A Phase I/II Study of Allogeneic Umbilical Cord Blood and Umbilical Cord Tissue-Derived Mesenchymal Stromal Cell Infusions in Children with Cerebral Palsy (IND 17921)

Principal Investigators:
Jessica Sun, MD
Robertson Clinical & Translational Cell Therapy Program
DTRI/Duke University
[redacted]
Joanne Kurtzberg, MD
Robertson Clinical & Translational Cell Therapy Program
DTRI/Duke University
[redacted]

Additional Investigators:

Laura Case, DPT, MS, PCS, C/NDT

[redacted]

Kathryn Gustafson, PhD

[redacted]

Joan Jasien, MD

[redacted]

Colleen McLaughlin, DNP, CPNP

[redacted]

Mohamad Mikati, MD

[redacted]

Gordon Worley, MD

[redacted]

Statistician:

Jesse Troy, PhD

[redacted]

STCL Lab Supervisor:

Barbara Waters-Pick, BS, MT (ASCP)

[redacted]

GMP Cell Processing Lab Supervisor:

Norin Meadows

[redacted]

TABLE OF CONTENTS

INVESTIGATOR SIGNATURE PAGE

A Phase I/II Study of Allogeneic Umbilical Cord Blood and Umbilical Cord Tissue-Derived Mesenchymal Stromal Cell Infusions in Children with Cerebral Palsy

I agree to conduct and supervise this clinical study in accordance with the design and specific provisions of this protocol; modifications to the study or protocol are acceptable only with a mutually agreed upon protocol amendment except when necessary to protect the safety of subjects. I agree to await IRB approval for the protocol and informed consent before initiating the study, to obtain informed consent from subjects prior to their enrollment in the study. I agree to report to responsible regulatory agencies and the IRB (when necessary) adverse events that occur in the course of this investigation. I agree to maintain accurate and adequate records in the case report forms as required by this protocol and maintain those records for the period of time required. I will make the study documentation available for safety oversight committee review and/or for other inspections as required. I agree to maintain study documentation for the period of time required. I agree to comply with all other requirements regarding the obligations of clinical investigators according to FDA regulations and guidance. I agree to ensure that all people assisting in the conduct of this study are informed in meeting the above commitments.

_____

(Investigator's printed name)

_____

(Investigator's signed name)

PROTOCOL SUMMARY

Protocol Number:	
Title:	A Phase I/II Study of Allogeneic Umbilical Cord Blood and Umbilical Cord Tissue-Derived Mesenchymal Stromal Cell Infusions in Children with Cerebral Palsy
Study Phase:	I/II
Study Site:	Single site; Duke University, Durham NC
Study Therapy, Dosage, and Route of Administration:	(1) Single intravenous infusion of a maximum of $10x10^7$/kg allogeneic umbilical cord blood (CB) cells (2) Three intravenous infusions of $2x10^6$/kg human umbilical cord tissue cells (hCT-MSC), manufactured from allogeneic umbilical cord donors
Objectives:	Primary Objective(s): 1. To determine the efficacy of repeated intravenous doses of hCT-MSC in children with cerebral palsy 2. To determine the effect size of change in GMFM-66 scores in subjects treated with allogeneic CB or hCT-MSC Secondary Objective(s): 1. To determine changes in brain connectivity via MRI imaging in these children and whether they correlate with clinical response
Research Participant Population:	90 evaluable children ages 2-5 years with hypertonic cerebral palsy due to stroke, periventricular leukomalacia or hypoxic ischemic encephalopathy
Study Design:	Randomized, open label
Safety Assessments/Endpoints:	Incidence of infusion reactions, bloodstream infections, graft versus host disease, and alloimmunization

ABBREVIATIONS

AE	Adverse Event
AlloCB	Allogeneic Umbilical Cord Blood
ASD	Autism Spectrum Disorder
CB	Umbilical Cord Blood
CBC	Complete Blood Count
CBU	Umbilical Cord Blood Unit
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CMV	Cytomegalovirus
CRF	Case Report Form
DMSO	Dimethyl Sulfoxide
DTI	Diffusion Tensor Imaging
EMG	Electromyography
FDA	Federal Drug Administration
fMRI	Functional Magnetic Resonance Imaging
GMP	Good Manufacturing Practice
GMFCS	Gross Motor Function Classification System
GMFM-66	Gross Motor Function Measure - 66
GvHD	Graft versus Host Disease
hCT-MSC	Human Cord Tissue-Derived Mesenchymal Stromal Cells
HIE	Hypoxic Ischemic Encephalopathy
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HTLV	Human T-Cell Lymphotropic Virus
ICH	International Conference on Harmonisation
IRB	Internal Review Board
IV	Intravenous
IVH	Intraventricular Hemorrhage
MCA	Middle Cerebral Artery
MRI	Magnetic Resonance Imaging
MSCs	Mesenchymal Stromal Cells
PVL	Periventricular Leukomalacia
SAE	Serious Adverse Event
STCL	Stem Cell Laboratory
SOP	Standard Operating Procedure
TNCC	Total Nucleated Cell Count
WBC	White Blood Cell

1.0 PURPOSE

The main purpose of this study is to estimate change in motor function 12 months after treatment with a single dose of allogeneic umbilical cord blood (AlloCB) or repeated doses of umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC) in children with cerebral palsy. In addition, this study will contribute much needed data to the clinical trials community on the natural history of the motor function in CP over short-term (less than 1 year) time periods relevant to the conduct of clinical trials and assess the safety of AlloCB and hCT-MSC infusion in children with cerebral palsy.

2.0 BACKGROUND AND HYPOTHESIS

2.1 Overview

Children with cerebral palsy face a lifetime of disability, resulting in enormous physical, emotional, and financial burdens to affected patients, their parents, and society at large. Typically caused by an in utero or perinatal injury to the developing brain, cerebral palsy is the most common – and most costly – chronic motor disorder of childhood. The cornerstone of cerebral palsy treatment relies on countless hours of physical and occupational therapies that are entirely supportive. There is no treatment available to repair the brain damage that caused the disabilities. Thus, a novel therapy that could promote repair of damaged brain tissue has potential to reduce societal burden and to greatly improve survival, function, and quality of life for patients with cerebral palsy.

Umbilical cord blood (CB) and mesenchymal stromal cells (MSCs) lessen the clinical and radiographic impact of hypoxic brain injury and stroke in animal models. In patients with leukodystrophies undergoing unrelated donor CB transplantation, donor cells have been observed engrafted in the brain post-transplant. In addition, we have observed ongoing myelination in the brains of these patients after initial demyelination caused by the underlying disease. The central hypothesis of our work is that CB cells or human cord tissue-derived MSCs (hCT-MSC), acting primarily through paracrine mechanisms, could serve as vehicles for emerging cellular therapies in patients with brain injuries. We conducted safety studies and recently completed phase II, randomized, double blind, placebo-controlled trial of autologous CB in children with cerebral palsy. In that study, children who were infused with $\geq 2x10^7$ cells/kg exhibited a greater degree of motor improvement than children who received lower doses or placebo. That study was limited by small sample size since many children with cerebral palsy do not have a banked autologous cord blood unit and by the inclusion of children 1-2 years of age for whom analysis of the predicted motor change score was not possible. We also conducted a phase I safety study of sibling CB infusion in 15 patients with cerebral palsy, indicating that allogeneic partially HLA-matched CB infusion is safe in this patient population.

Therefore, this study is a phase I/II trial of higher doses of ***allogeneic*** CB or hCT-MSC to confirm our previous findings and eliminate the restriction of having an autologous CB unit. The hypothesis of this trial is that adequately dosed allogeneic CB cells and hCT-MSC administered intravenously will, via cellular or trophic effects, induce repair of brain injury in children with cerebral palsy, thereby improving their functional abilities. In addition, data from the prior study indicates that brain connectivity as measured by MRI with diffusion tensor imaging (DTI) correlates with functional status and motor improvement in children with cerebral palsy, and therefore may be useful as a biomarker and predictor of response. This relationship will be further explored in this clinical trial.

2.2 Cerebral Palsy

Cerebral palsy is a form of acquired brain injury defined as a group of disorders of posture and movement attributed to non-progressive disturbances in the fetal or neonatal brain.[1] It is the most prevalent motor disorder of childhood, affecting 2 to 3 of every 1,000 live births.[2] In the United States, approximately 10,000 babies and infants are diagnosed with cerebral palsy each year. According to the United Cerebral Palsy Research and Education Foundation, approximately 764,000 children and adults in the United States have cerebral palsy (http://ucp.org/wp-content/uploads/2013/02/cp-fact-sheet.pdf). While some infants at risk can be identified at birth, many cases are silent in the newborn and only detected when the child exhibits paresis, spasticity and developmental delay in the first few years of life. Affected children have varying degrees of functional impairments ranging from mild limitations in advanced gross motor skills to severely limited self-mobility despite the use of assistive technology. Though cerebral palsy is a motor disorder, it can be associated with cognitive and sensory deficits and can have a detrimental impact on global childhood development by affecting the ability to explore, communicate, learn, and achieve independence.[3]

Cerebral palsy is one of the most disabling and costly chronic conditions of childhood, with medical costs for children with cerebral palsy estimated at 10 times higher than children without cerebral palsy[4] and an estimated annual cost of care of $8.2 billion for affected children in 2002.[5] Unfortunately, no curative therapy exists. Current treatment modalities focus on maximizing functional abilities and quality of life through several approaches. These include non-pharmacologic measures such as physical, occupational and speech therapies, orthotics, optimal nutrition, constraint therapy and use of adaptive devices; pharmacologic interventions such as oral pharmacological agents, botulinum toxin injections, and oral or intrathecal baclofen; and surgical options including dorsal rhizotomy and various orthopedic procedures. Most children with cerebral palsy require lifelong supportive care, adaptive therapy and equipment, and special education, utilizing greater shares of medical and societal resources as compared to typically developing children.

