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Umbilical cord blood cells for treatment of cerebral palsy; timing and treatment options

Courtney A. McDonald[1], Michael C. Fahey[2], Graham Jenkin[1] and Suzanne L. Miller[1]

Cerebral palsy is the most common cause of physical disability in children, and there is no cure. Umbilical cord blood (UCB) cell therapy for the treatment of children with cerebral palsy is currently being assessed in clinical trials. Although there is much interest in the use of UCB stem cells for neuroprotection and neuroregeneration, the mechanisms of action are not fully understood. Further, UCB contains many stem and progenitor cells of interest, and we will point out that individual cell types within UCB may elicit specific effects. UCB is a clinically proven source of hemotopoietic stem cells (HSCs). It also contains mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs), and immunosupressive cells such as regulatory T cells (Tregs) and monocyte-derived supressor cells. Each of these cell types may be individual candidates for the prevention of brain injury following hypoxic and inflammatory events in the perinatal period. We will discuss specific properties of cell types in UCB, with respect to their therapeutic potential and the importance of optimal timing of administration. We propose that tailored cell therapy and targeted timing of administration will optimize the results for future clinical trials in the neuroprotective treatment of perinatal brain injury.

Cerebral palsy is the most common physical disability in childhood caused by damage to the developing brain that occurs in the antenatal, perinatal, or early postnatal period. Cerebral palsy describes a complex set of motor symptoms, with disability ranging from mild motor coordination dysfunction through to significant hemiplegia or quadriplegia. The heterogeneity of cerebral palsy reflects a spectrum of neuropathologies that differentially affect the preterm or term infant brain. The motor disabilities that define cerebral palsy are also often coexistent with other serious problems—one in two children with cerebral palsy has intellectual disabilities including cognition, memory, learning, and behavior deficits; one in four has epilepsy; one in four cannot talk; and one in four are incontinent (1). Accordingly, cerebral palsy places a profound burden on families, health-care systems, and society. There is no cure for cerebral palsy and, although it is encouraging that the prevalence of the condition indicates a downward trend in some recently published figures, this trend is not apparent globally (2,3).

Stem cells have received widespread attention and interest for their potential to improve multiple conditions or disease states (4). This has resulted in many patients and families investing large amounts of money to travel overseas for stem cell treatment in the hope of finding a cure. People with cerebral palsy, and particularly the parents of infants or children with cerebral palsy, are keenly pursuing stem cell-related therapies. This is reflected in the worldwide statistics showing that cerebral palsy is ranked second (after multiple sclerosis) as the most common condition treated with stem cells (5). It does, however, remain that stem cell treatments for cerebral palsy are currently unproven, the optimal source of stem cells is not yet known, and stem cell treatment is not readily available and is often very costly, particularly if patients or families are traveling overseas to receive treatment. It is, therefore, the responsibility of the scientific and medical community to exercise caution at this stage, and to robustly examine stem cell efficacy, timing, and optimal cell type to guide clinical practice and inform community interest. In this review we will examine the current understanding of the use of stem cells in the treatment of the brain injury that underlies cerebral palsy, with a principal focus on the neuroprotective/neuroregenerative potential of umbilical cord blood (UCB) cells.

When considering stem cell therapies, given the large number of stem and progenitor cell types that are being tested in preclinical studies, it can be hard to determine which cell source may be best suited to a specific condition. For treatment of many disorders, and in particular perinatal brain injury, there are multiple advantages for the use of UCB mononuclear cells, including their low immunogenicity and, therefore, low risk of rejection and development of graft vs. host disease (6), making UCB cells a relatively safe source for transplantation. UCB is also readily available in large quantities, is usually discarded at birth, and can withstand long-term cryopreservation, maintaining up to 90% viable cell recovery post thaw (7). Most importantly for translation, UCB has been used clinically for almost 30 years (8) and is now routinely used to treat acute leukemia, aplastic anemia,

[1]The Ritchie Centre, Hudson Institute of Medical Research and Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia; [2]Department of Paediatrics, Monash University, Clayton, Victoria, Australia. Correspondence: Suzanne L. (suzie.miller@monash.edu)

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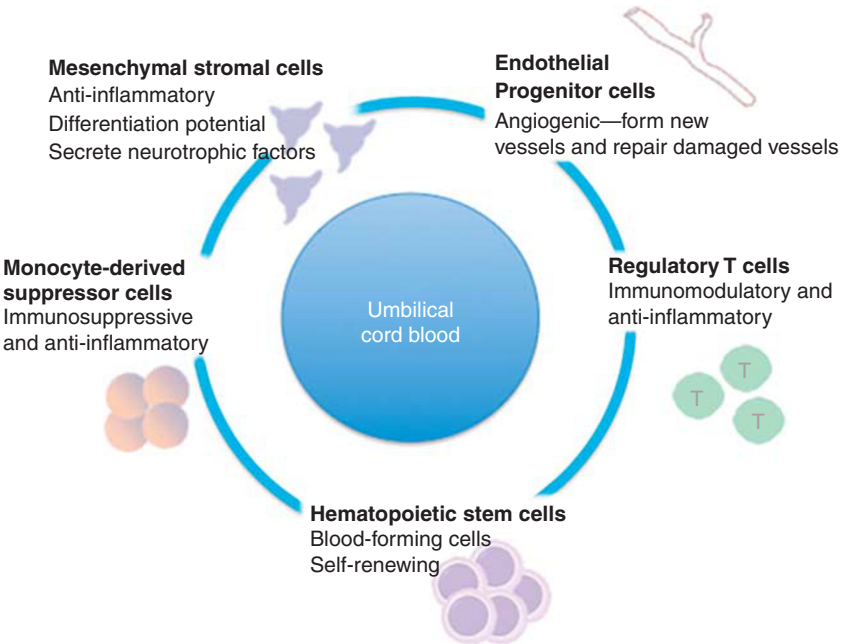

