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Feasibility of Autologous Cord Blood Cells for Infants with Hypoxic-Ischemic Encephalopathy

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Abstract

Objective—To assess feasibility and safety of providing autologous umbilical cord blood (UCB) cells to neonates with hypoxic-ischemic encephalopathy (HIE).

Study design—We enrolled infants in the Intensive Care Nursery who were cooled for HIE and had available UCB in an open-label study of non-cyropreserved autologous volume- and red blood cell-reduced UCB cells (up to four doses adjusted for volume and RBC content,$1 – 5 \times 10^7$cells/dose). We recorded UCB collection and cell infusion characteristics, and pre- and post- infusion vital signs. As exploratory analyses we compared cell recipients' hospital outcomes (mortality, oral feeds at discharge) and one year survival with Bayley III scores ≥ 85 in 3 domains (cognitive, language, and motor development) with cooled infants who did not have available cells.

Results—Twenty-three infants were cooled and received cells. Median collection and infusion volumes were 36 and 4.3 milliliters. Vital signs including oxygen saturation were similar before and after infusions in the first 48 postnatal hours. Cell recipients and concurrent cooled infants had similar hospital outcomes. Thirteen of 18 (74%) cell recipients and 19 of 46 (41%) concurrent cooled infants with known 1 year outcomes survived with scores ≥ 85.

Conclusions—Collection, preparation and infusion of fresh autologous UCB cells for use in infants with HIE is feasible. A randomized double-blind study is needed.

Keywords

Asphyxia; Neonatal encephalopathy; Umbilical cord blood; Neonate

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Moderate hypothermia improves outcomes for term and near-term infants born with moderate or severe hypoxic-ischemic encephalopathy (HIE), but in pivotal trials over 30% of cooled infants died or survived with impairment.[1–4] The high prevalence of poor outcome provides motivation to test additional interventions.[5] Hypothermia targets pathophysiology related to secondary energy failure, including excitatory neurotransmitter release and destructive apoptosis and 'continuum' cell death.[6,7] Additional interventions may focus on these mechanisms plus interactions between injury, injury response, and ongoing brain development.[8]

Nucleated umbilical cord blood (UCB) cells can differentiate *in vitro* into cells with characteristics of neurons, oligodendrocytes, astrocytes and microglial cells.[9–11] UCB cells have been used successfully in thousands of allogeneic transplants for cancer and genetic disease, including in infants with Krabbe Disease and Hurler Syndrome.[12, 13] Neonatal rodents injected with human UCB cells after hypoxic-ischemic injury have improved anatomic and neurobehavioral outcomes, most likely due to paracrine and trophic effects during the hours and days after injury, leading to speculation that UCB cells could be a useful adjunct intervention for human infants with HIE.[14–19]

We hypothesized that early infusion of autologous volume- and red blood cell (RBC)-reduced UCB cells in infants with HIE would, primarily via trophic and paracrine mechanisms, improve outcomes. To that end, we conducted a pilot feasibility and preliminary safety study of intravenous infusion of non-cryopreserved, RBC- and volume-reduced, autologous UCB cells in infants with moderate or severe HIE. Our objectives were to: (1) identify challenges to coordinating the multiple disciplines needed to collect, prepare and infuse cells in the first postnatal days; (2) characterize quality of UCB collections in high risk deliveries; and (3) report the cell recipients' response to infusions and their clinical outcomes at hospital discharge and one year of age.

Methods

We initiated this pilot study in January 2009. Infants admitted to the Duke Intensive Care Nursery (ICN) were eligible if they were ≥ 35 weeks gestation with HIE and met the ICN cooling criteria, which is based on the inclusion criteria used in the NICHD Neonatal Research Network (NRN) Hypothermia trial.[2, 20] Hypothermia criteria were met if infants had cord or first postnatal hour blood gas results with pH ≤ 7.0, or base deficit ≥ −16. If a blood gas in the first postnatal hour was unavailable, or if the cord or first postnatal hour blood gas pH was 7.01 – 7.15 or base deficit between −10 and −15, infants were eligible if they also had a history of an acute perinatal event and either an Apgar score at 10 minutes of ≤ 5 or need for positive pressure ventilation initiated at birth and continued for ≥ 10 minutes. Infants meeting criteria were then examined in 6 domains: level of consciousness, level of spontaneous activity, tone, posture, primitive reflexes, and autonomic function. If abnormal in 3 of 6 domains, or if the infant had seizures, the infant was treated with hypothermia and eligible for the study if cells were available.

