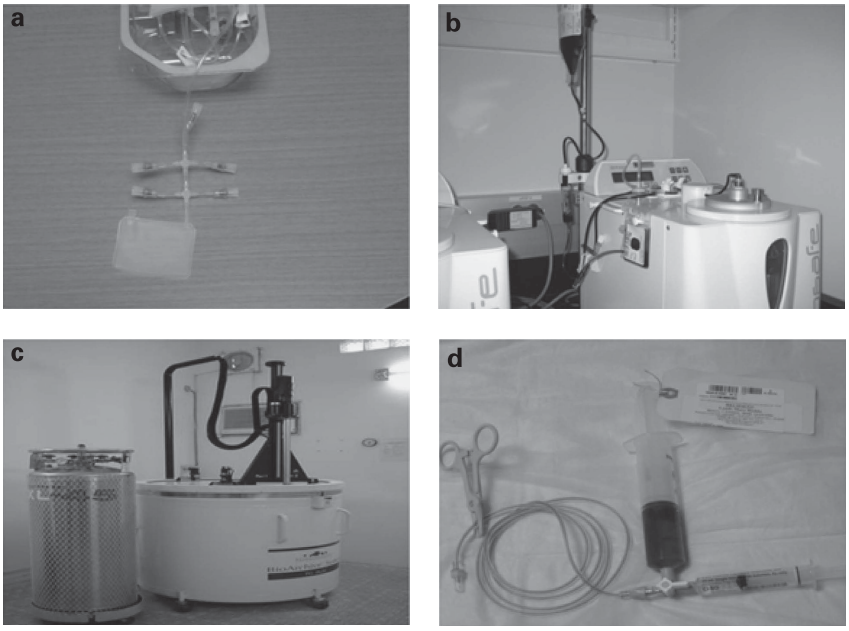

Figure 1. CB processing and cryopreservation. (**a**) Short term CB storage bag. (**b**) Automated system for CB red blood depletion and volume reduction. (**c**) Computerized CB liquid nitrogen storage tank. (**d**) Thawed CB that is ready to infuse into patients. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

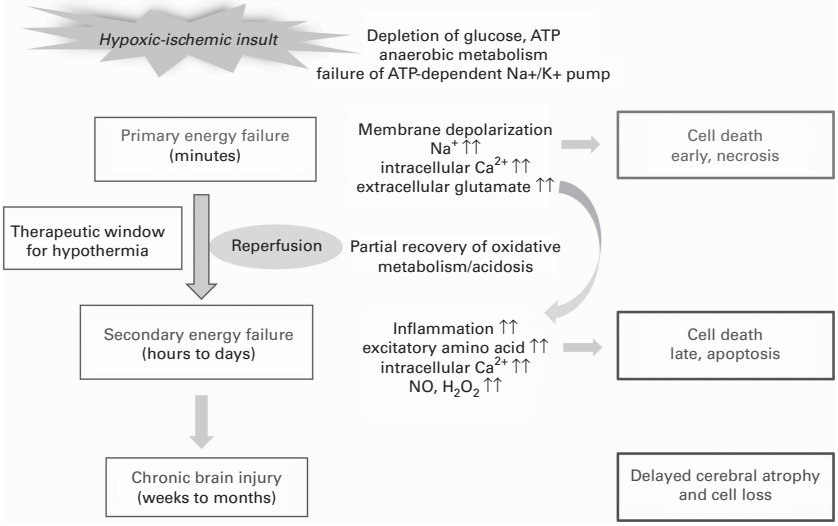

Figure 2. Proposed pathophysiology of HIE. Hypoxic ischemia triggers a cascade of events leading to cellular energy failure and ultimately cell death. Brain damage starts during initial cerebral ischemia, which results in deprivation of energy substrates, that is, oxygen and glucose to brain tissue, transforming the cells to anaerobic metabolism. With failure of the energy-dependent cell membrane ion channels, intracellular Na^+ accumulates and leads to rapid cell swelling and consequently cell necrosis. The damage then worsens during the recovery phase after resuscitation. During this latent phase, accumulation of excitatory neurotransmitter, uncontrolled opening of excitatory glutamate receptors, accumulation of intracellular calcium and subsequent activation of intracellular mechanisms have been observed, leading to secondary delayed energy failure and cellular apoptosis. After the secondary phase of injury there is another chronic phase of injury where there is further loss of brain cells. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

strategies aimed at modulating endogenous neurogenesis in combination with exogenous cell therapy to replace the damaged/lost cells, are likely to provide an improved treatment for neonatal HIE.

CLINICAL INTERVENTIONS FOR HIE: HYPOTHERMIA

Hypothermia, initiated during the phase between primary energy failure and secondary energy failure in perinatal animals with timed injury, has been successful in reducing brain damage, possibly through alteration or avoidance of the secondary energy failure. These results substantiate the presence of a therapeutic window in human newborns.[28,30,45]

Gunn *et al.*[46] induced hypothermia in fetal sheep at various times after injury and before and after onset of electrographic seizures in the animals, which predictably occur between 5.5–6 h after injury. Moderate hypothermia when induced by 90 min post reperfusion and continued until 72 h after ischemia, prevented brain injury and improved electroencephalographic recovery.[47] When hypothermia was delayed until 5.5 h after reperfusion, partial neuroprotection was seen.[48] With further delay until after seizures were established (8.5 h after reperfusion), there was no electrophysiological or overall histological protection with cooling.[49]

Potential mechanisms of action of hypothermia have been proposed by measuring how the physiologic and anatomic responses to HI were modified by moderate hypothermia (no less than 6 °C below normal). These include faster recovery of high energy phosphate pools, reduction in caspase activation and apoptosis, normalization (rather than decrease) of protein synthesis, reduction in free radicals and modulation of activation of microglia and cytokine production.[33,50,51] Another key mechanistic finding for hypothermia's potential as a therapeutic agent for HI brain injury was the reduction in the excitatory neurotransmitter glutamate when hypothermia was used compared with controls. In piglets, a 4 °C temperature reduction attenuated the increase in excitatory amino acids and nitric oxide concentrations in brain extracellular fluid following HI.[52]

Four pivotal multicenter clinical trials of hypothermia for neonates with moderate-to-severe HIE (CoolCap, NICHD Neonatal Research Network, Total Body Hypothermia and Infant Cooling Evaluation) have led to widening adoption of cooling as a treatment of choice for term and near-term neonates with HIE.[9] Four published meta-analyses, including a Cochrane review, demonstrated a consistent beneficial effect for moderate hypothermia with reduction of risk of the combined outcome, death or neurodevelopmental impairment at ~2 years (Figure 3). All meta-analyses of several main trials and well-described phase I and II randomized studies, which included outcome information, demonstrate consistent relative risk of death or disability of ~0.75 and a 95% confidence interval between 0.60 and 0.90.[53–56] The four trials had similar incidence of primary outcome among control groups, and similar incidence of primary outcome among the intervention groups (40–50%). It is however, important to note that hypothermia results in a reduction in risk of death or disability from 58% to only 47%, suggesting the need for adjunct therapies, such as stem cell regenerative therapy.