Both full-term and premature infants can develop cerebral palsy as a result of brain injury sustained during the prenatal, perinatal, or postnatal periods. Most affected full-term infants have sustained a prenatal neurologic insult, such as an *in-utero* stroke, or a hypoxic-ischemic injury at the time of delivery. The incidence of cerebral palsy is much higher in premature babies, affecting more than ten percent of very low birth weight infants (<1500g).[6] In this population, periventricular leukomalacia, which can be associated with ischemic injury or vasculitis is a common cause. Still, some children have no identifiable risk factors or etiology.

2.3 Rationale for Cellular Therapy

Human brain development is a complex and long process, beginning in the third week of gestation and extending through at least adolescence, probably into adulthood. While the majority of neurogenesis occurs in utero, synaptogenesis and myelination continue postnatally, with substantial growth and development during the first six years of life. Injury to the developing brain not only results in loss of normal brain cells and tissue, but also alters the neuro-environment, disrupting the intricate cellular and environmental interactions essential for healthy brain development. There is growing evidence that cell therapies have the ability to influence tissue damage and repair by signaling and activation of host cells via trophic and/or paracrine effects. While the exact mechanisms of neural sparing and/or recovery remain the subject of preclinical investigations, several

mechanisms have been hypothesized.[7,8] The survival potential of host neural cells may be enhanced by the delivery of trophic factors from infused and/or transplanted cells that provide anti-inflammatory and neuroprotective effects.[9-12] Brain plasticity may be increased by enhancing synaptogenesis, instigating endogenous repair mechanisms, stimulating angiogenesis resulting in neovascularization, and inducing migration and proliferation of endogenous neural stem cells.[13-15] Additionally, many neurologic conditions involve activation of proapoptotic signal transduction, which could be harnessed to attract cells to brain lesions in those diseases. Thus, CB/cord tissue-derived cells could also potentially act as a vehicle to deliver neuroprotective and restorative factors or signal endogenous cells to act in a targeted way toward damaged brain tissue.

In children with cerebral palsy, reorganization of central motor pathways has been demonstrated using transcranial magnetic stimulation (TMS), electromyography (EMG), and functional MRI (fMRI). In patients with a unilateral lesion involving the motor tracks, motor control of the affected limbs is primarily provided by abnormal corticospinal projections from either adjacent areas in the affected hemisphere or distant regions in the ipsilateral, healthy hemisphere,[16-18] demonstrating that an unaffected part of the brain is compensating for the injured portion. Changes in the primary source of cortical activation on fMRI have also been described in individual patients after virtual reality therapy [19] and constraint-induced movement therapy, in which the unaffected limb is restrained while the affected limb undergoes intensive motor training.[20] These observations indicate that neural plasticity plays an important role in the ability of children with cerebral palsy to recover from early brain injury. ***Modulating that plasticity via cellular therapy may enhance such recovery.***

2.4 Cell Types Utilized in this Trial

2.4.1 Allogeneic Umbilical Cord Blood (CB)

Many children who might benefit from CB therapies will not have their autologous CB banked. In order to extend these therapies to all patients, use of an allogeneic product will be necessary. Allogeneic CB has been used extensively in the field of hematopoietic transplantation. More recently, based on substantial preclinical data, CB has been investigated as a potential therapy for patients with brain injuries and neurological conditions. As most children and adults do not have access to autologous CB, allogeneic cells are frequently utilized in clinical trials of cellular therapies for neurologic conditions.

Allogeneic human CB as a source of cells for **hematopoietic reconstitution after myeloablative therapy** has a proven track record of over 30 years of use in the clinic, with over 35,000 transplants performed.[21] Allogeneic CB transplantation has been shown to be safe and has not been shown to cause tumors or cellular dysregulation. Compared to other cell sources, CB has the following advantages:

1. CB is an abundantly available source of stem cells that can be harvested at no risk to the mother or infant. It is routinely collected, cryopreserved, and banked.
2. Public CB donors are screened for risks of transmitting infectious agents through blood per CFR1271, subpart C donor screening regulations. Important infectious agents, particularly cytomegalovirus (CMV), are much less common in the newborn than adults, and are less likely to contaminate CB units.
3. CB units, cryopreserved and banked, are available on demand, and can be easily shipped and thawed for use when needed, eliminating delays and uncertainties that complicate bone marrow collection from unrelated donors.

4. CB lymphocytes are more immunotolerant of a new host. Thus, the intensity of graft-versus-host reactivity of fetal lymphocytes is less than that of adult cells, so transplantation of CB after myeloablation and immunosuppression results in less graft versus host disease (GvHD) than transplantation of bone marrow or other adult hematopoietic stem cell sources.
5. CB contains pluripotent stem cells that have demonstrated the ability to differentiate into numerous types of cells throughout the body, including in the brain. Thus CB may provide a source of cells for non-hematopoietic tissue repair or regeneration.

In this study, we propose to use allogeneic CB donor without the use of chemotherapy or other immunosuppressive therapies.

2.4.2 Mesenchymal Stromal Cells (MSCs)

MSCs are a heterogeneous group of undifferentiated, pluripotent cells that can be isolated from several different tissues including bone marrow, adipose tissue, and birth tissues (umbilical cord tissue, placenta). While MSCs can give rise to mesodermal tissue types including bone, cartilage, and fat, their primary mechanism of action is thought to result from immunomodulatory and other paracrine effects. MSCs have demonstrated a multitude of immunomodulatory effects on both humoral and cell-mediated immune responses. These include, but are not limited to, inhibiting B-, T-, NK, dendritic-cell, and microglial proliferation, decreasing pro-inflammatory cytokine production, and blocking neutrophil recruitment. In addition, numerous preclinical studies using MSCs transplantation for diseases of the central nervous system suggest that MSCs can act through release of different neurotrophic, anti-inflammatory, and anti-apoptotic factors to promote recovery the injured area and prevent further damage.[22-26]

Despite their ability to modulate the immune response, MSCs themselves have low immunogenicity. MSCs express low levels of MHC class I molecules on their surface and lack the expression of MHC class II and several costimulatory molecules. This allows MSCs to be used in the allogeneic setting across HLA barriers, without the need for donor-recipient HLA matching. In fact, in a review of 13 human studies of intravenous allogeneic MSC administration, including 1,012 mostly adult patients, there were no reports of infusional toxicity,[27] supporting the notion that MSCs are “immune-privileged” and can avoid immunological allorecognition. When utilized as a therapeutic cell, MSCs exert effects via trophic signaling. It is estimated that after infusion, MSCs survive in the recipient for up to 4 months. MSCs do not engraft in the recipient.

Safety of MSCs:

MSCs manufactured from bone marrow and adipose tissue have been studied in hundreds of clinical trials worldwide involving thousands of individuals and a wide variety of human conditions. Their safety has been demonstrated repeatedly, and is summarized in a 2012 systematic review and meta-analysis[27] of 36 clinical trials in 14 countries using MSCs in over 1,000 recipients with cardiovascular, neurological, oncologic, metabolic, gastrointestinal, and post-transplant conditions. Based on a combination of randomized controlled trials and uncontrolled studies with follow-up periods of weeks to up to 5 years, there was no association between MSC treatment and acute infusional toxicity, organ system complications, infection, death, or malignancy. The only side effect associated with MSC treatment was transient fever, which did not cause any long-term sequelae. Importantly, no malignancies were reported in patients without a prior history of cancer.

After intravenous administration, MSCs initially distribute to the lungs. While there is a theoretical risk of respiratory complications, the only randomized trial in which acute pulmonary reactions have been observed was conducted in patients with chronic ischemic heart failure who received intracoronary MSCs.[28] Thus, this risk seems to be limited to patients whose underlying condition makes them susceptible to developing pulmonary edema. Although a few cardiovascular studies delivering MSC via intramuscular injection have reported arrhythmias, the incidence was not statistically different from control groups in meta-analysis.[27]

Experience with MSCs in Children:
The most well-studied MSC product given to children to date is a bone marrow-derived product originally manufactured by Osiris as Prochymal® and subsequently acquired by Mesoblast as Remestemcel-L used to treat patients with GvHD after a hematopoietic stem cell transplant. Prochymal® was approved in Canada in 2012 for use in children with acute GvHD who have failed to respond to steroid treatment. A recent study of the product enrolled 241 children (median age 9.6 years) with refractory GvHD from 2007-2014.[29] Eight MSC doses ($2x10^6$ cells/kg/dose) were given intravenously over a four week time period, with four additional weekly infusions in patients who demonstrated a partial or mixed response. There were no incidences of ectopic tissue formation in 2434 total doses, with follow-up of 2-9 years. The most frequent severe adverse events were infections (24%) and respiratory disorders (16%), common issues in post-transplant patients. There were only 11 severe adverse events and one infusion reaction related to MSC treatment. Patients with primary steroid-refractory GvHD who had only been treated with steroids demonstrated an 81% response rate to MSCs. Overall, 65% of patients demonstrated an improvement in their GvHD, with higher response rates in patients with less severe disease. This demonstrates that repeated doses of MSCs are safe and well-tolerated in children, and suggests that they provide anti-inflammatory and immunomodulatory effects in the setting of GvHD.