UCB collection for donation to the Carolinas Cord Blood Bank (CCBB) for public banking within the Duke University Health System (DUHS) is routinely performed by dedicated, trained UCB collection staff and is restricted to deliveries of mothers who have given prior written informed consent for collection and have healthy term babies. If a CCBB donor mother delivered a baby with signs of HIE, CCBB staff collected UCB utilizing standard procedures, and UCB was deferred from public banking and instead, utilized if the sick infant was eligible for our study and the parents consented for study participation. For deliveries in which prior CCBB collection consent had not been obtained, the DUHS institutional review board (IRB) gave permission for obstetric staff to obtain verbal assent to collect UCB if in the perinatal period the obstetric caregiver thought the infant could meet HIE cooling criteria. If cells were available, and the infant met cooling criteria, parents were asked to provide written informed consent for the infant to be enrolled in the study. The study was approved by the Duke IRB

Cord blood was collected aseptically via *in utero* or *ex utero* techniques into cord blood collection bags (Pall, Medsep, Covina, CA) containing 35 mL of citrate phosphate dextrose anticoagulant provided by the CCBB.[21] UCB collections were made by trained obstetricians, midwives, or CCBB collection staff. Collection staff were present at both DUHS Birthing Centers (Duke University Hospital; DUH, and Duke Regional Hospital; DRH is an affiliated community hospital approximately 5 miles from DUH) during

weekdays for 8 – 12 hours per day. UCB collectors were also on site at 6 other regional centers not affiliated with the Duke Health System. UCB collected for outborn infants was sent with the infant on transport.

Collected UCB was transported at room temperature in validated shippers to the Duke Stem Cell Laboratory (SCL). There it was volume- and RBC reduced after 20–30 minute incubation with 6% Hespan (hetastarch, Hospira, Lake Forest, Illinois, USA) following established CCBB procedures using the Sepax 1 automated processing system (Biosafe, Geneva, Switzerland) if the unit contained > 30mL of UCB or manually if the unit was < 30 mL. Volume- and RBC-reduced UCB cells were deposited into a volume of 20.5 mL and aliquotted into individual dose syringes with primed connecting tubing and a syringe containing a normal saline flush. A specialized storage bag with 4 needleless injection ports for removing sterile aliquots for up to 4 doses was developed for use in this trial by Biosafe (Geneva, Switzerland). Total nucleated cell counts pre- and post-processing, post-processing CD34 cell content, Colony Forming Units (CFU), sterility and viability were assessed. Doses were engineered to contain $1–5\times10^7$ nucleated cells, with no more than 2 mL/kg of PRBC and a targeted dose volume ≤ 2 mL/kg/dose.

All infants were cooled to 33.5° C for 72 hours. [2, 20] Study staff carried pre-measured doses of cells to the ICN in labeled syringes from the Duke SCL. Infusions were started when cells and study staff were available for administration and monitoring. Infants were pretreated with hydrocortisone, 1 mg/kg IV 30 – 60 minutes prior to infusion. Infants received up to 4 infusions of $1 – 5 \times 10^7$ cells/kg, with the first dose as soon as possible after birth, and at 24, 48, and 72 postnatal hours. If the first dose was available after the first 12 postnatal hours, dose timing was adjusted to provide 3 infusions during the first 72 postnatal hours. After release of final guidance for public cord blood banking in 2011, the FDA implemented a requirement for regulation of all non-homologous uses of umbilical cord blood. Accordingly, we submitted an IND application for this study. With FDA approval of our IND application in July 2011, the protocol was modified to provide a maximum of 2 infusions of fresh cells in the first 48 postnatal hours. All infusions were administered in the Duke ICN. Infusate and subject identities were double-checked by research and clinical nursing staff. Infusions were monitored by research and clinical staff. Cells were infused over 15 – 20 minutes, followed by a 1 – 2 ml saline flush to clear the intravascular line. Unused cells were cryopreserved in the SCL after addition of 10% DMSO to unused cells in a Medsep dual compartment cryopreservation bag (Pall Medical, Covina CA). Cells were cryopreserved after controlled-rate freezing under liquid nitrogen in a Thermogenesis Bioarchive (Thermogenesis, Sacremento, CA) for long-term storage.