Figure 1. Principal stem and progenitor cells found in umbilical cord blood (UCB), and their main actions. A diagrammatic representation of the five key stem and progenitor cell subtypes found in UCB, that are likely to mediate the neuroprotective or neuroregenerative benefits of UCB. These include mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs), hematopoietic stem cells (HSCs), monocyte-derived suppressor cells (MDSCs), and regulatory T cells (Tregs). Listed below each cell type are their most commonly described features.

lymphomas, thalassemia, and sickle cell disease (9,10). Given the current clinical use and potential applications of UCB, the first UCB banks were established in the early 1990s ((ref. 11)) and, since then, public and private UCB banks have emerged around the world, and are very prevalent in high-resource countries.

UCB is a rich source of stem and progenitor cells including hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), endothelial progenitor cells (EPCs), and immunosuppressive cells, such as regulatory T cells (Tregs) and monocyte-derived suppressor cells (MDSCs; **Figure 1**) (12,13). UCB cells can influence local tissue repair via secretion of a range of important trophic factors such as cytokines (interleukin (IL)-6, IL-8, IL-10, and monocyte chemoattractant protein-1) (14), angiogenic factors (vascular endothelial growth factor and angiogenin) (15), and neurotrophic factors (brain-derived neurotrophic factor, nerve growth factor, glial cell line-derived neurotrophic factor) (14,16). Furthermore, many of the individual cell types composing UCB may act in a paracrine manner when transplanted, secreting soluble factors capable of direct stimulation and/or proliferation of neural stem cells (NSCs), or by stimulating endogenous cells to release reparatory factors (12).

The broad neuroprotective properties of UCB are also mediated by anti-inflammatory and anti-apoptotic properties, together with effects on cell survival and angiogenesis (13). UCB cells have now been shown to directly protect neurons (17), oligodendrocytes (18), and astrocytes (19) from undergoing apoptosis. Further, it has been demonstrated that, under certain *in vitro* conditions, UCB cells can differentiate down the neural lineage (15). As a result, UCB cells are currently being examined in clinical trials for a range of adverse conditions such as perinatal brain injury, established cerebral palsy, and adult stroke, in addition to their established benefits for blood-related diseases.

Rodent models of adult stroke or traumatic brain injury were the first to lay the foundation to investigate the potential of UCB cells to reduce brain injury. These studies provided promising data that UCB cells could migrate to sites of injury within the brain (20) and, when given intravenously, reduced functional deficits (21) and protected against white matter injury (18). In turn, subsequent studies in rats have explored the neuroprotective potential of UCB cells in perinatal brain injury. When UCB cells are given within the first 24 h after hypoxic–ischemic (HI) injury in term-equivalent rat pups, they improve both pathology and motor control, mediated by a reduction in neuronal degeneration, apoptosis, and microglial activation (22), as well as reducing spastic paresis and improving walking patterns (13,23,24). More recently, it has been shown that UCB-derived MSCs can augment the neuroprotective benefits of hypothermia in neonatal rats exposed to hypoxia–ischemia (25)—an important observation, given that therapeutic hypothermia is now the standard of care for term infants diagnosed with HI encephalopathy (HIE).

CLINICAL DATA SUPPORTING UCB THERAPY FOR CEREBRAL PALSY

The promising data derived from rodent studies on the treatment of brain injury with UCB cells initially underpinned

Table 1. Clinical trials using UCB for the treatment of CP and ischemic brain injury in the neonate