In a recent study, a German group pooled bone marrow cells from eight healthy donors to create an MSC cell bank.[30] They treated 26 children, ages 1-19 years old with steroid-refractory GvHD, with a total of 81 doses of MSCs from this cell bank (median 3 doses per patient, median $2.2x10^6$/kg MSCs IV per dose). Treatment was well-tolerated, with only one reported headache and one episode of nausea across all doses. Six patients died from causes unrelated to MSC treatment (disease progression, GvHD, sepsis, thromboembolism). Seventy-seven percent of patients responded to therapy, defined as a complete or partial remission of their GvHD.

Others are investigating the use of MSCs in patients with other conditions. There have been case reports of intracoronary administration of autologous bone marrow-derived MSCs in children, ages 4 months to 14 years, with severe dilated cardiomyopathy and/or heart failure with subsequent short- and intermediate-term improvement in clinical condition and B-type natriuretic peptide values.[31-33] Studies have also been conducted in patients with type 1 diabetes.[34] One such study administered umbilical cord-derived MSCs intravenously in 29 patients, including several children, with newly diagnosed type 1 diabetes.[35] Treatments were well-tolerated, and moderate improvements in some metabolic measures were observed. Early phase studies of MSCs are also underway in preterm infants with bronchopulmonary dysplasia. In the only published study, cells were delivered intratracheally and no significant side effects were reported in these young babies.[36] None of these early phase studies have reported toxicity related to the MSC therapy.

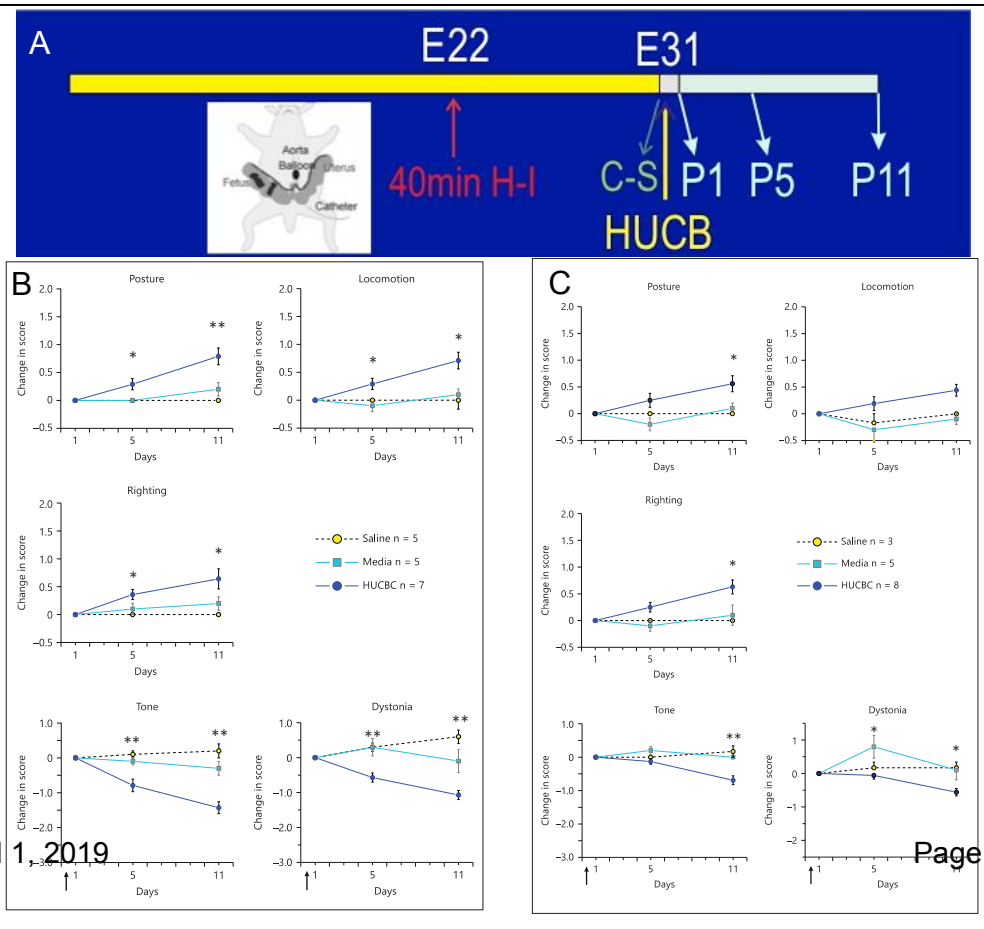
2.5 Animal Studies of Cell Therapy in Brain Injuries

Numerous animal models have demonstrated both neurological and survival benefits of cell therapies in the setting of stroke, ischemia, and intracranial hemorrhage.[37-40] These injuries differ in that some are focal (i.e. stroke) and others are global (i.e. hypoxia), but all are typically characterized by immediate damage to all neural cell types within the affected region accelerating a cascade of events that lead to demyelination and necrosis of brain tissue. Inflammation, apoptosis, neuronal and oligodendrocyte death, and astrocytosis are all operative in mediating damage resulting from these insults. Neuroprotection, neovascularization, and neuronal regeneration have all been demonstrated after cell administration in various models.[15,37,41]

2.5.1 Animal Studies in Hypoxic/Ischemic Brain Injury

Cellular therapy has been studied in models of hypoxic ischemic encephalopathy (HIE). In a neonatal rat model that results in severe cerebral damage and contralateral spastic paresis after unilateral carotid artery ligation on day seven of life, intraperitoneal CB mononuclear cells administered one day after the hypoxic event migrate to the area of brain damage and persist for at least two weeks. Although the extent of morphologic injury on gross pathology was not altered, animals who received CB mononuclear cells did not develop spastic paresis, indicating functional recovery.[39] In a baby rabbit model of HIE via uterine artery occluion,[42] Tan demonstrated that human CB administered nine days after the injury improved gross motor function in functional assays (figure 1).[43] A possible dose effect was also observed, with less improvement detected at lower doses.

Figure 1: Rabbit model of cerebral palsy and intravenous CB administration.
Panel A: Experimental model. The uterine artery is occluded 9 days prior to delivery, resulting in a cerebral palsy phenotype. Kits are delivered just prior to term and receive IV CB cells on the day of birth. Motor assessments are performed on days 1, 5, and 11. Panel B: CB administration improved neurobehavioral scores at days 5 and 11 in the severe group compared to saline and media. Panel C: CB administration improved neurobehavioral scores at days 5 and 11 in the mild group. *p<0.05, **p<0.01. (Drobyshevsky et al, Dev Neurosci, 2015)



In a murine model of HIE, bone marrow-derived MSCs delivered into the brain parenchyma resulted in reduced lesion size and improved behavioral outcomes even when treatment was started 10 days after the insult. Evidence of increased endogenous cell proliferation and decreased microglial proliferation was observed.[44]

2.5.2 Animal Studies in Stroke

The most extensively studied models involve brain damage resulting from permanent middle cerebral artery occlusion (MCAO) in adult rats or transient occlusion accompanied with hypoxia in neonatal rats or mice. Intravenous injection of CB can greatly mitigate the damage caused by such acute hypoxic/ischemic brain injury.[45]

In addition to evaluating treatment response and further elucidating mechanism of action, many preclinical studies have attempted to address other critical aspects of cell administration, including dose, timing, and route of delivery. In addition to functional outcomes, Vendrame examined anatomic measures of infarct volume after MCAO in the presence of increasing doses of CB cells.[37] At 4 weeks after infusion, infarct volume measurements revealed an inverse relationship between CB cell dose and damage volume, reaching significance at a dose of 10^7 cells, thereby demonstrating a dose-dependent relationship between CB cell dose, behavioral improvement, and neuronal sparing. Also in an MCAO model, Chen showed a greater improvement in somatosensory behavior and neurologic dysfunction when CB or bone marrow stromal cells were administered at one day versus seven days after the injury, suggesting that earlier may be better.[38] In fact, the majority of stroke models have evaluated stem cell therapy in the acute or subacute setting, immediately to several days after the insult. However, Shen demonstrated improvement in functional outcomes in rats treated intravenously with bone marrow MSCs one month after MCAO stroke. In this model, scar tissue was reduced and the number of proliferating cells and oligodendrocyte precursors in the area of injury were increased, possibly indicating neurogenesis and myelination.[46] This suggests that although the ideal timing of cellular therapy for stroke or other brain injury is still unknown, benefits may be attainable long after the injury is sustained. Models delivering cells via the intravenous, intra-arterial, intracerebral, and intranasal routes have not demonstrated that any one mode of delivery is significantly superior in terms of functional outcomes.[47-49]

2.5.3 Animal Studies in Intraventricular Hemorrhage (IVH)

Intraventricular hemorrhage (IVH) is common in preterm babies and often results in periventricular leukomalacia and subsequent development of cerebral palsy. Ballabh and colleagues developed a rabbit model of IVH by administering glycerol intraperitoneally to premature rabbit pups.[50] In this model, IVH is followed by the development of hydrocephalus and subsequent white matter demyelination. Intraventricular administration of human CB cells 24 and 72 hours after glycerol failed to prevent the hydrocephalus, but did reduce subsequent demyelination (Ballabh, personal communication, 2014). In a neonatal rat model of IVH induced by intraventricular injection of autologous blood, mice given umbilical cord tissue-derived MSCs intravenously two days after IVH exhibited less reactive gliosis and hypomyelination and significant behavioral improvement compared to control animals.[51]

2.5.4 Summary

In summary, xenogeneic infusion of human CB cells and cord tissue-derived cells in small animals following brain injury results in improved survival and functional outcomes.