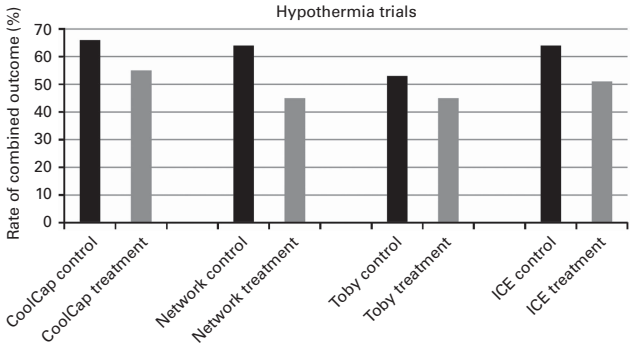

Figure 3. Incidence of the primary outcome in control and cooled groups of the four pivotal multicenter clinical trials of moderate hypothermia in neonates with HIE. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

In the NICHD Neonatal Research Network trial, mortality was reduced in the hypothermia group, 24% compared with 37% in

the control group (relative risk 0.68, 95% confidence interval: 0.43–1.01), and the risk of disabling CP was 19.2% for the hypothermia group and 30.0% among the control group for a relative risk of 0.68 and a 95% confidence interval of 0.38–1.22.[56] In the more recently published Total Body Hypothermia trial, mortality was the same for cooled and regular care, but among survivors, the incidence of CP was significantly decreased in cooled infants (28%) as compared with controls (40%) ($P = 0.03$).[57] The results of multicenter human clinical trials indicate that the critical period for initiation of cooling appears to occur within the first 6 postnatal hours; an on-going NICHD study is examining whether later initiation of cooling, after the first 6 postnatal hours, will be as effective (ClinicalTrials.gov # NCT00614744).

CLINICAL INTERVENTIONS FOR HIE: AUTOLOGOUS CB SCT

Earlier experience with allogeneic unrelated CB transplantation in patients with inborn errors of metabolism demonstrated that CB cells, both hematopoietic and non-hematopoietic in origin, can engraft in the central nervous system, providing the missing enzyme and facilitating neural cell repair.[58] Based on these observations, Sun *et al.*[59] conducted a pilot study to determine the safety and feasibility of i.v. infusion of autologous CB in 184 pediatric patients who had their CB privately banked at birth and who subsequently experienced various acquired neurological injuries, including 140 with CP. This investigation demonstrated the safety and feasibility of thawed and washed autologous CB infusions in the outpatient setting in these pediatric recipients.

Currently, Cotten *et al.* have initiated a clinical trial of autologous volume- and red cell-reduced CB infusions in term infants with HIE, along with concurrent whole body cooling. In this clinical trial, fresh red blood cells and volume-reduced CB cells were infused into neonates after diagnosis with HIE, and at 24, 48 and 72 postnatal hours, respectively (NCT00593242) (personal communication with Cotten). Cell doses ranged from 1×10^7 to 5×10^7cells per kilogram per dose of cells with 1–4 doses administered to each baby. After volume and red cell reduction, CB cells can be maintained at room temperature for a minimum of 48 h. Evidence suggests viability could extend beyond 72 h, but the current FDA-approved research protocol restricts study dosing to the first 48 h. Cells must be frozen if maintained beyond 48 h.[7] At Duke, there is an extended arm of the HIE study that allows for infusion of cryopreserved CB within the first two weeks of life. To date, one infant in the Duke study of CB cells for HIE has received thawed cells. Preliminary results of this experience have also demonstrated the feasibility and safety of autologous CB transplantation in neonates with HIE.[60] Currently >20 infants have been infused with no apparent safety risks attributable to use of fresh, red blood cells and volume-reduced CB cells revealed to date. After completion of a safety pilot (30 babies), a randomized trial utilizing cooling ± autologous CB infusions is planned. Table 1 summarizes the advantages and disadvantages of CB infusion in neonates with HIE.

This same group has initiated a prospective, randomized, placebo-controlled study of autologous CB infusions in older children (ages 1–6 years) with CP.

CB STEM CELL BIOLOGY

CB is a rich source of stem and progenitor cells. In addition to HSCs, non-hematopoietic stem cell populations exist in CB, including, but not limited to, MSCs and more primitive stem cell populations with multi-lineage differentiation properties, that is, unrestricted somatic stem cells, multi-lineage progenitor cells, embryonic-like stem cells and very small embryonic-like stem cells that have respectively been shown to differentiate into neural cells.[14,17,61–65] MSCs, which can also be isolated from BM and other sources, have been demonstrated to exert neuro-protection in HIE animal models.[66–70]

Cell lines with NSC properties have been established from HUCB following immune-depletion of the CD34-positive cells from CB and spontaneous aggregation and differentiation with stimulation by epithelial growth factor.[71] When such CB-NSCs were seeded on human-originated biodegradable scaffold, they were able to differentiate into neurons, form functional circuits and generate spontaneous field/action potentials.[72] Recently, Tracy *et al.*[73] demonstrated that oligodendrocyte progenitors could also be isolated and expanded *ex vivo* from human CB.

In addition, monocytes constitute about 5–10% of HUCB mononuclear cells and bear unique features in their immaturity in the immune and inflammation stimulatory functions, as compared with those originating from BM or peripheral blood.[74] Monocytes have also been shown to participate in angiogenesis via several mechanisms.[74] Such angiogenic and anti-inflammatory effects have likely had an important role in CB regenerative therapy, especially in ischemic diseases.

The composition of multiple populations of stem or progenitor cells underscores the regenerative potential of CB, meanwhile predicting their mechanisms of action to be multifactorial. This concept has been supported by recent preclinical study comparing the effectiveness of freshly isolated CB mononuclear cells with other neural committed stem/progenitors in experimental rats 3 days after focal brain injury.[75] The highest effectiveness, demonstrated by enhanced functional recovery and reduced lesion volume, was observed in the group treated with CB mononuclear cells.

ANIMAL MODELS OF HUMAN CB TRANSPLANTATION FOR HIE

Animal models of HIE include studies in rabbits (Tan), macaques (Juul) and rodents (Vannucci), etc.[76–80] Many of the animal studies reviewed here have been based on the Vannucci model in rodents. This model involves unilateral carotid artery ligation followed by a period of moderate hypoxia in neonatal rats or mice.[80] The procedure is typically conducted in the 7-day-old rodent (P7), an age at which cortical maturity roughly corresponds to 34–36 weeks gestation in humans.[81] Cortical maturity of the P12–13 rat is comparable to term humans.[82,83] Thus, the models reviewed so far represent an acute and localized HI insult and at an age equivalent to a somewhat preterm gestation human.

Table 2 summarizes the results of human CB transplantation in animal models of HIE. Meier *et al.*[84] first reported the results of HUCB mononuclear cell administration in P7 rats. In this study, i.p. injection of 1×10^7 HUCB mononuclear cells 24 h after HI led to a reduced spastic paresis 2 weeks after the insult. Migration of the transplanted cells to the damaged hemisphere was observed but there was no apparent evidence for neural cell differentiation or reduction in brain atrophy 21 days after administration. Recently, this group demonstrated that HUCB given 24 h after HI significantly preserved somatosensory function on the side of the lesion without structural evidence of improvement.[85]

Pimentel-Coelho *et al.*,[86] using 2×10^6 cryopreserved-thawed HUCB cells i.p. in P7 rats 3 h after HI, also showed homing or engraftment of trivial numbers of HUCB cells in the lesion and improvements in sensorimotor reflexes 4 days after HI, but not at 2 and 7 days. The improved outcomes were associated with reduced caspase-3 cleavage, activated microglia and macrophages at various brain regions. This study suggested that CB cells have anti-apoptotic and anti-inflammatory properties.