Statistical analyses

We used descriptive statistics to characterize subject demographics and feasibility measures which included volume of UCB collected, time between collection, preparation and infusion. We also collected total nucleated cell count before and after volume and RBC reduction, cell viability, $CD34^+$ cell count, colony forming units, and sterility from each unit. For acute safety, we compared vital signs and oxygen saturations at baseline and 15 minutes post-infusion using Wilcoxon signed-rank tests. We also compared adverse

outcomes including mortality, seizures, development of pulmonary hypertension as well as one-year outcomes in infants enrolled in the study and infants admitted to the Duke ICN treated with hypothermia for HIE during the same time period who did not have cells available (concurrent cooled infants). One-year neurodevelopmental outcomes were assessed utilizing the Bayley Scales of Infant and Toddler Development-3rd Edition (Bayley III) which includes assessment of cognitive, language, and motor developmental domains (means=100, standard deviation (SD) = 15 for the three domains).[22] We compared Bayley III scores for cell recipients with those from the concurrently cooled cohort. We also compared rate of survival with one year Bayley III scores ≥ 85 in all domains. In order to provide some comparison of severity of illness for the cell recipients and the concurrently cooled infants we calculated a score derived from the NICHD Hypothermia study's cohort that provides an estimate of likelihood of benefit from cooling (NICHD score). The score assigns values for blood gas results, posture, spontaneous activity, 5 minute Apgar score and presence of maternal chronic hypertension, pregnancy-induced hypertension, pre-eclampsia or eclampsia.[23] We used Fisher exact and Wilcoxon rank sum tests where appropriate. In addition, we used multivariable logistic regression to examine odds of mortality for cell recipients and concurrently cooled infants controlling for the NICHD score.

Results

Between January 1, 2009, and June 5, 2012, twenty-three infants were enrolled, received cells and are ≥ 1 year old. During the study period, 2 infants had cells collected and were cooled for HIE, but their parents declined enrollment. During the study period, 82 infants did not have cells collected and were admitted to the Duke ICN for HIE and were cooled. The only statistically different characteristic between groups was outborn status. Six of the cell recipients were born at Duke's community hospital affiliate, DRH, the rest were born at DUH. Over 80% of the concurrent cooled infants were outborn (Table I).

Parents of 5 enrolled infants had provided prior CCBB donor consent. Eighteen (78%) subjects' parents gave verbal approval for collection. For the two infants who had UCB collected, but whose parents declined study enrollment, UCB was discarded as medical waste.

Multidisciplinary Collaboration

During the study's first year, 2 infants were enrolled. Nine infants born in the DUHS system were admitted to the Duke ICN for hypothermia for HIE, but did not have UCB collected. After consultation with maternal-fetal medicine (MFM) colleagues in February 2010, a policy was implemented which included consideration of UCB collection at delivery for all "obstetric emergencies". For "obstetric emergencies" at DUH the paging system is activated to alert all members of the care team including obstetrics, neonatology and anesthesia. MFM research staff tracked obstetric emergencies to confirm that UCB was collected where appropriate and followed up with staff when it was not. MFM research faculty and staff provided daily reminders at labor and delivery work rounds and nursing shift sign-outs about the study. Their goal was to identify high-risk deliveries where obstetric staff was concerned that the immediate antenatal clinical picture could be associated with an infant that could

have HIE, and to remind staff of UCB collection for the study. Collection kits were placed in all delivery areas, operating suites and the Neonatology resuscitation equipment bag. In our community hospital collection kits were placed in the operating suites and we updated the nursing and medical staff about the study. With these efforts, between February 2010 and March 2011, UCB was collected at 64 high risk deliveries and 15 infants received study infusions. No eligible infant was missed.

Cord blood collection and infusion preparation

Volume of UCB collected ranged widely from 3 to 178 mL and contained a median of 4.8×10^8 total nucleated cells. On average, eighty-five percent of cells were recovered after processing. With a goal dose of $1 – 5 \times 10^7$cells/kg for each infusion, 3×10^7 cells would have to be available for a 3 kg infant to have one infusion at the lowest dose. Even with the lowest collected blood volume, cell numbers were adequate for at least one dose containing the target cell number. Viability of post-processing units was high. Relative amount of CD34 cells in units varied widely. Time between collection and arrival at the bedside and initiation of infusion ranged from 3.9 to 220 hours. For infants admitted to the DUH ICN on week days, when SCL staff were present, average time between collection and initiation of infusion was 6.5 hours (range; 3.9, 12). In 15 (65%) infants, excess cells were cryopreserved for potential future use.