Status	Study name	Sponsor	Study type	Primary outcome	Cell administration			Age	Inclusion criteria	# Patients	Clinical trial ID
					Source	Dose	Route				
Recruiting	Safety and Effectiveness of Cord Blood Stem Cell Infusion for the Treatment of CP in Children	Augusta University, USA	Blinded, crossover, placebo-controlled	Safety	Autologous	>10 M/kg body weight	IV	2–12 years	Any severity of CP	20	NCT01072370
Completed	UCB therapy for children with CP	Bundang CHA Hospital, Korea	Open label, single group assignment	Changes in motor performance, changes in gross motor function	Allogeneic +rehabilitation	Unknown	IV/IA	6 Months to 20 years	Any severity of CP	17	NCT01639404
Completed	UCB therapy for CP	Bundang CHA Hospital, Korea	Randomized, double blind, placebo-controlled	Changes in motor performance, changes in gross motor function	Allogeneic +rehabilitation	Unknown	IV/IA	6 Months to 20 years	Any severity of CP	37	NCT01528436
Completed	Allogenic UCB and erythropoietin combination therapy for CP	Bundang CHA Hospital, Korea	Randomized, double blind, placebo-controlled	Changes in motor performance, changes in gross motor function	Allogeneic+EPO	>30 M/kg body weight	IV/IA	10 Months to 10 years	Any severity of CP	105	NCT01193660
Recruiting	Granulocyte colony-stimulating factor and autologous cord blood infusion in CP	Hanyang University Seoul Hospital, Korea	Randomized, double blind	Safety with repeated G-CSF injections	Autologous	Unknown	Not clear	2–10 Years	Non-severe CP	88	NCT02866331
Active, not recruiting	Assessment of the safety of allogeneic UCB infusions in children with CP	Duke University Medical Center, USA	Open label, single group assignment	Safety	Allogeneic (sibling matched; first six HLA-matched, next nine half-matched)	Unknown	IV	12 Months to 6 years	Any severity of CP	15	NCT02599207
Completed	A randomized study of autologous UCB reinfusion in children with CP	Duke University Medical Center, USA	Randomized, double blind cross-over	Improvement of standardized measures of neurodevelopmental function at 2 years	Autologous	>10 M/kg body weight	IV	12 Months to 6 years	Any severity of CP	63	NCT01147653
Unknown	Allogeneic UCB therapy in children with CP	Bundang CHA Hospital, Korea	Open label, single group assignment	Cytokine analysis, changes in motor performance, changes in gross motor function, cognitive and motor neurodevelopment	Allogeneic (HLA mismatch)	>30 M/kg body weight	Not clear	Up to 15 years	Any severity of CP	18	NCT02025972
Recruiting	Stem cells in umbilical blood infusion for cp (scubi-CP)	Murdoch Childrens Research Institute, Australia	Open label, single group assignment	Safety	Allogeneic (sibling-matched)	>10 M/kg body weight	IV	1–16 Years	Any severity of CP	12	NCT03087110

Table 1 Continued

Status	Study name	Sponsor	Study type	Primary outcome	Cell administration			Age	Inclusion criteria	# Patients	Clinical trial ID
					Source	Dose	Route				
Active, not recruiting	Safety and effectiveness of banked cord blood or bone morrow stem cells in children with Cp (act for CP)	The University of Texas Health Science Center, Houston, USA	Randomized, double blind Cross-over	Safety	Autologous (UCB or bone marrow)	>10 M/kg body weight	IV	2–10 Years	Any severity of CP	20	NCT01988584
Unknown	Allogeneic UCB therapy with erythropoietin in children with CP	Bundang CHA Hospital, Korea	Randomized, double blind, placebo-controlled	Changes in motor performance, changes in gross motor function, cognitive and motor neurodevelopment	Allogeneic (HLA mismatch)+EPO	>30 M/kg body weight	Not clear	10 Months to 6 years	Any severity of CP	120	NCT01991145
Unknown	Combination therapy of cord blood and G-CSF for patients with brain injury or neurodegenerative disorders	Bundang CHA Hospital, Korea	Open label, single group assignment	Changes in motor performance, changes in gross motor function	Allogeneic+G-CSF	Unknown	Not clear	19 Years or older	Any severity of CP	10 (CP, ALS, Parkinsons, brain injury)	NCT02236065
Unknown	Autologous stem cells in newborns with oxygen deprivation	Hospital Universitario, Mexico	Open label, single group assignment	Safety at 1 week and 1 year clinical assessment	Autologous	Unknown	IV	Within first 48 h after birth	HIE	20	NCT01506258
Not yet recruiting	Autologous cord blood and human placental-derived stem cells in neonates with severe HIE (HPDSC+HIE)	New York Medical College, USA	Open label, single group assignment	Safety and tolerability	Autologous	Unknown	IV	Within first 7 days after birth	Severe HIE	20	NCT02434965
Recruiting	A multi-site study of autologous cord blood cells for HIE	Duke University Medical Center, USA	Randomized, double blind, placebo-controlled	Survival at 1 year, Bayley assessment	Autologous	Two doses of unknown concentration	IV	Within first 48 h after birth	Moderate to severe HIE	160	NCT02612155
Completed	Cord blood for neonatal HIE	Duke University Medical Center, USA	Open label, single group assignment	Safety and feasibility	Autologous	50 M/kg (up to four doses)	IV	Within first 14 days after birth	Moderate to severe HIE	52	NCT00593242
Completed	Autologous cord blood cells for brain injury in term newborns	National University Hospital, Singapore	Open label, single group assignment	Safety	Autologous	Unknown	Not clear	Within first 72 h after birth	Moderate to severe HIE	2	NCT01649648
Not yet recruiting	Neonatal HIE: safety and feasibility study of a curative treatment with autologous cord blood stem cells (NEOSTEM)	Assistance Publique Hopitaux De Marseille, France	Open label, single group assignment	Safety	Autologous	Unknown	Not clear	Within first 72 h after birth	HIE	20	NCT02881970