Optimal dose, timing, and route of administration as well as specifics regarding mechanism of action are still the subject of preclinical investigations, and may vary based on the type, degree, and time interval since the insult. Nonetheless, these studies suggest that dose may play an important role, that intervention in the chronic phase of injury can still be beneficial, and that intravenous administration may be adequate.

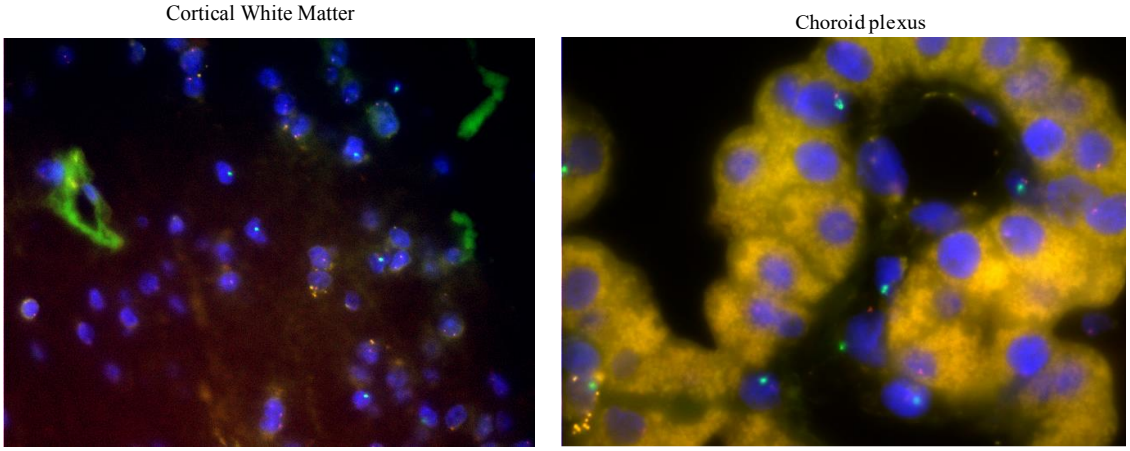
2.6 Human Studies of Cell Therapy in Neurologic Conditions

2.6.1 Umbilical Cord Blood in the Treatment of Inherited Metabolic Diseases

Allogeneic transplantation of human CB in patients with genetic lysosomal and peroxisomal storage diseases is effective in preventing or ameliorating the associated neurological damage.[52-55] The engraftment of donor cells into a patient with an inherited metabolic disease provides a constant source of enzyme replacement, thereby slowing or halting the progression of disease. Patients with these diseases, ranging in age from newborns to young adults, transplanted early in the course of their disease derive extensive benefits from the transplant procedure, which both extends life (for decades) and greatly improves neurologic functioning.[56-58] Infusion of CB cells in these cases are proceeded by immunosuppression to support the goal of durable engraftment.

In collaboration with Dr. Evan Snyder and with informed consent of her parents, we examined the brain of a 20-month-old female baby who died after sex-mismatched CB transplant for symptomatic Krabbe disease. Immunocytochemical staining for cell type-specific markers were combined with FISH using probes for X and Y chromosomes and DAPI staining to affirm host versus donor cells within the brain. Numerous male cells were found distributed throughout the forebrain, brainstem, cerebellum and cortex. Donor cells were also seen within blood vessels and transversing through and incorporated into the choroids plexus. Many stained with macrophage markers and a few donor-derived oligodendrocytes were seen. Globoid bodies, the pathological perivascular signature of Krabbe disease, were eliminated.[59] This observation, as well as animal studies that demonstrate donor-derived cells in the brain and multiple other organs after hematopoietic stem cell transplant, indicates that the transplanted stem cells are capable of repopulating more than just the hematopoietic system and are not limited by the blood-brain barrier.[60]

Figure 2: Brain sections of a female Krabbe patient who died 1o months post UCBT. XY Fish demonstrates male (donor, blue dots) cells engrafting in and differentiating in brain.



2.6.2 Safety of Autologous Cord Blood in Children with Brain Injuries

At our institution, we have already treated several hundred children ages one day to nine years with or at risk for cerebral palsy with *autologous* CB. The first 184 patients were described in 2010 as a Phase I study.[61] Between 3/2004 and 12/2009, 184 infants and children with HIE, cerebral palsy, congenital hydrocephalus and other brain injuries received 198 autologous cord blood infusions at Duke (14 patients received two infusions based on availability of larger cell doses). Autologous CBUs were obtained from 24 different cord blood banks: 149 (81%) CBUs came from 11 private U.S. banks (113 from two of the larger private U.S. banks), 13 (7%) CBUs from 11 international banks, and 22 (12%) CBUs from 2 public banks. The majority of parents had elected to store their child's cord blood privately when they were born. The median volume of cord blood collected was 60 mL (range 5-180 mL) and median TNC contained in the cord blood, as reported by the cord blood bank at the time of cryopreservation, was $4.7x10^8$ (range 0.3-33.8x10^8) total nucleated cells and 1.8 x10^6 (range 0-19.1 x10^6) CD34 cells. All infusions were administered through a peripheral IV after premedication with oral Tylenol 15mg/kg, IV Benadryl 0.5mg/kg and IV Solumedrol 0.5mg/kg. Median post thaw recovery of TNC was 82% (range 13-200%), and patients were dosed with a median of $2.0x10^7$ TNC/kg (range 0.1-13.3x10^7), $0.7x10^5$ CD34+ cells/kg (range 0.04-6.4x10^5), and $6.5x10^4$ CFU/kg (range 0-315x10^4).

Three patients (1.5%) experienced hypersensitivity reactions during their CB infusion characterized by wheezing with or without urticaria two to ten minutes after the IV infusion was initiated. The reactions resolved after discontinuation of the infusion and treatment with additional IV Benadryl and bronchodilators. The remainder of the CB cells were discarded for two of the infusions stopped prior to completion; one patient was able to restart and complete the CB infusion. One patient's mother experienced an allergic reaction consisting of urticaria, presumably due to contact with DMSO exhaled onto her face and neck by her child receiving a CB infusion. The reaction resolved with oral Benadryl. No adverse events have been reported. Specifically, no infections, autoimmune diseases, tumors, or other adverse events have been observed.

This series demonstrated safety and feasibility of autologous CB infusion, and anecdotal reports of improved function were common. However, there was potential for a considerable placebo effect as parents often endorsed dramatic benefit even shortly after infusion. Therefore, a randomized, double blind, placebo-controlled trial was performed to evaluate objective functional outcomes. This study is described in detail below.

2.6.3 CB Infusion in Babies with Hypoxic Ischemic Encephalopathy (HIE)

In phase I trial of newborns with HIE at birth conducted at Duke, fresh, non-cryopreserved autologous CB processed on Sepax 1 (Biosafe, Geneva) was infused in 1, 2, or 4 doses within the first 72 hours of life in babies with moderate-to-severe encephalopathy qualifying for systemic hypothermia.[62] These babies (n=39) were compared to a concomitant group of babies who were also cooled at Duke but did not receive CB cells (n=146). Infusions were found to be safe in these critically ill babies, and babies receiving cells had increased survival rates to discharge (100% vs. 89%, p=0.03). Of the 25 cell recipients with known one-year outcomes, 16 (64%) survived with Bayley III scores ≥ 85 in all three domains, and of the 63 cooled-only infants with known one year outcomes, 25 (40%) survived with Bayley III scores ≥ 85 in all three

domains (p = 0.04). A phase II randomized, placebo controlled, multicenter trial is currently underway.