Cord blood safety

No significant infusion reactions were noted. Heart rate, mean arterial pressure and oxygen saturation did not vary significantly before and after infusions for the first 2 infusions. Mean oxygen saturation was lower after the third infusion (96.5% before vs. 94.5% after infusion, $p < 0.05$) and fourth infusion (98.4% before vs. 97.4% after infusion, $p < 0.05$). One infant, born at 35 weeks gestation, had a cord blood pH 6.72 and had a spontaneous ileal perforation prior to infusion #4. One infant had a positive UCB culture for *E. coli* which was also present in the mother's blood culture. The infant's own postnatally acquired blood culture was negative, but the infant received 21 days of ampicillin and cefotaxime. One infant's line was occluded minutes after initiating cells, so that infusion was stopped. Subsequent infusions were completed for this subject without incident.

Comparison of hospital outcomes between cell recipients and concurrent cooled infants

Need for ECMO was similar for cell recipients and concurrently cooled infants. A higher portion of the concurrent cooled group was discharged home on seizure medications than cell recipients, but this difference was not significant. None of the cell recipients died during the HIE-related hospitalization compared with 11 (13%) of the concurrent cooled group. This difference was not statistically significant.

One year outcomes

Demographic characteristics of the cell recipients and concurrent cooled infants with available survival and Bayley III outcomes at one year were similar except for outborn status (32% among cell recipients and 91% among the concurrent cooled infants, $p < 0.001$) (Table IV; available at www.jpeds.com). Survival and Bayley III outcome data are available

for 19 (82%) cell recipients at 1 year. Four (17%) cell recipients did not return for 1 year follow-up. The primary provider for one infant whose family relocated to another state affirmed that this infant survived through the first postnatal year, but there has not been a Bayley III assessment, leaving 18 infants with developmental outcome data at one year. Of these, 13 (72%) had Bayley scores ≥85 in all three test domains. Two infants who received cells died after the one year follow-up visit. At one year both were severely impaired and could not be scored on the Bayley III. One infant died at 14 months of RSV pneumonia. This infant was also diagnosed after study intervention with a chromosome 17p12 deletion as well as Wolf-Parkinson-White syndrome. The second infant who died had severe encephalopathy with resultant cystic encephalomalacia. He was diagnosed with cytomegalovirus (CMV) during his 5th postnatal week. There were no replicating particles of CMV in the cord blood unit. He developed infantile spasms after discharge. At 14 months, he died of an acute gastroenteritis with hypovolemic shock.

Eleven of the concurrent cooled group died prior to discharge from their HIE-related complications. Survival and Bayley III data is available in 46 (56%) of the initial 82 concurrently cooled infants. Nineteen (41%) of those infants evaluated had Bayley III scores ≥ 85 in all three domains (Table V). On multivariable logistic regression controlling for the NICHD score, the OR for mortality or Bayley III scores < 85 for cells compared with no cells was 0.27 with a 95% CI [0.08, 0.92]. A higher NICHD score was associated with increased odds of mortality or low Bayley III scores (OR 1.08; 95% CI 1.01, 1.14).

Discussion

The information collected from our study provides evidence that collection, preparation, and intravenous infusion of autologous, volume- and RBC- reduced, non-cryopreserved cord blood cells within the first few postnatal days are feasible, and, in this small group, safe. The study reinforces the need for well-orchestrated multidisciplinary collaboration to collect, process and infuse UCB in order to have UCB cells available for infants with HIE.

Most UCB units collected for this study would not have met the standard required for inclusion in public banks. However, given the size of the babies for whom the cells were collected, sufficient cells for our selected target dose in the neonatal period were recovered from all UCB collections, even 3 mL. The automated system for cord blood processing we used could be placed and utilized in any hospital blood bank or neonatal unit which would facilitate clinical trial site participation. If UCB cells are to be tested in a large multicenter trial, training of obstetric providers, including reassurance that even small volume collections could provide target doses, as well as close collaborations related to timing of request for consent for collection, will be required.