Table 1 Continued

Status	Study name	Sponsor	Study type	Primary outcome	Cell administration			Age	Inclusion criteria	# Patients	Clinical trial ID
					Source	Dose	Route				
Recruiting	Neuroprotective effect of autologous cord blood combined with therapeutic hypothermia following neonatal encephalopathy	Children's Hospital of Fudan University, China	Randomized, single blinded	Mortality and disability rate	Autologous +hypothermia	Three doses of unknown concentration	Not clear	Within first 72 h after birth	HIE or cerebral infarction	60	NCT02551003
Recruiting	Autologous cord blood cell therapy for neonatal encephalopathy	Neonatal Encephalopathy Consortium, Japan	Open label, single group, multicenter	Safety	Autologous	Three doses of unknown concentration	IV	Within first 14 days after birth	Moderate to severe HIE	6	NCT02256618
Unknown	Autologous UCB transfusion for preterm neonates	Ain Shams University, Egypt	Open label, single group assignment	Duration of mechanical ventilation	Autologous	Unknown	Not clear	Within first 14 days after birth	Preterm (<33 wks), RDS, IVH	60	NCT01121328

CP, cerebral palsy; HIE, hypoxic–ischemic encephalopathy; HLA, human leukocyte antigen; IA, intra-arterial; IV, intravenous; IVH, intraventricular hemorrhage; RDS, respiratory distress syndrome; UCB, umbilical cord blood; wks, weeks.
Information obtained from *ClinicalTrials.gov*.

the commencement of clinical trials to investigate the therapeutic potential for UCB cells for cerebral palsy. Currently, there are 21 clinical trials investigating UCB therapy for cerebral palsy, or brain injury in neonates (with HIE), including six completed studies (*clinicaltrials.gov*; **Table 1**). Recently, a meta-analysis was performed investigating all clinical trials that had published the use of stem cells as a therapy for established cerebral palsy (26). The meta-analysis examined all stem cell sources, including olfactory ensheathing cells, neural progenitor cells, and allogeneic UCB. Only randomized controlled trials and controlled clinical trials were considered, of which five trials met the selection criteria. The authors concluded that, when children with cerebral palsy were treated with stem cells, there was a significant intervention effect at a short-term (6 months) follow-up. Most interestingly, the effect was greatest for UCB cells, compared with any other cell types (26).

Although such analysis is encouraging, only a small number of clinical trials that all have relatively small sample sizes have been completed using UCB cells for treatment of cerebral palsy. In addition, many of the listed clinical trials are open-label, single-group studies that have the primary outcome of safety (12 of 21), with 5 of 21 being randomized controlled trials (**Table 1**). Safety studies are important and are the necessary first step to progress any new therapy through ethics and governance bodies, and six completed studies now report safety in >200 patients. Critical questions that now remain include—are UCB cells efficacious to reduce cerebral palsy? What is their mechanism/s of action? When is the best time to treat? How many cells and doses should be administered? What is the best cell type? And should we use autologous or allogeneic cells? Taken together, these questions are difficult to answer in a timely manner in a clinical setting.

POTENTIAL OF UCB FOR REDUCING PERINATAL BRAIN INJURY AND CEREBRAL PALSY; PRECLINICAL LARGE ANIMAL EVIDENCE

Preclinical studies in animal models that use a controlled and standardized insult are considered gold standard in the development of therapies from bench to bedside. In addition, a review by Bennet *et al.* (27) recommended that studies in large animal models are crucial to confirm the safety and, most importantly, efficacy of cell therapies to reduce perinatal brain injury and cerebral palsy. To date, just a handful of studies have assessed the efficacy of UCB cells in large animal models of perinatal brain injury (**Table 2**). These studies are heterogenous in their use of animal models (sheep and rabbits), their cell source (human or ovine UCB cells), route of administration, and developmental timing of injury (preterm or term). However, it is notable that results from these large animal studies indicate that early administration of UCB cells, soon after HI perinatal brain injury, confers neuroprotective benefits for brain biochemistry (magnetic resonance spectroscopy (MRS) measures), neuropathology, and functional outcomes (28–30).

Table 2. Large animal studies investigating the efficacy of UCB cells for cerebral palsy

Cell source	Animal model	Cell administration				Histopathological assessment	Neurobehavioral/clinical assessment	Reference
		Timing	Route	Dose (total)	Dose (cells/kg)			
Human UCB-MNCs, xenogeneic	Rabbit—preterm HI	4 h After birth, 9 d after insult	Intravenous	2.5×10^6 or 5×10^6	45×10^6 or 90×10^6	No histological assessment. Assessed presence of hUCB cells by MRI and PCR. No evidence by MRI, human signal detected by PCR	Improved posture, righting reflex, locomotion, tone, and dystonia	Drobyshevsky *et al.* (29)
Ovine UCB-MNCs, autologous	Sheep—term HIE	12 h after birth	Intra-arterial (brachial artery)	100×10^6	25×10^6	Reduced apoptosis, microglial inflammation, and astrogliosis in all brain regions examined. Minimal evidence of cells in the brain	Improved NAA:lactate ratio as measured by MRS. Mildly improved feeding, posture, and ability to stand	Aridas *et al.* (28)
Ovine UCB-MNCs, allogeneic	Sheep—preterm HI	12 h or 5 d after birth	Intravenous	50×10^6	25×10^6	Oligodendrocytes and myelinated axons protected. Decreased apoptosis and activated microglia. Minimal evidence of cells in the brain	No neurobehavioral or clinical assessments	Li *et al.* (30)

d, day; MNC, mononuclear cells; MRS, magnetic resonance spectroscopy; NAA, N-acetyl aspartate; UCB, umbilical cord blood.