Yasuhara *et al.*[87] demonstrated that i.v. administration of 15 000 HUCB cells 7 days after HI in P7 rats, with or without mannitol administration, was associated with improvement in motor asymmetry and coordination 7 and 14 days later. The time spent on rotarod test showed a small improvement with CB cells and a slightly greater improvement when combined with mannitol. This same group had previously shown no improvement in rotarod tests from intra-hippocampal

Table 2. A summary of CB transplantation in animal models of HIE

Human CB transplantation in animal models of HIE			
Animal model *Cells and dose* *Timing and route*	*Engraftment* *Date of tests*	*Behavioral tests* *Date of tests*	*Overall observation and explanation*
P7 Wistar rat[84,85] Hypoxia: 80 min HUCB MNCs 10 million 24 h post HI i.p.	Detection of many human cells in the ipsilateral hemisphere at the area of activated microglia 3 days 2 weeks post injection	Footprint analysis[84,85]: 14[84] and 40 days[85] post injection Cylinder test: 40 days post injection	Reduced spastic paresis[84] Recovery of cortical processing and sensorimotor behavior[85] No reduction in brain atrophy No human cell differentiation
P7 Lister–Hooded rat[86] Hypoxia: 90 min HUCB MNCs 2 million 3 h post HI i.p.	Detection of a few human cells in the cerebral cortex and striatum ipsilateral hemisphere 2 days post injection	Negative geotaxis reflex: 2, 4 and 7 days post injection Cliff aversion reflex: 2, 4 and 7 days post injection Gait: 4, 7 and 10 days post injection	Improvement in negative geotaxis and cliff aversion reflex tests at day 4 post transplantation, but not day 2 or day 7 No alteration in gait reflex test Reduced caspase-3-mediated cell death in the damaged striatum Reduced microglial activation
P7 Sprague-Dawley rat[87] Hypoxia: 2.5 h HUCB MNCs ± Mannitol 15 000 7 days post HI i.v. (jugular vein)	Detection of a few human cells in ipsilateral hippocampus 14 days post injection No difference in the number of cells between mannitol and vehicle treatment	EBST Rotarod treadmill 7 and 14 days post injection	Mannitol upregulated CNS growth factors in HI injured animals transplanted with HUCB Improvement in motor function following HUCB and mannitol treatment
P7 Wistar rat[88] Hypoxia: 2 h HUCB MNCs 10 million 24 h post HI i.v. (jugular vein)	Detection of a few cells in both ipsilateral and contralateral hemispheres 24 h, 1week and 3 weeks after HI	Morris water maze Open-field activity Cylinder rearing test Grid walking test Tapered/ledged beam walking test 3 weeks post HI	No significant behavioral and morphologic improvement

Abbreviations: CNS = central nervous system; EBST = elevated body swing test; HI = hypoxia-ischemia; HIE = hypoxia-ischemia encephalopathy; HUCB = human umbilical cord blood; MNCs = mononuclear cells.

administration of 200 000 human multipotent progenitor cells derived from BM, implying a superior function of CB cells.[69] Only a few transplanted CB cells (2–25 per brain) were found in the entire brain following HUCB administration, suggesting that the mechanism of action is more from a neurotrophic effect, perhaps on endogenous cells.[87] Mannitol has been used to facilitate the delivery of various drugs directly to the brain, by producing osmotic shrinking of the endothelial cells and mechanical separation of the tight junctions that form the blood–brainbarrier (BBB). This study by Yasuhara *et al.* demonstrated a cooperative increase on the levels of neural growth factor in the central nervous system 3 days after CB infusion and mannitol treatment, suggesting a potential therapeutic benefit of combined BBB permeation via mannitol and CB infusion for the treatment of neonatal HIE; however, mannitol is not used clinically in treatment after HIE.[87]

De Paula *et al.*[88] found that i.v. injection of 1×10^7 HUCB cells 24 h after HI in P7 rats was associated with small number of cells in the brain and no significant improvement in either volume of infarction or behavioral tests, including locomotion, balance, limb asymmetry and spatial memory 3 weeks later. The authors suggested that the degree of brain damage, injected cell dose, length of follow-up for evaluation and route of delivery might have influenced the outcome.

Human CB transplantation studies have also been conducted in adult animals following experimentally induced ischemic injury.[89–96] Beneficial effects have generally been observed determined by behavioral improvements in most cases, but also by the reduction in lesion size in some studies. Owing to the poor survival of the transplanted cells and little evidence for neural differentiation, bystander effects have been postulated to be the main mechanisms for functional recovery after CB transplantation, including release of neurotrophic factors to stimulate endogenous neurogenesis, prevention of cell loss and immunomodulation (Figure 4).[17,97,98] It has also been suggested that stem cell engraftment in the brain may not be critical for functional recovery provided that neurotrophic factors secreted by exogenous cells could reach the ischemic brain.[93] However, in most of the animal studies, human CB cells were injected without prior or concomitant immunosuppression. Although immunologic tolerance of CB cells has been documented, possibly mediated by diminished cytotoxic responses of the host,[99,100] and were further supported by the sporadic detection of the human cells in the ipsilateral brain of the above experimental animals,[84–88] the potential effect of immunosuppressive treatment on the engraftment of the human cells has not been extensively investigated. Therefore, such xenotransplantation studies do not recapitulate the clinical setting of autologous CB transplantation in neonates with HIE. Nevertheless, the functional recovery in the experimental animals, possibly mediated by trophic effects of CB cells, substantiate the ongoing investigation of CB cell therapy for neonatal HIE.

ANIMAL MODELS OF OTHER SCT FOR HIE

Other stem cells that have been tested in animal models of HIE include multipotent progenitors (MAPCs), MSCs and NSCs (Tables 3 and 4). Multiple sources of multipotent stem cells, either syngeneic or allogeneic, injected at different days and via different

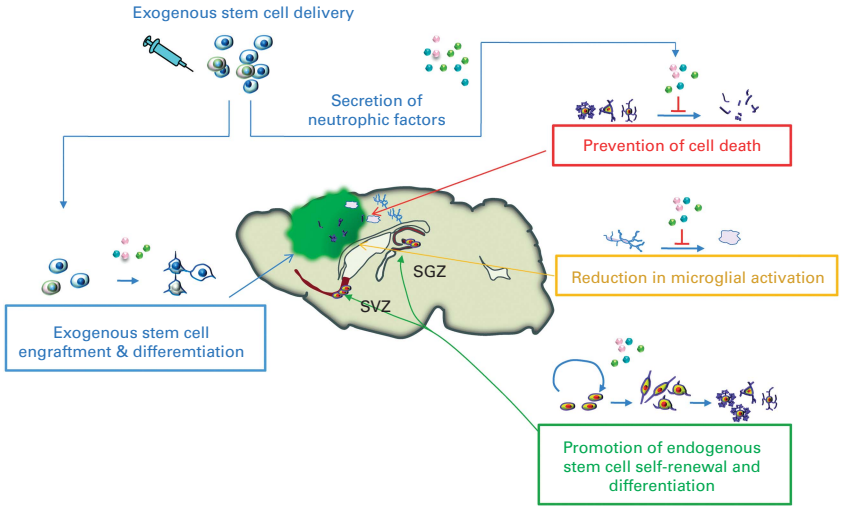

Figure 4. Stem cell regenerative therapy for the rat brain following HI. NSCs are primarily present in the subventricular zone (SVZ) of the lateral ventricle wall and the subgranular zone (SGZ) of the hippocampal dentate gyrus. The endogenous neurogenesis following HI insult has been demonstrated to be limited to repair the damaged brain. In the stem cell regenerative therapy, various sources of exogenous stem cells are administered, either by direct local delivery, or via systemic injection. The mechanisms of action have mainly been attributed to the release of growth factors and cytokines by the transplanted cells to stimulate endogenous neurogenesis, prevent cell loss and immunomodulation. Migration of the transplanted cells to the site of injury has been demonstrated. However their differentiation to desirable neural cell types has been limited.