2.6.4 CP-AC Study: A Phase II Randomized, Placebo-Controlled, Crossover Study of Autologous CB Infusion in Children with Cerebral Palsy

Table 1: Characteristics of Patients and Autologous CB Units	**Autologous CB Group (N=32)**	**Placebo Group (N=31)**
Patient Characteristics		
Age, years – median (range)	2.1 (1.1-6.2)	2.3 (1.1-7.0)
Sex – no. (%)		
Male	20 (62.5)	22 (71)
Race – no. (%)		
Caucasian	27 (84.4)	28 (90.3)
Type of Cerebral Palsy – no. (%)		
Hypotonic Quadraplegia	1 (3.1)	3 (9.7)
Spastic Diplegia	6 (18.8)	6 (19.4)
Spastic Hemiplegia	15 (46.9)	15 (48.4)
Spastic Quadraplegia	10 (31.3)	7 (22.6)
GMFCS Level* – no. (%)		
I/II	21 (65.6)	21 (67.7)
III/IV	11 (34.4)	10 (32.3)
Bayley Cognitive Score (n=43) [T] **– median (range)**	85 (55-110)	90 (55-110)
Cord Blood Characteristics – median (range)		
Collection Volume, mL	66 (4.5-146)	
Pre-Cryo TNCC, x10^8	4.4 (1.1-15.5)	
Pre-Cryo Cell Dose, x10^7/kg	3.00 (1.11-8.68)	
Cell Dose Infused, x10^7/kg	1.98 (0.75-4.83)	

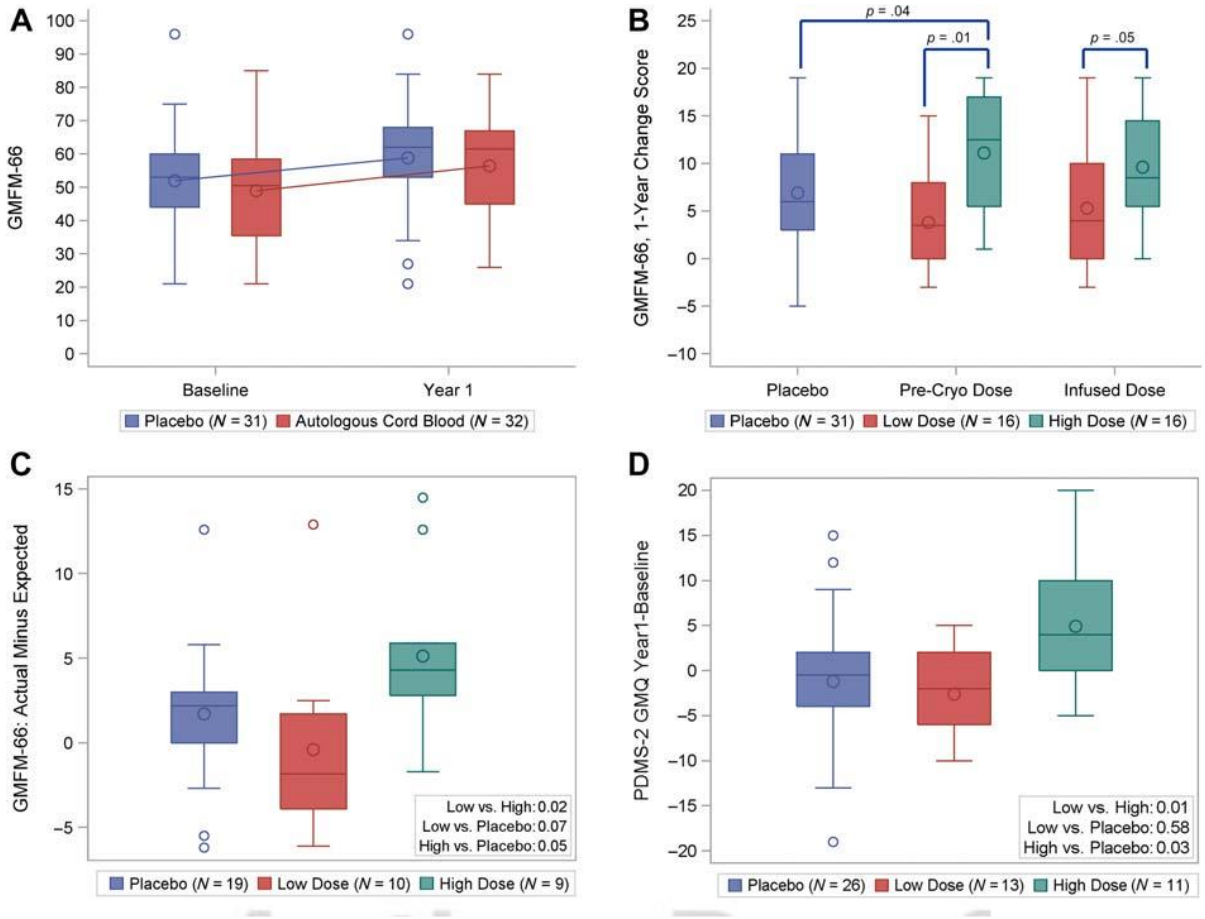
CD34+ Dose Infused, x10^5/kg	0.60 (0.11-3.90)	
CFU Dose Infused, x10^5/kg	3.91 (0.04-36.21)	

The CP-AC study was a prospective, randomized, double blind, placebo controlled crossover study of a single intravenous infusion of autologous CB in children ages 1-6 years with cerebral palsy.[63] The Gross Motor Classification System (GMFCS) was utilized to classify the level of motor function at study entry and follow-up. Children were eligible if they were (1) GMFCS level 2-4 or (2) GMFCS level 1 with hemiplegia if they used their affected hand as an assist only. Children with known genetic conditions, intractable seizures, or severe microcephaly were ineligible. Autologous CB units had to have a documented precryopreservation total cell dose of 1-5x10^7/kg, negative sterility culture, and negative maternal infectious disease screening. Subjects were evaluated at baseline, one-, and two-years with motor evaluations and brain MRI. They were randomized to the order in which they received CB and placebo infusions (given one year apart). The primary endpoint was change in Gross Motor Function Measure-66 (GMFM-66) score one year after the initial infusion (CB or placebo). Infusions, dosed at 1-5x10^7/kg based on the precryopreservation total nucleated cell count (TNCC), were administered intravenously over 5-10 minutes in the outpatient setting after premedication with Tylenol, Benadryl, and Solumedrol. Subjects received IV fluids and were monitored for 2-4 hours post-infusion.

Results: Sixty-three patients were enrolled and randomized to an initial infusion of autologous CB (n=32) or placebo (n=31). Median age at initial infusion was 2.1 years. Etiology of CP was classified into the following categories: periventricular leukomalacia (n=17), *in utero* stroke or bleed (n=27), ischemic injury (n=7), or other (n=12). One-third of patients had moderately severe GMFCS levels (III-IV) at study entry. The two treatment groups were balanced with respect to age, gender, race, type and severity of cerebral palsy. Patient and CB characteristics are shown in Table 1. Despite negative pre-cryopreservation cultures, one CB unit grew β-hemolytic strep at the time of thaw. There were no clinical infections. One subject had transient infusion reactions consisting of hives +/- low-grade fever after each infusion; an additional dose of Benadryl was administered after the first reaction.

Analysis of the 63 patients at one year showed no difference in GMFM-66 change scores between placebo and treated groups (6.9 vs. 7.5, p=0.72). However, treated subjects who received above the median infused cell dose of 1.98x10^7/kg demonstrated improvement in GMFM-66 change scores compared to subjects who received lower cell doses (p=0.05) (Figure 3). The infused cell dose was not correlated with age (p=0.70) or type (p=0.32) or severity (p=0.46) of cerebral palsy.

Figure 3: GMFM-66 Scores by Randomized Treatment Assignment and Infused Cell Dose
Panel A: Distribution of GMFM-66 score at baseline and 1 year in patients randomized to placebo and autologous cord blood. Lines connect the group means (circles) over time. Panel B: GMFM-66 change scores based on median cell doses (Precryopreservation doses: Low, <3x10^7/kg vs. High, >=3x10^7/kg; Infused doses: < 1.98x10^7/kg vs. High: >= 1.98x10^7/kg). Panel C: One year Observed-Expected GMFM-66 scores in patients ≥2 years of age at baseline based on infused cell dose. Panel D: PDMS-2 gross motor quotient change scores based on infused cell dose.

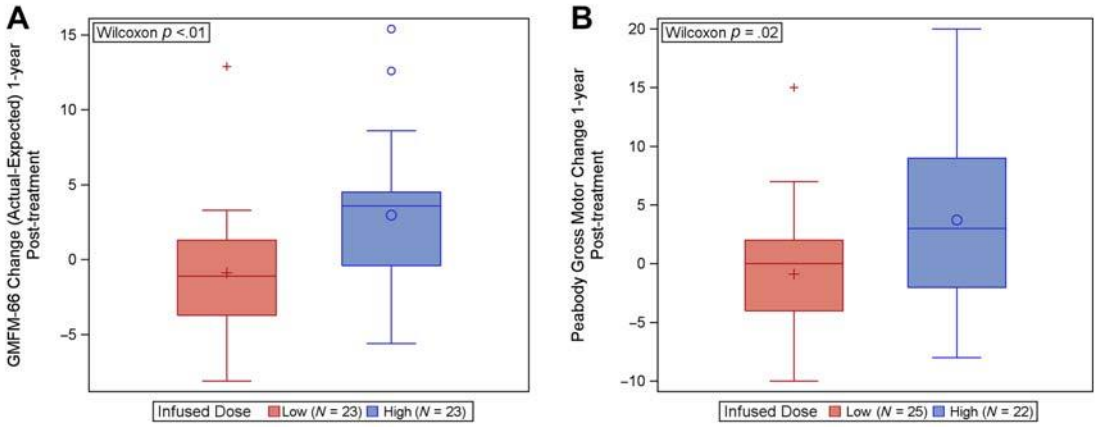


Using a subject's baseline GMFM-66 score, GMFCS level, age, and published percentiles,[64] we calculated the expected one-year GMFM-66 score for each patient. Since such percentile values are only available for children ages ≥2 years, one-year old subjects (n=25) were excluded from this analysis. In the evaluable patients (n=38), the difference between the actual one-year GMFM-66 score and the expected one-year GMFM-66 score was then calculated. In the entire autologous CB group, the median actual-expected difference in GMFM-66 scores was 1.7 (range -6.1-14.5), versus 2.2 (range -6.2-12.6) in the placebo group. When the CB group was analyzed by infused cell dose, subjects who received above the median infused cell dose of $1.98x10^7$/kg (n=9) improved a median of 4.3 points *greater* than expected (p=0.05 vs placebo), whereas subjects who received below the median infused dose improved a median of 1.9 points *less* than expected (p=0.02 vs high dose; p=0.07 vs placebo, figure 3C).