Drobyshevsky *et al.* (29) was the first published study assessing UCB therapy in a large animal (rabbit) model of perinatal brain injury. In this study, human UCB mononuclear cells were administered in a preterm model of HI brain injury. Importantly, they compared two doses of cells to determine a dose response; a low dose (2.5 million, equivalent to ~45 million human UCB cells/kg) and a high dose (5 million, equivalent to ~90 million cells/kg). Neurobehavioral assessment performed at 5 and 11 days after birth showed that the high dose of UCB had greater efficacy than the lower dose. Unfortunately, the experiments were performed separately and, therefore, no direct comparisons could be made between the two doses and any conclusions made on the effect of dosage needs to be confirmed. Moreover, this study used a xenogeneic transplantation model where human UCB cells were given to rabbits. Whereas previous studies have used human cells in rodent models (22,23) and have been shown to be efficacious and likely to evade major histocompatibility complex incompatibility, there is still an increased risk of immunological reaction and clearance of the cells before they have had time to be effective. For this reason, studies investigating autologous and allogeneic transplantation from the same species are necessary.

Aridas *et al.* (28) examined the use of autologous UCB therapy in a lamb model of birth asphyxia and HIE. A significant finding from this study was the restoration of normal brain biochemical profiles with UCB treatment, as assessed using MRS, particularly for lactate: N-acetyl aspartate (NAA) ratio (**Figure 2**). Clinically, MRS is often assessed at 5–7 days after perinatal asphyxia and an increase in the ratio of lactate:NAA is considered a reliable biomarker of HIE and predicts death or disability at 12 months of age (31). In the study of Aridas *et al.*, UCB mononuclear cells were

Figure 2. Umbilical cord blood (UCB) improves the brain biochemical profile of lactate:N-acetyl aspartate (NAA) after asphyxia. Magnetic resonance spectroscopy (MRS) was performed at 12 and 72 h following a severe asphyxic insult at birth in term lambs. UCB was administered intra-arterially at 12 h, immediately before the first magnetic resonance spectroscopy (MRS). Lambs that received UCB therapy demonstrated a significant decrease in brain lactate:NAA from 12 to 72 h, whereas lactate:NAA continued to increase in the non-treated asphyxia lambs. Adapted from Aridas *et al.* (28).

administered intra-arterially (via the brachial artery) at 12 h after a severe asphyxic insult at birth, and MRS was initially performed at 12 h, within minutes of UCB cells being given, when an altered brain biochemical profile had already been established before cell administration. MRS was then performed again at 72 h post asphyxia, and it was observed that UCB therapy reduced the lactate:NAA ratio by more than 50% compared with results at 12 h and compared with untreated asphyxia lambs, indicating a reversal of injurious pathways after UCB therapy (**Figure 2**, adapted from (ref. 28)). Histopathological analysis was performed on tissue collected 72 h post HI and, in all regions of the brain assessed, autologous UCB therapy reduced caspase-3-induced apoptosis, microglial inflammation, and astrogliosis (29). A limitation of this study was that animals were only maintained for 72 h after birth asphyxia and, therefore, no long-term outcomes were available. Further research of long-term outcomes of such treatment is, therefore, warranted.

A second sheep study by Li *et al.* (30) used a preterm HI model of brain injury and administration of allogeneic term UCB at two time points, either at 12 h or at 5 days after HI injury. The most significant findings in this study related to white matter brain regions, where UCB therapy at 12 h was able to protect oligodendrocytes and axonal myelination compared with HI injury alone, while also significantly reducing the number of activated microglia within the white matter. UCB therapy at 5 days was not protective for oligodendrocytes or microglia, but did reduce cell death. In addition, this study is the only large animal study so far to examine the effects of UCB on oxidative stress levels, showing that UCB therapy at 12 h significantly reduced circulating markers of oxidative stress at 48 h, compared with HI animals. A further study by Li *et al.* (32) compared the neuroprotective benefits of UCB obtained from preterm vs. term cord blood, in consideration that cellular makeup of the stem/progenitor cells of interest (as per **Figure 1**) is altered during gestation (13). This study showed that UCB cells from term or preterm cord blood are effective at reducing cerebral inflammation and white matter brain injury induced via a HI insult in preterm fetal sheep, but secondary mechanisms of neuroprotection are different.

A significant limitation to all of the large animal studies discussed here is they were conducted over a relatively short experimental period. In part, this is contributed by the challenges of maintaining these animals in a neonatal intensive care setting over a prolonged period, and the associated financial constraints of maintaining large animals. Consequently, large animal studies may not address the crucial need for long-term follow-up data. Another limitation that arises with large animal studies is deciphering the mechanism/s of action of UCB cells. With the limited availability of cross-reactive antibodies and molecular probes available for sheep and rabbits, compared with rodent and human studies, it makes it difficult to fully characterize pathways that therapies may be modulating. This includes what cell types within UCB may be the most *effective* cells to treat a specific condition, which is a consideration when moving toward "off-the-shelf" standardized products.