Specific evidence identifying cellular mechanisms for how UCB cells influence neurologic outcome are sparse. Emerging evidence suggests that, in addition to secondary energy failure and the immediate period of repair which has been the target of cooling, hypoxic ischemic injury leads to weeks to months of vulnerable endogenous repair and development of new functional pathways.[25] Three UCB cell studies of postnatal day 7 rodents subjected to hypoxia and unilateral carotid ligation show improved neurobehavioural outcome.[14–16]

There is some evidence of cell entry into the CNS, but none of the studies suggests persistence of human UCB cells in the CNS. One study found decreased caspase 3 activation in the area surrounding the most significant injury, along with possibly linked decrease in microglial activation and macrophage infiltration.[15] In another study which combined cells with mannitol, neural growth factor, glial cell line derived neurotrophic factor and brain derived neurotrophic factor (BDNF) were higher in cell recipients 3 days after cell infusion in the area of greatest injury, suggesting a paracrine effect.[16] One animal study failed to show neurobehavioral or biochemical benefit.[26] However, this study used 1/100th the cell dose used in the other 3 experiments. In subsequent work, this group demonstrated improved spatial memory recovery after using a dose 10 times higher than in their earlier experiments.[27] In recent work with a rat model of hypoxic-ischemic injury on postnatal day 7, intraperitoneal or intrathecal transplantation of human UCB cells 24 hours after injury accelerated regression of inflammatory microglial activation related events and narrowed the perilesional astrocytic wall associated with such injuries.[28] This group also demonstrated that intraperitoneal dosing of human UCB cells 24 hours after hypoxic-ischemic injury in rat pups resulted in reduced caspase-3 expression, as well as increased BDNF and vascular endothelial growth factor (VEGF), which could reduce inflammation and apoptosis, and aid angiogenesis post injury.[29]

Even though we are reporting initial results of autologous UCB cell infusions in the first postnatal days, other investigators have reported use of UCB cells in young children with cerebral palsy (CP). The Duke Blood and Marrow Transplant Program conducted a phase I safety and feasibility study of autologous UCB cell infusions in 184 children age 6 days to 9.5 years old with acquired neurological diseases. Seventy-six percent had CP. No neurodevelopmental results were formally evaluated, but the infusions were feasible and safe. Three patients had infusion reactions, all responsive to medical therapy and stopping the infusion.[30] An ongoing, randomized study of autologous cell infusions in children with spastic CP, which includes neurodevelopmental, neuromotor, neurophysiologic and imaging follow-up to access outcomes is recruiting at Duke (ClinicalTrials.gov: NCT01147653). Papadopoulos reported transfusing autologous UCB cells into two young children with spastic diplegia without apparent harm, along with granulocyte colony stimulating factor injections. They noted some improvement in function in the years following cell infusion.[31] Korean investigators have reported results of 96 subjects,10 months to 10 years old, with CP who received either allogeneic UCB cells matched for at least four of six human leukocyte antigen (HLA) types and erythropoietin, erythropoietin alone, or placebo. The UCB cell recipients received cyclosporine for 3 weeks following the study infusion. Although all groups showed improvements in functional measures over time, the cell recipients had significantly higher scores on developmental and functional tests 6 months after study interventions.[32] The Korean study study suggests a potential role for allogeneic transplant. According to the the Worldwide Network for Blood and Marrow Transplantation, a global association of donor registries and cord blood banks, over 2 million units of UCB nucleated cells have been collected in public and private banks. If UCB cells prove to have benefit in the treatment of acquired brain injuries in newborns and young children, development of an allogeneic ‘off-the-shelf’ product, with appropriate immune-suppression would make UCB cell-based intervention available to all infants and children in need of this therapy.

The doses used in our pilot study were based on the dose range presently used and shown to be efficacious in allogeneic transplant after myeloablative chemotherapy. The effective dose in the autologous setting is not known and could be higher or lower than that used in allogeneic transplantation. The variations noted in range of hematopoetic CFU's and CD34 cells collected may be a surrogate for the cells that could influence efficacy, but with the numerous types of progenitor cells included in cord blood, using one cell type to indicate potential efficacy for neurologic repair is not known or practicable at this time.[18] Donega demonstrated improved outcome with higher doses of mesenchymal cells in 9 day old mice with hypoxic ischemic injury.[33] Similar dose ranging studies are needed for UCB cells.