routes, have generally resulted in behavioral improvements in some, if not all, of the functional tests on selected days.[67–70,101–103] Alleviation of brain tissue injury, however, was only observed in some of the studies; the difference may be related to the severity of brain injury: a better outcome seems to be associated with a shorter period of hypoxia treatment.[67,101,103] Engraftment of the multipotent stem cells has also been observed. However, differentiation has mainly been limited to GFAP$^+$ astrocytes.[101–103]

The inefficiency to undergo *in vivo* differentiation has also been observed following delivery of NSCs in the injured postnatal neocortex in animal models of HIE. Lee *et al.*[104] reported that the majority of the donor cells remained undifferentiated or became astroglia, with only about 5% and 4% differentiation into neurons and oligodendrocytes, respectively. In an adult ischemic brain injury model, under the condition of immunosuppression either transiently or throughout the experiment, Nodari *et al.*[105] reported that immortal human NSCs could differentiate toward astroglial and neuronal lineage, but not oligodendrocytes in the ischemic brain. Thus, it appears that the injured brain microenvironment does not favor engraftment and regeneration of the transplanted cells. Strategies aimed at optimizing the microenvironment for both endogenous and exogenous neurogenesis would potentially enhance the benefits of stem cell regenerative therapy. In a previous study, mouse NSCs were seeded onto a polyglycolic acid polymer scaffold and implanted into the infarction cavities of mouse brains at 1 week after the HI injury.[106] This manipulation promoted reciprocal interactions between host and transplanted NSCs, and resulted in a substantial reduction in parenchymal loss and reconstitution of anatomical connections. Other strategies include delivery of stem cells overexpressing neurotrophic factors and lineage predisposition of stem cells before transplantation.[104,107–110]

Route of stem cell administration

Migration of various types of stem cells to the site of injury has been documented in HIE animal studies following either systematic or local injection.[70,84,111,112] Considering the volume of CB and the young age of the neonates with HIE, i.v. infusion is a safe and effective method of administration, even though it means that only a limited number of cells may arrive in the damaged brain.[113] As compared with i.v. injection, carotid artery injection of neural progenitors, mainly conducted in the adult stroke model, resulted in a higher level of cellular survival and engraftment in the injured brain.[113,114] However, the risk of approach via carotid injection has been reduction in cerebral blood flow and consequently microstrokes.[114,115] Such an approach, via the carotid, is much too risky to consider at this early stage of clinical trials of cells for neonates with HIE. Nasal administration may represent another novel route of stem cell delivery to the ischemic brain in newborns.[101] Intranasal injection of MSC 10 days after HI in the P9 mice resulted in engraftment of MSCs in the affected hemisphere, improved sensorimotor function and a reduction in brain lesion size.[101]

The method involving intra-cerebral injection of stem cells has been utilized in numerous animal studies, especially with expanded cell populations such as NSCs and MSCs. A large number of cells can be delivered locally to desired brain regions, which may result in a direct effect on endogenous neurogenesis.[116] Local delivery of multipotent progenitor cells into the hippocampus, the region that controls motor function and meanwhile is highly vulnerable to HI insult, has been demonstrated to ameliorate the motor deficits in the rat model of HIE.[69] The benefits have been partially attributed to direct stimulation of endogenous neurogenesis in the stem cell niche (dentate gyrus) that is adjacent to the injection site.[69] However, it has to be noted that the same authors also reported a similar beneficial effect of multipotent progenitor cells in the rat HIE model, following i.v. injection.[70] Meanwhile, the safety of such an invasive procedure has to be carefully evaluated before its application in clinical studies.

Timing of SCT

It has been reported that endogenous cell death is prominent in the first day after HI insult and the endogenous repair mechanisms start to be active by day 2 and peaked at day 3.[67] Therefore, the first few days after the HI insult may represent the window of maximum growth-promoting capacity in the brain and may also favor the regenerative potential. Supportively, transplantation of 100 000 murine MSCs to the ipsilateral hemisphere of the P9 mouse at day 3 post HI resulted in not only functional improvements, but

Table 3. A summary of multi-potent SCT in animal models of HIE

Multi-potent SCT in animal models of HIE			
Animal model *Cells and dose* *Timing and route*	*Engraftment* *Date of tests*	*Behavioral tests* *Date of tests*	*Overall observation and explanation*
P7 Sprague–Dawley rat[69] Hypoxia: 2.5 h Rat MAPCs syngeneic or allogeneic 200 000 7 Days post HI Intra-hippocampal with immunosuppression	Detection of graft survival in the hippocampal region 14 days post injection	EBST Rotarod treadmill 7 and 14 days post injection	Significant behavioral improvement was only found at day 14, but not day 7 No difference between syngeneic and allogeneic MPC treatment
P7 Sprague–Dawley rat[70] Hypoxia: 2.5 h Rat MAPCs 200 000 7 Days post HI Intra-hippocampal or i.v. (jugular vein)	0.51 ± 0.15% and 0.18 ± 0.02% graft survival of intracerebral and i.v. injection, respectively Some neuronal differentiation was detected 14 days post injection	EBST Rotarod treadmill 7 and 14 days post injection	Both i.v. and intracerebral injection led to a significant improvement in behavioral tests at day 14 Both routes of injection promoted significant cell preservation in the hippodampal region
P9 C57Bl/6 mice[117] Hypoxia: 45 min Mouse MSCs syngeneic 100 000 3 Days post HI Intracerebral	$<$1% of the newly divided cells were from donor cells	Cylinder test 10 and 21 days post HI	Behavioral improvement on both days Increased proliferation and neuronal/oligo differentiation from the endogenous cells on both days Reduced lesion size and $MAP2^+$ MBP^+ area loss on day 21 post injection Reduced microglial activation
P9 C57Bl/6 mice[68] Hypoxia: 45 min Mouse MSCs syngeneic 100 000 3 and 10 days post HI Intracerebral	Not shown	Cylinder test 10, 21 and 28 days post HI Rotarod tread mill 21 and 28 days post HI	A single MSC treatment on day 3 improved sensorimotor function and reduced lesion size A repeated treatment on day 10 further enhanced the benefits, maybe via different pathways
P9 C57Bl/6 mice[101] Hypoxia: 45 min Mouse MSCs syngeneic 500 000 10 Days post HI Intranasal	Detection of few MSCs in the olfactory bulb and subventricular zone of both hemistpheres and ipsilateral hippocampus No neural differentiation 18 days post injection	Cylinder test 10, 21 and 28 days post HI	Restoration of sensorimotor function Reduced neuronal and white matter loss
P7 Sprague–Dawley rat[102] Hypoxia: 3.5 h Human BM MSCs 1 million 3 Days post HI Intracardiac	Detected in the whole brain, with no preference to the injured hemisphere May differentiate into $GFAP^+$ astrocytes 6 weeks post injection	Rotarod treadmill Cylinder test 14, 20, 30 and 40 days post injection	No reduction in the lesion size No significant effect on rotarod test Improved the performances on the cylinder test
P7 Sprague–Dawley rat[103] Hypoxia: 2.5 h HUCB MSCs 100 000 3 Days post HI Intracerebroparenchymal with immunosuppression	Detection at the injection site and hippocampus Some differentiation to astrocytes, but not neurons 7 days post injection	Modified neurological severity scores 1, 7, 14, 21 and 28 days post injection	Alleviation of brain tissue injury Improvement in the neurological tests at days 14, 21 and 28