OPTIMAL TIMING OF UCB THERAPY

A common finding between all three large animal studies reported above was that, when UCB cells were given as an early intervention therapy, within hours after HI injury, UCB reduced brain injury. However, each study employed a different administration time point from hours to days after the HI insult, so what lessons can be taken from this? In both the term and preterm sheep studies, cells were administered at 12 h after injury and significant protection of the gray and white matter, respectively, was observed (28,30). Delaying UCB administration to 5 days after HI insult reduced their efficacy, but significant neuroprotection was still observed (30). In contrast, in the rabbit study UCB cells were administered 9 days after preterm HI insult, which was 4 h after birth. This is a clinically relevant time point, considering that therapeutic intervention for infants born preterm may not occur until some hours after birth, when the baby has been stabilized. In addition, the exact timing of treatment will still be dependent on the time required for diagnosis of encephalopathy and, for the clinical trials, will include the time required to gain parental consent. Nevertheless, these are encouraging results, and should guide the development of early intervention therapy for the treatment of infants born preterm and with high risk of brain injury.

OPTIMAL ADMINISTRATION ROUTE; DO CELLS HAVE TO REACH THE BRAIN?

An important consideration raised by the Drobyshevsky study (30) relates to the administration protocol, as the authors found that their high dose of human UCB cells (5 million) was initially associated with a high mortality rate. They noted, however, that mortality was decreased when they increased the duration of cell infusion. A potential reason for this high mortality rate could be because of the route of administration that was used. In their study, the route chosen was intravenous; therefore, the "first pass" of the cells was via the pulmonary circulation, before the cells traveled through the rest of the body. There is good evidence in rodent models of stem cell administration that this "first pass" is critical because of the high risk of cell entrapment within the lung vasculature, leading to subsequent pulmonary embolism and, consequently, high mortality rates (33,34). This risk increases as the number of cells given is increased (35). In comparison, the Aridas study (29) used administration via the brachial artery, resulting in cells being delivered initially directly into the ascending aorta. This means that the cells passed via the brain before reaching the pulmonary circulation, potentially increasing the chance of entry of cells into the brain. The challenges with this route include the increased risk of cerebral embolism in the capillary network, which may have catastrophic effects, including hemorrhage. However, there was no evidence of any increased risk of brain bleeds following brachial administration of UCB cells in this

study (28). It should, however, be considered that the brachial artery route would not be used clinically, and this must be taken into account when determining the optimum route and dose for administration of UCB. Reassuringly, data from the clinical trials assessing safety have clearly shown that judicial intravenous administration of UCB, with doses up to 50 million cells/kg body weight to babies (36) and children (37,38) is safe and feasible, and pulmonary embolism has not been reported.

All studies reported herein examined potential engraftment of cells within the brain, using different techniques such as immunofluorescence, MRI, and PCR, and all studies concluded that UCB cell engraftment is a relatively rare event and involves only small numbers of cells. Aridas *et al.* investigate the presence of fluorescently labeled UCB cells in the brain 60 h after administration and found a very small number of cells within the subcortical white matter, hippocampus, and cortex (29). Li *et al.* also used fluorescently labeled cells and only found minimal presence of cells within the brain at 10 days post administration (31). Drobyshevsky *et al.* examined cell engraftment via MRI, and found no evidence of cells within the brain. They also used PCR to identify the presence of a human gene and detected a faint signal, but they were not able to quantify the number of cells present and concluded that it was likely to be small in number (30). Given that all studies used methods that involved passive labeling of cells, where the signal would be diluted with each population doubling, there is the possibility that the low rate of engraftment may be partly due to loss of signal if studied some time after administration. Studies using transduction with lentiviral labels might be a more robust approach. It is pertinent to note that a study in an adult rodent model of stroke showed that, even when UCB cells were given together with a blood–brain barrier permeabilizer (mannitol), with the aim of increasing cell entry into the brain, results showed that presence of UCB cells in the central nervous system is not necessary for neuroprotection (39). It was, however, demonstrated that UCB+mannitol co-treatment increased brain levels of neurotrophic factors, which correlated with a reduction in brain infarct in this model. These findings are strongly indicative that the benefits of UCB therapy are because of the release of trophic factors directly by the administered cells, or via stimulation of endogenous cells, which mediate cerebral repair.

EVIDENCE FOR THE EFFICACY OF OTHER STEM-LIKE CELLS

In this review we have concentrated on cells obtained from UCB; however, it is important to note that other cells with stem-like properties are being examined, and show promise, for their neuroprotective potential in the immature brain. NSCs are multipotent cells endogenously produced principally in the subventicular zone (SVZ) of the developing brain, and continue to be found in the SVZ in the adult brain. They possess the ability to self-renew and capacity to differentiate into neurons and glial cells. In neonatal rats exposed to HI, hNSCs were administered into the forebrain at 24 h post HI, and were shown to mediate microglial response, enhance axonal sprouting of neurons, and improve motor functions in rats at 1 month of age (40). Chen *et al.* (41) have studied the effects of clinical administration of neural stem-like cells, differentiated from autologous bone marrow-derived MSCs, to 30 children with cerebral palsy. They showed that NSC-like cell administration remained safe at 6 months post treatment, and gross motor function measures indicated improvement in motor ability at 3 and 6 months post treatment. Another cell type of interest is amnion epithelial cells (AECs), obtained from the amniotic membrane after removal of the placenta at birth. Human (h)AECs demonstrate low immunogenicity on xenogeneic administration and, indeed, rather than eliciting an adverse immune reaction in response to administration, hAECs prevent activation of both innate and adaptive immune pathways, suppress pro-inflammatory cytokines, and mediate macrophage recruitment (reviewed in (ref. 12)). In light of their strong immune modulatory and anti-inflammatory effects, the neuroprotective role of hAECs has been examined in response to inflammation-induced (42–44) and HI-induced preterm brain injury in large animal (sheep) models (45). In these studies, hAECs reduced white and gray matter brain injury, mediated predominantly by the anti-inflammatory effects of these cells, together with stabilization of the blood–brain barrier. Given the impressive anti-inflammatory and immunomodulatory benefits of hAECs, a Phase 1 safety trial is now underway to assess whether the administration of hAECs to infants with bronchopulmonary dysplasia is beneficial (ACTRN12614000174684). Neurological assessment is not an outcome measure in the current trial, but is a critical inclusion as a secondary outcome measure in future trials, given the association between bronchopulmonary dysplasia and adverse neurodevelopmental outcome (46). There are currently no clinical trials listed to specifically examine the neuroprotective effects of hAECs.