Although there may be some theoretical advantage to alternative sites of administration, to minimize risk at this point in our study, we chose to use the intravenous route. Intravenous administration may result in cells being trapped in other organs such as the lung, or in other organs with hypoxic-ischemic injury such as the liver, kidney or heart, although while there, like mesenchymal stem cells, they still may produce factors that facilitate optimal response after injury.[34] Donega used intranasal dosing.[33] Intrathecal administration of UCB derived cells and autologous mesenchymal cells from bone marrow in children with CP has been reported.[35, 36]

Timing of cell infusion is also a major question. We chose to target infusions in the first 72 postnatal hours because neonatal animal studies suggest treating proximal to the time of injury provides good results[14], but benefits have been noted even with combinations of infusions in the first hours to seven days post injury.[15, 16] In a rodent model of neonatal hypoxic-ischemic injury, a well-designed dose ranging and timing study found that intranasal dosing 3 and 10 days after injury improved outcomes, but dosing at 17 days post injury had minimal effect.[33] In our study, early infusion prevented exposure of newborn infants to DMSO, which is used in the process of cell cryopreservation. Given the latent phase of injury and repair, perhaps dosing after cooling using washed (to minimize exposure to DMSO at infusion), thawed cells may be helpful. This practice has been shown to be safe in the cerebral palsy study with infusion of autologous thawed and washed cells.[30]

The study of cord blood cells for neurologic injury must also address extensive false claims and dangerous outcomes of "cell therapies". The FDA began licensing public cord blood banks distributing unrelated, banked cord blood units for allogeneic transplantation in 2012. The use of autologous cord blood is currently not regulated in a similar fashion, but all non-homologous use of cells for indications other than those approved by the FDA should be studied under an investigator's IND, which we obtained for this study.

Although our study has given useful information to plan next-phase studies, we acknowledge that our comparison group for in-hospital and one-year outcome is not an ideal control group. The higher prevalence of birth at the tertiary center among cell recipients may be a proxy for more aggressive and immediate perinatal care and earlier initiation of hypothermia than outborns. Also, our follow-up rate among these concurrently cooled infants was low, which may be due in part to the fact that these infants were more likely to be born at outlying centers, which may have hindered their ability to return for follow-up assessments, and our developmental testing was at one year. That said, it is important to note

that in a meta-analysis of three of the pivotal trials of hypothermia, the rates of 2 year survival without cerebral palsy and with a mental developmental index score of more than 84, a psychomotor development index score of more than 84, and normal vision and hearing measured at two years ranged from 25 – 43% in the hypothermia groups, similar to that noted in our concurrent cooled cohort with available one-year follow-up.[35]

Collection, preparation and infusion of fresh autologous cord blood for use in infants with HIE is feasible, with extensive cooperation required among leadership and staff from the intensive care nursery, the cord blood bank, and the labor and delivery ward. Infusions did not lead to clinically significant problems attributable to cells. A randomized phase II study to provide further safety, feasibility and efficacy information among a wider number of sites is warranted.

Acknowledgments

Funding information is available at www.jpeds.com (Appendix).

Acronyms/Abbreviations

BDNF	brain derived neurotrophic factor
CCBB	Carolinas Cord Blood Bank
CFU	colony forming units
CMV	cytomegalovirus
CNS	central nervous system
DRH	Duke Regional Hospital
DUHS	Duke University Health System
HIE	hypoxic ischemic encephalopathy
ICN	intensive care nursery
IRB	institutional review board
MFM	Maternal fetal medicine
NGF	neuronal growth factor
NICHD	Eunice Kennedy Shriver National Institute of Child Health and Human Development
NRN	Neonatal Research Network
RBC	red blood cell
UCB	Umbilical cord blood
VEGF	vascular endothelial growth factor