Abbreviations: EBST = elevated body swing test; HI = hypoxia–ischemia; HIE = hypoxia–ischemia encephalopathy; HUCB = human umbilical cord blood; MAPCs = multipotent progenitors.

also increased cell proliferation and differentiation (toward neurons and oligodendrocytes) and reduction in the lesion volume at 21 days after the insult.[67] Meanwhile, a recent report on NSC transplantation indicated that engraftment of stem cells was maximal 3–7 days following HI, however, the functional outcomes were not reported.[104]

In animal models of HIE, stem cells have been transplanted 3 h, 24 h, 3 days, 7 days and 10 days, respectively, post the HI insult, with different degrees of beneficial outcome.[67,68,70,84,86,87,106] As most of these studies have more than one variable, it is difficult to conclude the best timing for stem cell therapy. It is possible that the function of stem cells varies at different stages of brain injury. Early interventions are likely important to reduce neurological injury by suppressing inflammation and apoptotic cell death, while transplantation in a chronic stage may improve long-term neurological functions. Consistently, a recent study comparing the effects of single (day 3 following HI insult) and repeated (second treatment on day 10) MSC treatment indicated that each MSC treatment resulted in distinct functional outcomes.[68] The first MSC

Table 4. A summary of NSC and other cell transplantation in animal models of HIE

NSC and other cell transplantation in animal models of HIE			
Animal model *Cells and dose* *Timing and route*	*Engraftment* *Date of tests*	*Behavioral tests* *Date of tests*	*Overall observation and explanation*
P7 CD-1 mouse[106] Hypoxia: 2 h Murine NSCs in PGA scaffold 1–2 million 7 days post HI Into the infarct cavity	Differentiation of donor cells into neurons, astrocyte and oligodendrocytes at 2 weeks post injection	Not tested	Formation of integrated parenchyma within the infarction cavity Reciprocal interactions between donor cells and host were formed by 6 weeks of transplantation Long-distance neuronal projections were established in both cerebral hemispheres Diminished astroglial scarring and mononuclear infiltration
P7 CD-1 mouse[110] Hypoxia: 2 h Murine NSCs overexpressing neurotrophin (NT) 3 300 000 3 days post HI Infarction site	Percentage of donor-derived neurons was increased from 5% (for the parental NSCs) to 10–20% in the infarct and to 81.4% in the penumbra. 0.4% expressed oligodendrocyte markers, 1% expressed astroglial marker and 17.2% undifferentiated	Not tested	NT-3 likely functioned not only on donor cells in autocrine/ paracrine effect, but also on host cells to enhance neuronal differentiation
P9 Wistar rat[108] Hypoxia Encapsulated baby hamster kidney cells overexpressing GDNF 2 days before HI intracerebral	Not determined	8-arm radial maze 7–12th, for 24 days Choice reaction time 10–15th, for 30 days Water maze task 16th, for 5 days	Restoration of cerebral volume reduction Improved performances in all three learning and memory tasks GDNF—capsule implantation exerted a long-lasting and significant neuroprotective effect against HI insult
P7 Sprague–Dawley rat[118] 90 min hES derived NSCs 300 000 Intracerebral	Detection of NSC graft at 4 weeks post injection by bioluminescent imaging 12.8% expressed nestin and $>40\%$ expressed neuronal markers. Astrocyte differentiation was also observed	Cylinder test Rotorod test 4 weeks post injection	A trend to reduce the infarct volume but was not a significant difference Improved sensorimotor behavior Enhanced axonal sprouting Modulation on endogenous microenvironment: gene transcription and microglial activation

Abbreviations: GDNF = glial cell line-derived neurotrophic factor; hES = human embryonic stem cell; HI = hypoxia–ischemia; HIE = hypoxia–ischemia encephalopathy; NSC = neural stem cells; PGA = polyglutamic acid.

injection on day 3 stimulated the formation/survival of new neurons and oligodendrocytes, while the second injection at day 10 further enhanced sensorimotor improvement and recovery of white matter injury.[68] There also appeared to be a crosstalk between the brain milieu, for example, neural and endothelial cells, and the transplanted cells, as determined by the differential genomic responses of MSCs to the extracts from the ischemic mouse brains with or without prior MSC injection.[68]

CONCLUSION

Neonatal HIE has been associated with a high risk of death and major neurological disabilities. Hypothermia initiated within the first 6 postnatal hours has produced consistent beneficial outcomes among neonates with HIE. However, there is still a large percentage of neonates with HIE that died or developed significant disabilities. The safety and feasibility of autologous CB transplantation in pediatric patients with acquired neurological disorders has recently been demonstrated. The ongoing clinical trial on autologous CB cells in infants with HIE within a few hours or days after diagnosis will further reveal hints of the potential for therapeutic efficacy of CB in the acute phase of HIE. In addition, animal studies have generally demonstrated benefits of different types of stem cells, with varied dosing, timing and methods of administration. CB, owing to its complex composition, may have multifactorial benefits. As the function of stem cells could be influenced by the progression of the disease and the endogenous microenvironment, a combined therapy should be considered in the future in the clinical setting. Multiple dosing, supplementation of additional stem cell sources and modulations on the host microenvironment will likely improve the repair and regeneration of the damaged brain. Additional studies to determine best dose, best administration route and best cell source(s) will be required to determine the potential benefit of CB therapy in infants with HIE.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Erin Morris, RN, for her excellent assistance in the preparation of the manuscript. This work was supported in part by the Pediatric Cancer Research Foundation.