FUTURE UCB CELL THERAPY—THE RIGHT CELLS AT THE RIGHT TIME

Traditionally, for the therapeutic use of UCB, the red blood cells and plasma are removed and the mononuclear cell fraction is administered. This fraction contains many different cell types with a variable mix of stem and progenitor cells. As previously noted, these cells include HSCs, EPCs, and MSCs (47,48). UCB is also an excellent source of potent immunoregulatory cells, including Tregs and MDSCs, **Figure 1** (49,50). It is believed that these cells are together, the major contributors to the therapeutic effect of UCB. However, each individual UCB sample (unit) has different proportions of these cells types and, furthermore, the proportion of these cells changes throughout gestation, meaning that preterm UCB is different in cell content compared with that of term UCB (51,52). There is also evidence that complications during pregnancy, such as intrauterine growth restriction, pre-eclampsia, and chorioamnionitis can change the proportion of stem and progenitor cells and, importantly, their characteristics (53). Of particular note, it has been shown

that UCB collected from intrauterine growth restriction pregnancies has a reduced proportion of EPCs, and that those cells present were functionally impaired (54). Unfortunately, we still do not understand how each of these cell types individually contributes to neurorepair in the studies undertaken so far, and this information will be critical to obtain as we move toward (i) identifying which UCB samples will be the most potent and efficacious in specific clinical circumstances, and (ii) developing new specific single cell-type therapies (including allogeneic) that may be more effective, but require expansion before use.

HSCs are characterized as CD34+ cells, with the ability to self-renew and differentiate into multiple blood cell lineages (55). Until recently, the principal application of UCB centered around HSCs, and their use in treating patients with hematopoietic conditions, such as childhood cancers (55). Compared with adult bone marrow, HSCs found in UCB have longer telomeres and increased colony-forming capacity (56,57). CD34+ cells derived from UCB have also been shown to significantly improve behavioral outcomes and increase neurogenesis after stroke in both adult and neonatal models (20,58,59). MSCs can also be isolated from UCB and, after HSCs, are the most well-studied cell type in UCB. MSCs are potent immunomodulatory cells that secrete an array of neuro- and angiotrophic factors that make them favorable cell types for treatment of neurological conditions (60). In culture, MSCs can also be directed toward, and may spontaneously form precursor and mature neurons, and astroglial cells (61,62). However, MSCs are present in term UCB samples at a very low frequency and number, with only 10–30% of human term UCB samples containing MSCs (63). EPCs can be isolated from UCB, adult peripheral blood, and bone marrow. They have the ability to form mature endothelial cells and play a critical role in promoting growth of new blood vessels and stabilizing damaged vessels (64). Both *in vivo* and *in vitro* data suggest that EPCs can also protect neural cells after a HI insult (65,66). Although it is considered that the main neuroregenerative benefit of EPCs is in their ability to induce angiogenesis, they can act in a paracrine manner to secrete factors that create a favorable niche for the differentiation of other progenitor cells (67). Tregs in UCB have a predominantly naive phenotype, with an enhanced proliferative potential compared with adult-derived Tregs (68). Tregs are potent immunosuppressive cells that normally maintain self-tolerance, prevent autoimmunity, and can inhibit transplant rejection and regulate immune responses during infections (69). In a stroke model, Tregs have been shown to reduce neuroinflammation and infarct size, and improve long-term neurological function (70). MDSCs are a recently discovered immunosuppressive cell, found in UCB at much higher numbers than in the peripheral blood of adults (71). Their role in neurological conditions has not yet been explored in any detail; however, their presence is linked to increased recovery in a multiple sclerosis model (72). With respect to perinatal brain injury, a recent study in which HI was induced in neonatal rodents demonstrated that depletion of monocytes from UCB at transplantation was associated with a reduction in motor improvement and microglial suppression, suggesting that there is a cell type within the monocyte population that has neuroprotective potential (73). Each of the UCB cell types mentioned—HSCs, MSCs, EPCs, Tregs, and MDSCs—demonstrate individual characteristics that are likely to contribute to neuroprotection and repair in the perinatal setting of brain injury. The therapeutic potential of MSCs for neonatal brain injury has been more thoroughly examined than has other cell types (reviewed in (ref. 74)), and, consequently, clinical trials are currently taking place for the administration of UCB-derived MSCs to infants with HIE and children with cerebral palsy. However, it is not yet known whether individual cell types can be isolated and expanded to provide therapeutic efficacy in the treatment of perinatal brain injury or cerebral palsy, or whether these cells work best alone or in a synergistic manner within the whole mononuclear cell fraction of UCB. Evidence supports that each of the individual stem/progenitor cell types shown in **Figure 1** contributes to specific neuroprotective benefits of UCB, including anti-apoptotic, anti-inflammatory, and antioxidant effects, along with vascular remodeling and the release of neurotrophic factors to support endogenous repair. However, we should also keep in mind that it may be the synergistic effects of all of these mechanisms of action that provide an optimal and combined neuroprotective strategy for perinatal brain injury. Further animal studies should examine and compare individual UCB-derived cells for their neuroprotective potential, compared with the whole UCB mononuclear fraction, with particular reference to the timing of cell administration to target specific injurious processes.