We then used the two year data to further explore the effect of cell dose by comparing the difference between observed and expected GMFM-66 scores one year after CB infusion in all subjects who were ≥2 years old when they were treated (n=46), regardless of when the infusion was given (baseline or one year). In this analysis, the dose relationship from the primary analysis was confirmed: subjects who received $\geq 2x10^7$ cells/kg (n=23) improved a median 3.6 points (IQR -0.4 to 4.5) *greater* than expected, whereas subjects who received $<2x10^7$/kg did not improve beyond expectation (median -1.1, IQR -3.7 to 1.3, p=0.003, figure 4A).

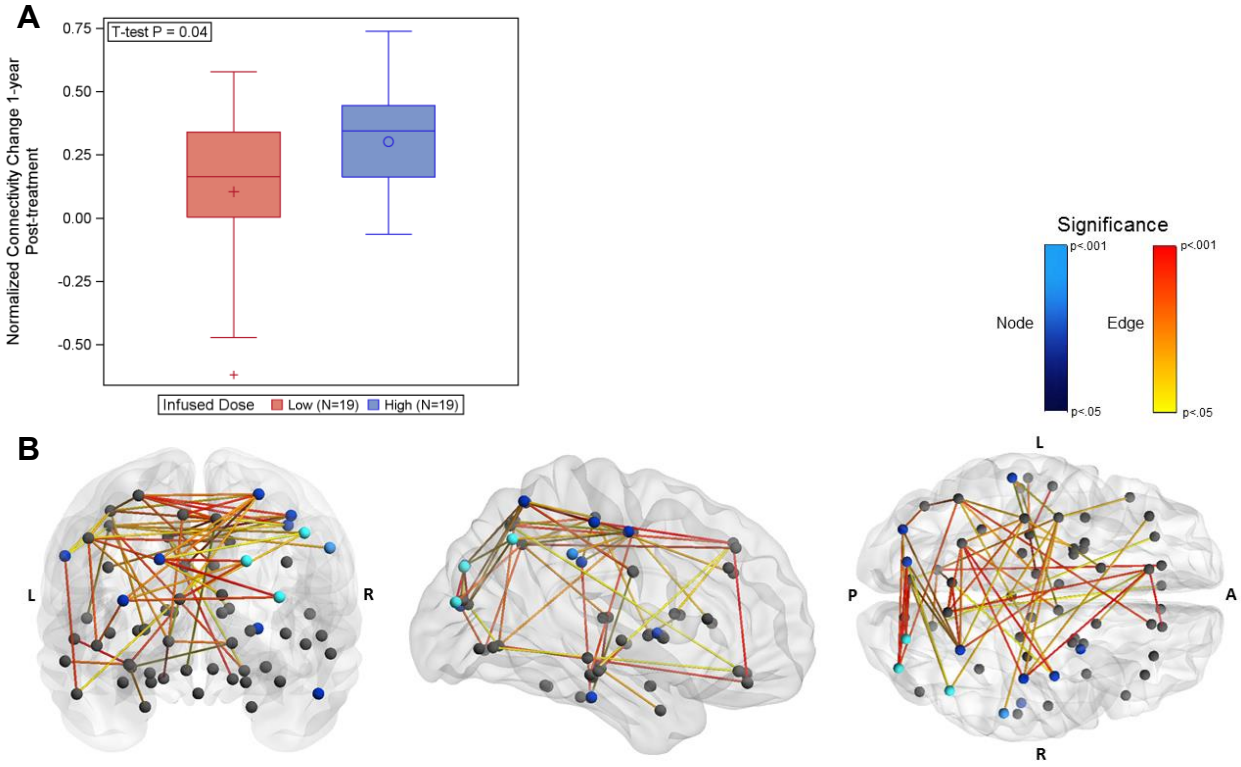
Fifty patients were also eligible for analysis of the Peabody Developmental Motor Scales-2 (PDMS-2), which assesses motor skills in children from birth through age 5, one year post initial treatment. The median one-year change from baseline in the Gross Motor Quotient was 1.0 in the autologous CB group and -0.5 in the placebo group (p=0.39). When the subjects treated with autologous CB were analyzed by infused cell dose (>/< $1.98x10^7$/kg), a significant change was detected in the Gross Motor Quotient both at one year post-randomization (figure 3D) and one year post-CB infusion, regardless of the timing of the infusion (3.0 vs. 0, p= 0.02, figure 4B).

Figure 4: Gross Motor Function One Year after CB Infusion by Cell Dose
High dose >=$2x10^7$/kg, low dose <$2x10^7$/kg. Panel A: Observed-Expected GMFM-66 scores one year after treatment in patients ≥2 years of age at the time of CB infusion. Panel B: PDMS-2 gross motor quotient change scores based on infused cell dose.



Imaging Results: MRI data was analyzed to explore relationships between change in GMFM-66 scores, total brain connectivity, and cell dose. Accurate anatomical image parcellation could not be obtained in approximately one-third of subjects due to substantial morphologic brain abnormalities, leaving 23 treated patients and 15 placebo patients with usable connectivity data. There were no statistically significant differences in type of cerebral palsy, GMFCS level, or age between patients with and without analyzable images. As previously described,[65] there was a moderate correlation between change in GMFM-66 score and total connectivity at one-year in all analyzable subjects (n=38, Spearman r=0.53; 95% CI: 0.25, 0.73; p<0.001). Total connectivity change was not related to baseline GMFCS level, typography of cerebral palsy, or gender, but was inversely correlated with age (Spearman r=-0.52; 95% CI: -0.72, -0.23; p=0.001). In the two-year analysis when all evaluable subjects were examined by cell dose, patients who received ≥$2x10^7$ TNCC/kg (n=19) demonstrated a statistically significant greater increase in normalized whole brain connectivity one year after treatment than children who received lower doses (n=19; p=0.04, figure 5). In the sensorimotor network, nodes with significant increases in connectivity that correlated with improvement in GMFM-66 scores included the pre- and post-central gyri, basal ganglia, and brain stem.

Figure 5: Brain Connectivity via MRI/DTI
Panel A: Change in normalized whole brain connectivity one year after treatment. Panel B: Connectome representation. The nodes and edges included are those that demonstrated significantly increased improvement in children receiving high doses compared to those receiving low doses, as indicated by the color chart, with insignificant nodes shown in gray.



Results of this trial suggest that when administered in sufficient doses (≥1.98x10^7/kg), autologous CB may improve motor function in young children with cerebral palsy. The study was limited by small sample size of the dosing groups and the inclusion of children 1-2 years of age for whom analysis of the predicted motor change score was not possible.

2.6.5 Allogeneic Sibling CB in Cerebral Palsy

Given the benefit in motor improvement observed in the CP-AC study utilizing autologous CB, and recognizing that most children do not have a suitably qualified autologous CB unit available, we conducted a phase I clinical trial to evaluate the safety of fully HLA-matched or haploidentical ***allogeneic*** sibling CB infusion in children with cerebral palsy (ClinicalTrials.gov ID NCT02599207). Fifteen children (9 female, 5 male), ages 1-6, with spastic CP were enrolled and treated. Children were eligible if they were (1) GMFCS level 2-4 or (2) GMFCS level 1 with hemiplegia if they used their affected hand as an assist only. Children with known genetic conditions, intractable seizures or severe microcephaly were ineligible. Sibling CB units had to have a precryopreservation total nucleated cell count (pTNCC) ≥2.5x10^7/kg, negative sterility cultures, negative maternal infectious disease screening and be a ≥4/8 HLA match with the participant.

Participants were evaluated at baseline and 6 months with functional evaluations (GMFM, Peabody), brain CT, and laboratory studies. On the day of infusion, sibling CB units were thawed and washed. After premedication with Tylenol, Benadryl and Solumedrol, participants received a dose of ≥2.5x10^7/kg cells, based on the pTNCC, intravenously over 5-10 minutes in the outpatient setting. Participants received IV fluids and were monitored for 1-2 hours post-infusion. Safety assessments were conducted 24 hours, 2 weeks, and 3, 6 and 12 months post-infusion.

The median baseline age of participants was 3.7 years (range 1.4-6). Sibling CB infusions had a median TNCC of 4.3x10^7/kg (range 1.8-5.2) and median CD34 dose of 0.6x10^5/kg (range 0.1–1.8). Four CB infusions were full HLA matches, 11 were haploidentical. All CB units had negative post-thaw sterility cultures. There were no acute infusion reactions and no unexpected imaging findings. No platelet antibodies, donor-specific HLA antibodies, or donor cells were detected in peripheral blood six months after infusion. With a median follow-up of 11 months, there were a total of 34 adverse events in 13 participants. Most (19/34) were attributed to common childhood infections, and none were related to the CB infusion. The only serious adverse event was unrelated to the infusion and occurred in a participant with a history of seizures who was hospitalized for a prolonged febrile seizure 5 months after CB infusion. Six months after infusion, participants improved a mean of 4.8 (SD 2.5) points on the GMFM-66 and 1 (SD 2.9) point on the Peabody Gross Motor Quotient. This study confirms that partially or fully-HLA matched ***allogenenic*** sibling CB infusion is safe and feasible in young children with CP.