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Appendix

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Table 1

Baseline Characteristics

	Cell recipients Mean (range) or N (%) N = 23	**Concurrent cooled Mean (range) or N (%) N = 82**	**P**
Gestational Age, wks	38 (34, 40)	39 (34, 41)	0.10
Birthweight, kg	3130 (2120, 4660)	3310 (1850, 4840)	0.67
Abruption	1 (5)	9 (12)	0.44
Cord prolapse	3 (14)	2 (3)	0.08
Non-reassuring fetal status	9 (41)	23 (32)	0.45
Cesarean delivery	16 (70)	48 (59)	0.47
Outborn	**6 (26)**	**73 (88)**	**< 0.001**
SGA[a]	1 (5)	7 (8)	0.45
LGA	2 (9)	6 (7)	0.99
Males	10 (44)	56 (68)	0.05
African American[b]	10 (50)	32(40)	0.52
5 minute Apgar ≤ 5	19 (83)	61 (75%)	0.58
10 minute Apgar ≤ 5	14 (61)	42 (57%)	0.81
Cord pH	6.99 (6.72, 7.26)	6.96 (6.48, 7.44)	0.82
Base Deficit	17 (8, 32)	18 (3, 36)	0.70
Cord pH < 7	13 (57)	47 (61)	0.44
Base deficit > 16	18 (78)	65 (78)	0.99
NICHD score[c]	23 (15, 39)	20 (8, 59)	0.64
NICHD score ≥ 30	5 (22)	23 (27)	0.79
Seizures	5 (22)	29 (35)	0.17

[a] Small and large for gestational age measurements were defined using growth charts from U.S. births.[24]

[b] 20 cell recipients and 80 concurrently cooled with race reported.

[c] NICHD score is an estimate of likelihood of positive outcome with cooling

Table 2

Characteristics of Cord Blood Collections

	Median (range) or N (%)
Volume Collected, mL	36 (3, 178)
# Cells Collected ($\times 10^8$)	4.8 (0.99, 48.3)
# Cells post processing ($\times 10^8$)	4.1 (0.97, 31.2)
Viability	99 (92, 100)
CFU ($\times 10^5$)	14.2 (0.8, 75.3)
CD34 ($\times 10^6$)	0.03 (0.004, 0.158)
Time to first infusion, hours	25 (4, 220)
≤ 6 hours	6 (26)
> 6 hours	17 (67)
Number of infusions	
4	11 (48)
3	3 (13)
2	6 (26)
1	3 (13)
Infusion volume, mL	4.3 (1.1, 10)

Table 3

Hospital Outcomes

	Cell recipients N = 23 (%)	**Concurrent cooled N = 83 (%)**	**P**
ECMO	3 (13)	5 (6)	0.37
Deaths	0 (0)	11 (13)	0.12
Seizure meds at discharge	4 (17)	21 (30)	0.19
100% Oral feeds at discharge	19 (83)	57 (79)	>0.99

Table 4

Baseline Characteristics of Cell Recipients and Concurrent Cooled Infants with Known Survival and One Year Bayley III Scores

Characteristics	Cell recipients Mean (range) or N (%) N = 19	Concurrent cooled Mean (range) or N (%) N = 46	P
Gestational Age, wks	38 (34, 40)	39 (34, 41)	0.08
Birthweight, kg	3230 (2360, 4660)	3270 (2100, 4840)	0.88
Abruption	1 (5.3)	5 (11)	0.66
Cord prolapse	2 (11)	2 (5)	0.58
Non-reassuring fetal heat rate	8 (42)	14 (33)	0.57
Cesarean delivery	13 (68)	26 (57)	0.42
Outborn	**6 (32)**	**43 (91)**	**< 0.001**
SGA	0 (0)	4 (9)	0.32
LGA	2 (11)	4 (9)	>0.99
Males	10 (52)	33 (70)	0.25
African American*	9 (50)	11 (25)	0.12
5 minute Apgar ≤ 5	15 (79)	33 (73)	0.76
10 minute Apgar ≤ 5	12 (63)	28 (68)	0.77
Cord pH (range)	6.97 (6.65, 7.26)	6.94 (6.48, 7.44)	0.92
Base Deficit	18 (10, 32)	18.5 (3, 31)	0.96
Cord pH < 7	13 (68)	28 (65)	>0.99
Base deficit > 16	15 (79)	36 (77)	1.0
NICHD score	23 (16, 39)	23 (9, 49)	0.90
NICHD score ≥ 30	3 (16)	14 (30)	0.35
Seizures	4 (21)	18 (38)	0.25

[a] Small and large for gestational age measurements were defined using growth charts from U.S. births.[24]

[b] 20 cell recipients and 80 concurrently cooled with race reported.

[c] NICHD score is an estimate of likelihood of positive outcome with cooling

Table 5

Survival with Bayley III Scores ≥ 85 in 3 domains

	Cells N = 18	**Cooled only N = 46**	**p**
*Survived to 15 months	16 (89)	35 (76)	0.25
Survival with all 3 Bayley domain scores ≥ 85	13 (72)	19 (41)	0.05