With reference to the optimal timing of UCB therapy, we have already discussed data from large animal studies that support the use of UCB mononuclear cell therapy, within the first days (9 days) after the initial insult, as being neuroprotective, and earlier intervention (12 h) post-HI insult, having a greater benefit (30). These findings are supported by results in adult rodents wherein treatment with UCB at 14 days post stroke does not confer any benefit (59). This is an important consideration when assessing the efficacy of UCB cells in current clinical trials, in which the vast majority of trials (**Table 1**) treat children through to adults (aged 6 months to 20 years) who have been diagnosed with cerebral palsy. To the best of our knowledge, there are no published animal studies in which UCB cells have been administered at an age equivalent to childhood, in terms of brain development, after perinatal brain injury. Nevertheless, some benefits of late-intervention UCB therapy are supported in the meta-analysis undertaken by Novak *et al.* (26).

The mechanisms by which UCB cells may mediate neurorepair in children with already established cerebral palsy are not known; however, we and others speculate that any positive effects observed could be contributed by a combination of factors, including release of neurotrophic factors, stimulating proliferation, and recruitment of neural progenitor cells, and increasing brain structural connectivity

(75–77). There are many questions that remain to be answered with respect to the optimal timing of stem cell therapy for treatment of neonatal brain injury, and, hopefully, to prevent cerebral palsy. Such questions will be answered only via a coordinated and complimentary approach in animal studies and human clinical trials.

CONCLUSIONS

This review of the current literature supports that UCB cell therapy demonstrates excellent potential to protect or repair brain injury in the young brain, as evidenced by results in animal studies and clinical trials. There does, however, remain much to learn about how individual UCB cells mediate specific neuroprotective contributions for perinatal brain injury. Indeed, in this review we have not explored in detail that the etiology and subsequent perinatal brain injury that causes cerebral palsy is heterogenous, with compromise that may have been antenatal, perinatal, or postnatal (or a combination of these), in infants who may have been born preterm or at term and, thus, are at different stages of brain development (78). The individual cell types contained in UCB have great anti-inflammatory, anti-apoptotic, and antioxidant potential, or may mediate repair of cerebral architecture (e.g., via vascular remodeling or re-establishment of neuronal networks), with each of these benefits important for cerebral repair under various adverse conditions. It does, however, remain unknown whether optimal neuroprotective benefit is gained when these cells are given in combination as the mononuclear cell fraction of UCB, perhaps complimenting each other's mechanisms of action, or if cells could be individually isolated and expanded as an "off-the-shelf" product for targeted therapeutic intervention. Current dogma suggests that autologous cell therapy is the gold-standard for treatment of cerebral palsy; however, it is known that the proportion of the cells of interest differ widely within individual cord blood samples, and so methodologies to standardize UCB treatment regimes for maximum efficacy may be required in the future. It is likely that the questions posed in this review are best addressed using animal models of perinatal brain injury, mirroring clinical scenarios of preterm white matter injury, intrauterine inflammation, fetal growth restriction, and term HIE in order to characterize optimal UCB cell therapy.

Despite the remaining questions, there is substantial evidence from preclinical and clinical trials to demonstrate that UCB therapy to prevent or treat cerebral palsy is safe and feasible. Small and large animal studies demonstrate that UCB cells are efficacious as a neuroprotective therapy, mediated via anti-inflammatory, immunomodulatory, angiogenic, antioxidant, neurotrophic factor release, and anti-apoptotic actions. Results from human clinical trials to date are also promising. Information obtained from small and large animal studies shows that early intervention of UCB cells within the first few days after the onset of injury is likely to be most efficacious, and indeed the earlier cells can be administered, the better the result. This is encouraging for conditions such as HIE or perinatal stroke that can be detected early, thus allowing timely intervention. The optimal timing for other complications in which a sentinel event is not evident, or occurs antenatally, remains relatively unknown. It is certainly encouraging that initial insights from clinical trials to treat established childhood cerebral palsy show promise. Indeed, a significant benefit of therapeutic intervention for the treatment of neonatal brain injury or cerebral palsy in childhood is that the developing brain shows remarkable neuroplasticity. In summary, we should be cautiously optimistic that UCB cell therapy will have a role in the prevention or repair of the brain injury that underlies cerebral palsy in the future. However, we should also be working in a coordinated manner toward consensus on an optimal dose, cell type, mode of delivery, timing of administration, and the potential for targeted therapy in both infants and children who are diagnosed with brain injury.

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