REFERENCES

1 Graham EM, Ruis KA, Hartman AL, Northington FJ, Fox HE. A systematic review of the role of intrapartum hypoxia-ischemia in the causation of neonatal encephalopathy. *Am J Obstet Gynecol* 2008; **199**: 587–595.
2 American Congress of Obstetricians and Gynecologists and American Academy of Pediatrics. *Neonatal Encephalopathy and Cerebral Palsy. Defining the*

Pathogenesis, 2003, American College of Obstetricians and Gynecologists, Washington, DC.
3 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976; **33**: 696–705.
4 Robertson CM, Finer NN, Grace MG. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J Pediatr* 1989; **114**: 753–760.
5 Shankaran S, Woldt E, Koepke T, Bedard MP, Nandyal R. Acute neonatal morbidity and long-term central nervous system sequelae of perinatal asphyxia in term infants. *Early Hum Dev* 1991; **25**: 135–148.
6 Badawi N, Felix JF, Kurinczuk JJ, Dixon G, Watson L, Keogh JM *et al.* Cerebral palsy following term newborn encephalopathy: a population-based study. *Dev Med Child Neurol* 2005; **47**: 293–298.
7 Azzopardi D, Strohm B, Edwards AD, Halliday H, Juszczak E, Levene M *et al.* Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch Disease Child Fetal Neonatal Ed* 2009; **94**: F260–F264.
8 *National Institute for Health and Clinical Excellence*. Therapeutic hypothermia with intracorporeal temperature monitoring for hypoxic perinatal brain injuries: interventional procedure guidance 347.
9 Cotten CM, Shankaran S. Hypothermia for hypoxic-ischemic encephalopathy. *Expert Rev Obstet Gynecol* 2010; **5**: 227–239.
10 Rodriguez-Gomez JA, Lu JQ, Velasco I, Rivera S, Zoghbi SS, Liow JS *et al.* Persistent dopamine functions of neurons derived from embryonic stem cells in a rodent model of Parkinson disease. *Stem Cells* 2007; **25**: 918–928.
11 Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat Med* 2006; **12**: 1259–1268.
12 Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W *et al.* Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 2008; **321**: 1218–1221.
13 Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. *Stroke* 2007; **38**(Suppl): 817–826.
14 Schira J, Gasis M, Estrada V, Hendricks M, Schmitz C, Trapp T *et al.* Significant clinical, neuropathological and behavioural recovery from acute spinal cord trauma by transplantation of a well-defined somatic stem cell from human umbilical cord blood. *Brain*, 2012; 135(Pt 2): 431–446.
15 Prasad VK, Kurtzberg J. Emerging trends in transplantation of inherited metabolic diseases. *Bone Marrow Transplant* 2008; **41**: 99–108.
16 Prasad VK, Mendizabal A, Parikh SH, Szabolcs P, Driscoll TA, Page K *et al.* Unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 pediatric patients from a single center: influence of cellular composition of the graft on transplantation outcomes. *Blood* 2008; **112**: 2979–2989.
17 Liao Y, Geyer MB, Yang AJ, Cairo MS. Cord blood transplantation and stem cell regenerative potential. *Exp Hematol* **39**: 393–412.
18 Kurtzberg J, Cairo MS, Fraser JK, Baxter-Lowe L, Cohen G, Carter SL *et al.* Results of the cord blood transplantation (COBLT) study unrelated donor banking program. *Transfusion* 2005; **45**: 842–855.
19 McLean C, Ferriero D. Mechanisms of hypoxic-ischemic injury in the term infant. *Seminars Perinatol* 2004; **28**: 425–432.
20 Johnston MV, Fatemi A, Wilson MA, Northington F. Treatment advances in neonatal neuroprotection and neurointensive care. *Lancet Neurol* 2011; **10**: 372–382.
21 Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. *Pediat Neurol* 2009; **40**: 215–226.
22 Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD *et al.* Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48 h studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 1994; **36**: 699–706.
23 Freeman JP. Brain injury in the term neonate. *Semin Perinatol* 2004; **28**: 415–424.
24 Mehmet H, Yue X, Squier MV, Lorek A, Cady E, Penrice J *et al.* Increased apoptosis in the cingulate sulcus of newborn piglets following transient hypoxia-ischaemia is related to the degree of high energy phosphate depletion during the insult. *Neurosci Lett* 1994; **181**: 121–125.
25 Fellman V, Raivio KO. Reperfusion injury as the mechanism of brain damage after perinatal asphyxia. *Pediatr Res* 1997; **41**: 599–606.
26 Liu XH, Kwon D, Schielke GP, Yang GY, Silverstein FS, Barks JD. Mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic-ischemic brain damage. *J Cereb Blood Flow Metab* 1999; **19**: 1099–1108.
27 Tan WK, Williams CE, During MJ, Mallard CE, Gunning MI, Gunn AJ *et al.* Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischemic injury in late gestation fetal sheep. *Pediatr Res* 1996; **39**: 791–797.
28 Gluckman PD, Guan J, Williams C, Scheepens A, Zhang R, Bennet L *et al.* Asphyxial brain injury--the role of the IGF system. *Mol Cell Endocrinol* 1998; **140**: 95–99.
29 Roth SC, Edwards AD, Cady EB, Delpy DT, Wyatt JS, Azzopardi D *et al.* Relation between cerebral oxidative metabolism following birth asphyxia, and neurodevelopmental outcome and brain growth at one year. *Dev Med Child Neurol* 1992; **34**: 285–295.
30 Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. *Brain Res Bull* 1998; **46**: 281–309.
31 Ferriero DM. Neonatal brain injury. *N Engl J Med* 2004; **351**: 1985–1995.
32 Hu BR, Liu CL, Ouyang Y, Blomgren K, Siesjo BK. Involvement of caspase-3 in cell death after hypoxia-ischemia declines during brain maturation. *J Cereb Blood Flow Metab* 2000; **20**: 1294–1300.
33 Edwards AD, Yue X, Squier MV, Thoresen M, Cady EB, Penrice J *et al.* Specific inhibition of apoptosis after cerebral hypoxia-ischaemia by moderate post-insult hypothermia. *Biochem Biophys Res Commun* 1995; **217**: 1193–1199.
34 Rothstein RP, Levison SW. Gray matter oligodendrocyte progenitors and neurons die caspase-3 mediated deaths subsequent to mild perinatal hypoxic/ischemic insults. *Dev Neurosci* 2005; **27**: 149–159.
35 Geddes R, Vannucci RC, Vannucci SJ. Delayed cerebral atrophy following moderate hypoxia-ischemia in the immature rat. *Dev Neurosci* 2001; **23**: 180–185.
36 Stone BS, Zhang J, Mack DW, Mori S, Martin LJ, Northington FJ. Delayed neural network degeneration after neonatal hypoxia-ischemia. *Ann Neurol* 2008; **64**: 535–546.
37 Miller SP, Ferriero DM. From selective vulnerability to connectivity: insights from newborn brain imaging. *Trends Neurosci* 2009; **32**: 496–505.
38 Imitola J. Prospects for neural stem cell-based therapies for neurological diseases. *Neurotherapeutics* 2007; **4**: 701–714.
39 Martino G, Pluchino S. The therapeutic potential of neural stem cells. *Nat Rev Neurosci* 2006; **7**: 395–406.
40 Laywell ED, Rakic P, Kukekov VG, Holland EC, Steindler DA. Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc Natl Acad Sci USA* 2000; **97**: 13883–13888.
41 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.
42 Plane JM, Liu R, Wang TW, Silverstein FS, Parent JM. Neonatal hypoxic-ischemic injury increases forebrain subventricular zone neurogenesis in the mouse. *Neurobiol Dis* 2004; **16**: 585–595.
43 Felling RJ, Snyder MJ, Romanko MJ, Rothstein RP, Ziegler AN, Yang Z *et al.* Neural stem/progenitor cells participate in the regenerative response to perinatal hypoxia/ischemia. *J Neurosci* 2006; **26**: 4359–4369.
44 Yang Z, You Y, Levison SW. Neonatal hypoxic/ischemic brain injury induces production of calretinin-expressing interneurons in the striatum. *J Comp Neurol* 2008; **511**: 19–33.
45 Blaschke AJ, Staley K, Chun J. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. *Development* 1996; **122**: 1165–1174.
46 Gunn AJ, Gunn TR. The 'pharmacology' of neuronal rescue with cerebral hypothermia. *Early Hum Dev* 1998; **53**: 19–35.
47 Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* 1997; **99**: 248–256.
48 Gunn AJ, Gunn TR, Gunning MI, Williams CE, Gluckman PD. Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep. *Pediatrics* 1998; **102**: 1098–1106.
49 Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatr Res* 1999; **46**: 274–280.
50 Thoresen M, Bagenholm R, Loberg EM, Apricena F, Kjellmer I. Posthypoxic cooling of neonatal rats provides protection against brain injury. *Arch Dis Child Fetal Neonatal Ed* 1996; **74**: F3–F9.
51 Fukuda H, Tomimatsu T, Watanabe N, Mu JW, Kohzuki M, Endo M *et al.* Post-ischemic hypothermia blocks caspase-3 activation in the newborn rat brain after hypoxia-ischemia. *Brain Res* 2001; **910**: 187–191.
52 Thoresen M, Satas S, Puka-Sundvall M, Whitelaw A, Hallstrom A, Loberg EM *et al.* Post-hypoxic hypothermia reduces cerebrocortical release of NO and excitotoxins. *Neuroreport* 1997; **8**: 3359–3362.
53 Edwards AD, Azzopardi DV. Therapeutic hypothermia following perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed* 2006; **91**: F127–F131.
54 Schulzke SM, Rao S, Patole SK. A systematic review of cooling for neuroprotection in neonates with hypoxic ischemic encephalopathy - are we there yet? *BMC Pediatr* 2007; **7**: 30.
55 Shah PS, Ohlsson A, Perlman M. Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review. *Arch Pediatr Adolesc Med* 2007; **161**: 951–958.