2.6.6 Allogeneic Unrelated Donor CB in Children with Cerebral Palsy

A study of allogeneic CB was conducted in 105 Korean children with cerebral palsy.[66] This study had three groups: allogeneic CB + cyclosporine + erythropoietin; erythropoietin alone; and a control group. One severely affected patient died in her sleep 14 weeks after CB administration, and this was determined to be unrelated to the CB infusion. Eight other patients experienced serious adverse events requiring hospitalization (pneumonia–4, seizure–1, influenza–2, urinary tract infection–1), but the distribution did not differ between groups. Non-serious adverse events that were more common in the CB group were pneumonia and irritability. At one year of follow-up, there were no reported prolonged or delayed serious adverse events. The authors reported greater improvements in cognitive and select motor functions in children who received CB and erythropoietin versus controls. There was no CB-only group for comparison.

The same group subsequently conducted a study of allogeneic CB versus placebo in 36 children ages 6 months to 20 years with cerebral palsy.[67] In this study, the CB group showed greater improvements in gross motor performance at 6 months compared to the placebo group, and motor outcomes were positively correlated with the dose of CB cells.

In a Russian study, 80 patients ages 1-12 years with cerebral palsy received 1-6 intravenous infusions of allogeneic CB with an average dose of 2.5x10^8 viable cells per infusion.[68] Most patients who received four or more infusions showed improvement in tone, motor, and/or cognitive function, but there was no control group for comparison. In their series, factors that impacted treatment response included age, severity of brain damage, and number of CB infusions.

In summary, data from early phase clinical trials demonstrate that CB infusion is safe in young children with cerebral palsy and suggest that it may be effective in improving motor function when given intravenously at adequate doses.

2.6.7 MSCs in Cerebral Palsy

MSCs derived from both bone marrow and umbilical cord tissue have been investigated in cerebral palsy in a few small studies in China.[69-71] In one study, autologous bone marrow-MSCs were injected intrathecally and/or into brain parenchyma of 46 children ages six months to 15 years with cerebral palsy.[70] The only side effects reported were transient low-grade fever and wound aches. That study demonstrated a statistically significant change in GMFM-66 percentile at one, six and 18-months post-treatment, though there was no placebo or natural history cohort for comparison.

In a case report, one patient with cerebral palsy was treated with seven doses of 5-10x10^6 MSCs generated from the umbilical cord tissue of her younger sibling and administered via the intravenous and intrathecal routes.[69] This patient also experienced temporary low-grade fever after the intrathecal injections but no other side effects. She was followed for 28 months after the final treatment, and demonstrated motor improvements during that time.

In another study, eight twin pairs of children with spastic cerebral palsy received four intrathecal injections of allogeneic, unrelated umbilical cord tissue-derived MSCs.[71] Improvements in GMFM-88 scores were noted six months after treatment. Interestingly, the improvement within each member of the twin pair was correlated, suggesting a possible hereditary component to the response to therapy.

2.6.8 Allogeneic CB in Stroke

The CoBIS study was an IRB approved, FDA IND sponsored, prospective, open-label, multi-center, Phase 1 safety study of a single intravenous infusion of non-HLA matched, ABO matched, ***allogeneic*** CB in 10 adults ages 18-80 years old. CB units were selected by ethnicity, blood type, and ability to supply a dose of 0.5 – 1.5 x 10^7 TNCC/kg. Eligible patients included those experiencing a recent, acute cortical, hemispheric, ischemic stroke in the middle cerebral artery (MCA) distribution as detected by MRI as a diffusion weighted abnormality and are enrolled if their National Institutes of Health Stroke Scale (NIHSS) is 8-15 (right hemisphere) or 8-18 (left hemisphere). Participants who received tPA or undergo mechanical perfusion are eligible for inclusion. Participants were not pre-treated with immunosuppressive drugs. The primary endpoint was safety as assessed by the frequency and severity of adverse events within 24 hours of CB infusion and during the 12 months post CB infusion. Secondary outcome measures included Modified Rankin Scale (mRS), NIHSS, the Barthel Index (BI), and European Quality of Life (EQ-5D-3L), Patient Health Questionnaire Scale (PHQ8), Telephone Interview for Cognitive Status (TICS), and a self-reporting survey of rehabilitation therapy. MRI was used to evaluate changes in the brain 3 months post infusion.

There were no serious adverse events in any of the 10 participants enrolled and treated with allogeneic unrelated donor non-HLA matched, ABO matched CB within 10 days of their stroke. This safety data suggests intravenous infusion of unmatched, ***allogeneic***, CB cells is well tolerated. A randomized, placebo controlled Phase 2 study is underway with the goal of using unrelated non-HLA matched CB to down-regulate inflammation and promote neuroprotection and neurorepair in patients with ischemic stroke.

2.6.9 MSCs in Stroke

Several small studies using systemically administered autologous bone marrow-derived MSCs have been conducted in adult patients with acute or chronic stroke, with no significant side effects.[72] A phase II study of an allogeneic MSC product (MultiStem) was recently conducted in 126 adult patients with stroke (65 treated, 61 placebo).[73] MSC therapy was well-tolerated. While there was no difference between placebo and treated patients in measures of stroke recovery, the treatment group had a lower rate of mortality and infections, associated with down regulation of inflammatory biomarkers including IL-6. Patients who received MSCs earlier (24-36 hours post-stroke vs. 36-48 hours post-stroke) demonstrated more favorable recovery than patients who received later treatment or placebo.

2.6.10 Unrelated Donor Cells in Autism Spectrum Disorder (ASD)

At Duke, we are currently conducting a phase II randomized, double-blind, placebo controlled trial of autologous or allogeneic CB infusion in young children with ASD (DukeACT). Participants are randomized to the order in which they receive CB and placebo infusions, given six months apart. Children who have an adequate autologous CB unit stored are assigned to receive an autologous CB infusion; those without an available autologous CB unit are assigned to receive an infusion of allogeneic, unrelated donor CB from the Carolinas Cord Blood Bank with the same donor screening and HLA matching requirements set forth in this protocol.

A blinded safety analysis was performed after the first 100 participants were enrolled in the DukeACT study. Patients who enroll in DukeACT are randomized in a 2:1 ratio to CB (autologous or allogeneic) or placebo. With up to 12 months of follow-up, the safety profile of CB infusions has been similar to our prior experience. There have been no Serious Adverse Events, graft versus host disease, or product-related infections. As of August 2017, there were a total of 166 Adverse Events (AEs) reported in 100 participants. One hundred and twenty-two events (73.5%) were Mild, 42 (25.3%) were Moderate, and 2 (1.2%) were Severe. Six events (3.6%) in 3 subjects (3.0% of enrolled patients, 4.4% of subjects reporting events) were Related to study product. The six related events are coded as Immune System Disorders/Allergic Reaction to Excipient. One Severe event reported as “Hypoxia, Transient” was related to an allergic reaction to the study product. The other reported Severe event was a “Fall” Unrelated to the study product. Overall, 22/38 subjects (57.9%) with an autologous cell source had AEs and 40/62 (64.5%) of subjects with an allogeneic cell source had AEs, the vast majority of which were related to common childhood ailments. The most frequently occurring Unrelated events were: Pyrexia (16 events), Upper Respiratory Infection (11 events), Otitis Media (8 events), and Aggression (8 events).

In China, 37 autistic children, ages 3-12 years, were enrolled in a study utilizing allogeneic CB mononuclear cells (CBMNC) and/or umbilical cord-derived mesenchymal stem cells (UCMSC).[74] The children were divided into three groups: CBMNC (n=14), CBMNC+UCMSC (n=9), and control (n=14). All children received standard rehabilitation therapy. Cells were given in four doses, 5-7 days apart, via intravenous and/or intrathecal administration. In the cell recipients, transient fever (5/23) was the only reported adverse event related to the therapy. Compared to control patients at six months, they observed greater improvements in the Childhood Autism Rating Scale (CARS) in the CBMNC+UCMSC group and in the Clinical Global Impression scale (CGI) in both cell groups. This was a small, non-randomized study designed primarily to assess safety. Nonetheless, the functional data suggests the potential for benefit.