56 Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2007 (e-pub ahead of print 17 October 2007; doi:10.1002/14651858.CD003311).
57 Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E *et al.* Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009; **361**: 1349–1358.
58 Escolar ML, Poe MD, Provenzale JM, Richards KC, Allison J, Wood S *et al.* Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *N Engl J Med* 2005; **352**: 2069–2081.
59 Sun J, Allison J, McLaughlin C, Sledge L, Waters-Pick B, Wease S *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.
60 Cotten CM, Goldberg R, Smith PB, Grotegut C, Goldstein RF, Fisher KA *et al.* Autologous cord blood cells for infants with hypoxic-ischemic encephalopathy (HIE): a feasibility study. *Pediatric Academic Societies.* Denver, CO, 2011, pp E-PAS20113833.327.
61 McGuckin C, Jurga M, Ali H, Strbad M, Forraz N. Culture of embryonic-like stem cells from human umbilical cord blood and onward differentiation to neural cells in vitro. *Nat Protoc* 2008; **3**: 1046–1055.
62 Kucia M, Halasa M, Wysoczynski M, Baskiewicz-Masiuk M, Moldenhawer S, Zuba-Surma E *et al.* Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. *Leukemia* 2007; **21**: 297–303.
63 van de Ven C, Collins D, Bradley MB, Morris E, Cairo MS. The potential of umbilical cord blood multipotent stem cells for nonhematopoietic tissue and cell regeneration. *Exp Hematol* 2007; **35**: 1753–1765.
64 Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N *et al.* A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med* 2004; **200**: 123–135.
65 Greschat S, Schira J, Kury P, Rosenbaum C, de Souza Silva MA, Kogler G *et al.* Unrestricted somatic stem cells from human umbilical cord blood can be differentiated into neurons with a dopaminergic phenotype. *Stem Cells Dev* 2008; **17**: 221–232.
66 Xia G, Hong X, Chen X, Lan F, Zhang G, Liao L. Intracerebral transplantation of mesenchymal stem cells derived from human umbilical cord blood alleviates hypoxic ischemic brain injury in rat neonates. *J Perinat Med* **38**: 215–221.
67 van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Mesenchymal stem cell treatment after neonatal hypoxic-ischemic brain injury improves behavioral outcome and induces neuronal and oligodendrocyte regeneration. *Brain Behav Immun* **24**: 387–393.
68 van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Repeated mesenchymal stem cell treatment after neonatal hypoxia-ischemia has distinct effects on formation and maturation of new neurons and oligodendrocytes leading to restoration of damage, corticospinal motor tract activity, and sensorimotor function. *J Neurosci* **30**: 9603–9611.
69 Yasuhara T, Matsukawa N, Yu G, Xu L, Mays RW, Kovach J *et al.* Behavioral and histological characterization of intrahippocampal grafts of human bone marrow-derived multipotent progenitor cells in neonatal rats with hypoxic-ischemic injury. *Cell Transplant* 2006; **15**: 231–238.
70 Yasuhara T, Hara K, Maki M, Mays RW, Deans RJ, Hess DC *et al.* Intravenous grafts recapitulate the neurorestoration afforded by intracerebrally delivered multipotent adult progenitor cells in neonatal hypoxic-ischemic rats. *J Cereb Blood Flow Metab* 2008; **28**: 1804–1810.
71 Buzanska L, Jurga M, Stachowiak EK, Stachowiak MK, Domanska-Janik K. Neural stem-like cell line derived from a nonhematopoietic population of human umbilical cord blood. *Stem Cells Dev* 2006; **15**: 391–406.
72 Jurga M, Lipkowski AW, Lukomska B, Buzanska L, Kurzepa K, Sobanski T *et al.* Generation of functional neural artificial tissue from human umbilical cord blood stem cells. *Tissue Eng Part C Methods* 2009; **15**: 365–372.
73 Tracy ET, Zhang CY, Gentry T, Shoulars KW, Kurtzberg J. Isolation and expansion of oligodendrocyte progenitor cells from cryopreserved human umbilical cord blood. *Cytotherapy* 2011; **13**: 722–729.
74 Sanberg PR, Park DH, Kuzmin-Nichols N, Cruz E, Hossne Jr NA, Buffolo E *et al.* Monocyte transplantation for neural and cardiovascular ischemia repair. *J Cell Mol Med* 2010; **14**: 553–563.
75 Gornicka-Pawlak el B, Janowski M, Habich A, Jablonska A, Drela K, Kozlowska H *et al.* Systemic treatment of focal brain injury in the rat by human umbilical cord blood cells being at different level of neural commitment. *Acta Neurobiol Exp* 2011; **71**: 46–64.
76 Tan S, Drobyshevsky A, Jilling T, Ji X, Ullman LM, Englof I *et al.* Model of cerebral palsy in the perinatal rabbit. *J Child Neurol* 2005; **20**: 972–979.
77 Derrick M, Drobyshevsky A, Ji X, Tan S. A model of cerebral palsy from fetal hypoxia-ischemia. *Stroke* 2007; **38**(Suppl): 731–735.
78 Jacobson Misbe EN, Richards TL, McPherson RJ, Burbacher TM, Juul SE. Perinatal asphyxia in a nonhuman primate model. *Dev Neurosci* 2011; **33**: 210–221.
79 Juul SE, Aylward E, Richards T, McPherson RJ, Kuratani J, Burbacher TM. Prenatal cord clamping in newborn Macaca nemestrina: a model of perinatal asphyxia. *Dev Neurosci* 2007; **29**: 311–320.
80 Rice 3rd JE , Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. *Ann Neurol* 1981; **9**: 131–141.
81 Vannucci RC, Connor JR, Mauger DT, Palmer C, Smith MB, Towfighi J *et al.* Rat model of perinatal hypoxic-ischemic brain damage. *J Neurosci Res* 1999; **55**: 158–163.
82 Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev* 1979; **3**: 79–83.
83 Romijn HJ, Hofman MA, Gramsbergen A. At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? *Early Hum Dev* 1991; **26**: 61–67.
84 Meier C, Middelanis J, Wasielewski B, Neuhoff S, Roth-Haerer A, Gantert M *et al.* Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr Res* 2006; **59**: 244–249.
85 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.
86 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* **19**: 351–358.
87 Yasuhara T, Hara K, Maki M, Xu L, Yu G, Ali MM *et al.* Mannitol facilitates neurotrophic factor up-regulation and behavioural recovery in neonatal hypoxic-ischaemic rats with human umbilical cord blood grafts. *J Cell Mol Med* **14**: 914–921.
88 de Paula S, Vitola AS, Greggio S, de Paula D, Mello PB, Lubianca JM *et al.* Hemispheric brain injury and behavioral deficits induced by severe neonatal hypoxia-ischemia in rats are not attenuated by intravenous administration of human umbilical cord blood cells. *Pediatr Res* 2009; **65**: 631–635.
89 Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE *et al.* Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke* 2001; **32**: 2682–2688.
90 Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S *et al.* Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *J Neurosci Res* 2003; **73**: 296–307.
91 Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T *et al.* Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke* 2004; **35**: 2390–2395.
92 Newman MB, Willing AE, Manresa JJ, Davis-Sanberg C, Sanberg PR. Stroke-induced migration of human umbilical cord blood cells: time course and cytokines. *Stem Cells Dev* 2005; **14**: 576–586.
93 Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. *Stroke* 2004; **35**: 2385–2389.
94 Newcomb JD, Ajmo Jr CT, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Timing of cord blood treatment after experimental stroke determines therapeutic efficacy. *Cell Transplant* 2006; **15**: 213–223.
95 Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD *et al.* Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev* 2005; **14**: 595–604.
96 Jiang L, Womble T, Saporta S, Chen N, Sanberg CD, Sanberg PR *et al.* Human umbilical cord blood cells decrease microglial survival in vitro. *Stem Cells Dev* **19**: 221–228.
97 de Paula S, Greggio S, DaCosta JC. Use of stem cells in perinatal asphyxia: from bench to bedside. *J Pediatr* **86**: 451–464.
98 Pimentel-Coelho PM, Mendez-Otero R. Cell therapy for neonatal hypoxic-ischemic encephalopathy. *Stem Cells Dev* **19**: 299–310.
99 Risdon G, Gaddy J, Broxmeyer HE. Allogeneic responses of human umbilical cord blood*Blood Cells* 1994; **20**: 566–570, discussion 571–572.
100 Rainsford E, Reen DJ. Interleukin 10, produced in abundance by human newborn T cells, may be the regulator of increased tolerance associated with cord blood stem cell transplantation. *Br J Haematol* 2002; **116**: 702–709.
101 van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Nasal administration of stem cells: a promising novel route to treat neonatal ischemic brain damage. *Pediatr Res* 2010; **68**: 419–422.
102 Lee JA, Kim BI, Jo CH, Choi CW, Kim EK, Kim HS *et al.* Mesenchymal stem-cell transplantation for hypoxic-ischemic brain injury in neonatal rat model. *Pediatr Res* 2010; **67**: 42–46.
103 Xia G, Hong X, Chen X, Lan F, Zhang G, Liao L. Intracerebral transplantation of mesenchymal stem cells derived from human umbilical cord blood allevi-

ates hypoxic ischemic brain injury in rat neonates. *J Perinat Med* 2010; **38**: 215–221.
104 Lee IS, Jung K, Kim M, Park KI. Neural stem cells: properties and therapeutic potentials for hypoxic-ischemic brain injury in newborn infants. *Pediatr Int* **52**: 855–865.
105 Nodari LR, Ferrari D, Giani F, Bossi M, Rodriguez-Menendez V, Tredici G *et al*. Long-term survival of human neural stem cells in the ischemic rat brain upon transient immunosuppression. *PLoS One* 2010; **5**: e14035.
106 Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat Biotechnol* 2002; **20**: 1111–1117.
107 Katsuragi S, Ikeda T, Date I, Shingo T, Yasuhara T, Ikenoue T. Grafting of glial cell line-derived neurotrophic factor secreting cells for hypoxic-ischemic encephalopathy in neonatal rats. *Am J Obstet Gynecol* 2005; **192**: 1137–1145.
108 Katsuragi S, Ikeda T, Date I, Shingo T, Yasuhara T, Mishima K *et al*. Implantation of encapsulated glial cell line-derived neurotrophic factor-secreting cells prevents long-lasting learning impairment following neonatal hypoxic-ischemic brain insult in rats. *Am J Obstet Gynecol* 2005; **192**: 1028–1037.
109 Lee HJ, Lim IJ, Lee MC, Kim SU. Human neural stem cells genetically modified to overexpress brain-derived neurotrophic factor promote functional recovery and neuroprotection in a mouse stroke model. *J Neurosci Res* **88**: 3282–3294.
110 Park KI, Himes BT, Stieg PE, Tessler A, Fischer I, Snyder EY. Neural stem cells may be uniquely suited for combined gene therapy and cell replacement: evidence from engraftment of Neurotrophin-3-expressing stem cells in hypoxic-ischemic brain injury. *Exp Neurol* 2006; **199**: 179–190.
111 Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC *et al*. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proc Natl Acad Sci USA* 2004; **101**: 11839–11844.
112 Park KI, Hack MA, Ourednik J, Yandava B, Flax JD, Stieg PE *et al*. Acute injury directs the migration, proliferation, and differentiation of solid organ stem cells: evidence from the effect of hypoxia-ischemia in the CNS on clonal "reporter" neural stem cells. *Exp Neurol* 2006; **199**: 156–178.
113 Lappalainen RS, Narkilahti S, Huhtala T, Liimatainen T, Suuronen T, Narvanen A *et al*. The SPECT imaging shows the accumulation of neural progenitor cells into internal organs after systemic administration in middle cerebral artery occlusion rats. *Neurosci Lett* 2008; **440**: 246–250.
114 Li L, Jiang Q, Ding G, Zhang L, Zhang ZG, Li Q *et al*. Effects of administration route on migration and distribution of neural progenitor cells transplanted into rats with focal cerebral ischemia, an MRI study. *J Cereb Blood Flow Metab* 2010; **30**: 653–662.
115 Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG *et al*. Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. *Stroke* 2008; **39**: 1569–1574.
116 Modo M, Stroemer RP, Tang E, Patel S, Hodges H. Effects of implantation site of stem cell grafts on behavioral recovery from stroke damage. *Stroke* 2002; **33**: 2270–2278.
117 van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. Mesenchymal stem cell treatment after neonatal hypoxic-ischemic brain injury improves behavioral outcome and induces neuronal and oligodendrocyte regeneration. *Brain Behav Immun* 2010; **24**: 387–393.
118 Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R *et al*. Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. *Stroke* 2010; **41**: 516–523.