2.6.11 MSCs in Other Neurologic Conditions

A few clinical trials of autologous MSC therapy have been conducted in patients with multiple sclerosis. Of 25 patients with progressive multiple sclerosis treated with a single intrathecal injection of autologous bone marrow-derived MSCs, the disease stabilized in half of patients over a one-year time period.[75] Side effects, all transient and self-limited including low-grade fever, nausea/vomiting, lower limb weakness, and headache, were likely related to the intrathecal route of administration and were consistent with those reported from other small studies of intrathecal MSC treatment in patients with multiple sclerosis.[76,77] Another study that treated 10 patients with progressive visual deficits due to multiple sclerosis with a single intravenous dose of autologous bone marrow-derived MSCs demonstrated improvements in visual acuity, visual evoked response latency, and optic nerve area.[78]

One clinical trial has been conducted in seven patients with Parkinson's Disease who received a single dose of autologous bone marrow-derived MSCs transplanted to the subventricular zone using stereotaxic surgery. With a follow-up of 10 to 36 months, three patients demonstrated an improvement in disease symptoms. Two patients also reported subjective improvement of symptoms and reduction in drug dosage.[79]

2.7 Source of Unrelated CB Units for this Trial

The Carolinas Cord Blood Bank (CCBB) is one of the largest public cord blood banks in the nation. Established in 1998 with support from the National Heart and Blood Institute of the NIH, the CCBB has over 30,000 CB units in inventory and has distributed over 2,500 CB units for transplant to date. In 2012 the CCBB received approval from the FDA for its BLA application to market DUCORD, a stem cell product derived from umbilical cord blood, for use in transplants between unrelated donors and recipients. DUCORD is approved for use in hematopoietic stem cell reconstitution for patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment. The CCBB currently collects from 10 hospital sites (8 in North Carolina, 1 in Atlanta, GA and 1 in Boston, MA). It also accepts CB donations from mothers delivering in any hospital in North Carolina and Atlanta through a kit donation program.

2.8 Specifications for Qualification CB Units

Based on established criteria utilizing allogeneic CB for hematopoietic stem cell transplantation and our experience in treating more than 600 children with autologous CB for neurological conditions, we have established the following criteria to qualify banked CB units for cell therapy studies. CB units utilized for this current study will be obtained from the Carolinas Cord Blood Bank. It is the first choice to utilize CB units from the Carolinas Cord Blood Bank. However, if a qualifying CB unit cannot be found from the Carolinas Cord Blood Bank, another accredited bank may be used to locate a matching CB unit with the following criteria listed below:

The CB unit must have:

1. Pre-cryopreservation total nucleated cell count (TNCC) documented and at least 12 x 10^7/kg. We target CB units that are at least 12 x 10^7/kg, however, we will accept units that are at least 10 x 10^7/kg.
2. Pre-cryopreservation viability ≥85% of total cells and ≥70% of CD34+ cells (if performed)
3. Pre-cryopreservation sterility culture performed and negative

4. Maternal infectious disease screening as follows: Testing must include negative results for Hepatitis B, Hepatitis C, HIV, HTLV, and syphilis. Additional screening, which is dependent on the timing of the CB collection, may be performed based on local and national regulations. Units from mothers who have a positive CMV antibody screen may be used.
5. Test sample available for identity confirmation and potency testing
6. HLA typing performed and meets study-specific parameters
7. CD45+ viability ≥40% and CD34+ viability ≥70% on thawed test sample

These same criteria will be utilized for this clinical trial and, along with procedures for CB administration, are detailed again in section 6.0.

2.9 Source of MSCs for this Study: hCT-MSC

hCT-MSC is a third party MSC product manufactured from allogeneic donor digested umbilical cord tissue that is expanded for two passages in culture, cryopreserved, stored in the vapor phase of liquid nitrogen, and banked. The umbilical cord tissue is donated by healthy mothers delivering healthy full term babies after a normal pregnancy with written informed consent. The cells are manufactured, cryopreserved and stored in the Robertson CT2 GMP laboratory (Duke University, Durham, NC).

Umbilical cord tissue is an attractive source of MSCs as it is readily available and easily obtained without consequence to the donor, is non-controversial, has a higher proliferative potential than MSCs from other postnatal sources.[80] Numerous preclinical studies have not demonstrated any evidence of tumorigenicity or toxicity of cord tissue-derived MSCs.[81] A summary of early phase clinical trials published in English that utilized cord tissue-derived MSCs is shown in Table 1. Among these 36 studies including 695 patients and at least 1,416 doses of cord tissue-derived MSCs with follow-up ranging from three months up to six years, no severe adverse events were reported. Several more clinical trials of cord-tissue derived MSCs in various disease conditions are underway (clinicaltrials.gov).

Table 1: Umbilical Cord Tissue-Derived MSCs Studied in Humans

Report	Indication	# of treated patients	Ages, yr (range or mean)	Route of administration	Dose	# of doses (interval)	Duration of f/u (months)	AEs due to CT-MSCs
Li, 2016[74,82]	Autism	20	3-14	IT	$1x10^6$/kg	2 (5-7 days)	6	transient fever (n=4)
Xie, 2016[83]	Hypoxic Ischemic Encephalopathy	12	45-68	IV	$1x10^8$	1	6	none
Wang, 2016[84]	Juvenile Idiopathic Arthritis	10	2-15	IV	$4x10^7$	2 (3 months)	12	none
Chen, 2016[85]	Psoriasis	2	26-35	IV	$1x10^6$/kg	1, 5	48, 60	none
Cai, 2016[34]	Type I Diabetes	21	5-28	Intra-arterial (pancreatic artery)	$1.1x10^6$/kg	1	12	none
Hu, 2016[86]	Type 2 Diabetes	31	42-63	IV	$1x10^6$/kg	2 (monthly)	36	none
Hua, 2016[87]	Spinal Cord Injury	1	25	IT	$1x10^7$	4 (3 days)	12	none
Kim, 2016[88]	Atopic Dermatitis	34	29	Subcutaneous	$2.5\text{-}5x10^7$	1	3	none
Qin, 2016[89]	Diabetic Foot	28	N/A	Endovascularly	N/A	N/A	3	none
Wang, 2016[90,91]	Lupus	40	17-54	IV	$1x10^6$/kg	2 (weekly)	12-72	none
Zhao, 2015[92]	Heart Failure	30	53.2	Intracoronary	NR	1	6	none
Liang, 2015[93]	Radiation Myelitis	1	37	IV, IT	$5.2x10^7$ IV, $1.1x10^7$ IT	1	18	none
Wang, 2015[71]	Cerebral Palsy	16	4-12	IT	$1\text{-}1.5x10^7$	4	6	NR
Li, 2015[94]	Coronary Artery Occlusion	15	N/A	Intracoronary	$3\text{-}5x10^6$	1	24	none
Rajput, 2015[95]	Duchenne Muscular Dystrophy	11	5-18	IV & IM	$1x10^6$/kg	4 (monthly)	36	none
Gu, 2015[96]	Lupus Nephritis	58	12-55	IV	$1x10^6$/kg	1	12	none
Pan, 2015[97]	Bone Marrow Necrosis	1	11	Intrabone	$2x10^7$	1	38	none
Wu, 2015[98]	GvHD	24	14-44	IV	$0.5\text{-}1x10^6$/kg	1	1-24	none
Zhu, 2015[99]	Leukemia, undergoing haplo HSCT	25	4-17	IV	$1\text{-}1.4x10^6$/kg	1	3-25	none
Miao, 2015[100]	Neurological disorders	88	2-68	IT	NR	4-6 (5-7 days)	NR	transient HA, fever, back/leg pain

Report	Indication	# of treated patients	Ages, yr (range or mean)	Route of administration	Dose	# of doses (interval)	Duration of f/u (months)	AEs due to CT-MSCs
Li, 2015[101]	Becker Muscular Dystrophy	3	6-46	IV	3-5x10^7	1	3	none
Wang, 2015[102]	Hemorrhagic Cystitis	7	11-38	IV	0.8-1.6x10^6/kg	1-3	NR	none
Cheng, 2014[103]	Spinal Cord Injury	10	35.3	Spinal Cord Injection	2x10^7	2 (10 days)	6	none
Chang, 2013[36]	Bronchopulmonary Dysplasia	9	10 days	Intratracheal	1-3x10^7	1	3	none
Li, 2014[104]	Multiple Sclerosis	13	42	IV	4x10^6/kg	3 (2 weeks)	12	none
Kong, 2014[105]	Type 2 Diabetes	18		IV	N/A	3	6	slight fever
Wang, 2013[106]	Traumatic Brain Injury	20	5-48	IT	1x10^7	4 (5-7 days)	6	HA, dizziness
Wang, 2013[107]	Primary Biliary Cirrhosis	7	33-58	IV	0.5x10^6/kg	3 (monthly)	12	none
Zhang, 2013[108]	HIV	7	26-49	IV	0.5x10^6/kg	3 (monthly)	12	none
Jin, 2013[109]	Spinocerebellar Ataxia	16		IV & IT	N/A	N/A	12	none
Wu, 2013[110]	Leukemia, undergoing CBT	8	3-12	IV	2-10x10^6/kg	1	8-27	none
Jiang, 2013[111]	Stroke	4	40-59	Intra-arterial (MCA)	2x10^7	1	6	none
Hu, 2013[35]	Type I Diabetes	15	17.6	IV	1.5-3.2x10^7	1	24	none
Shi, 2012[112]	Liver Failure	24	24-59	IV	0.5x10^6/kg	3 (monthly)	18	none
Zhang, 2012[113]	Liver Cirrhosis	30	25-64	IV	0.5x10^6/kg	3 (monthly)	12	none
Qu, 2009[114]	Bone Nonunion	36	36	Intrabone	1x10^6 - 1x10^7	1	13	none
Totals: 36 studies		**695**	**0-68**	**IV: 362 IT: 162 Other: 199**		**>1,416**	**3-72**	**4 studies (3 IT route)**

Abbreviations: HA-headache, IM-intramuscular, IT-intrathecal, IV-intravenous, N/A-not available, NR- not recorded

2.10 Study Rationale and Hypotheses

As described above, previous studies suggest that adequately dosed autologous CB infusion can improve motor function in children with cerebral palsy. As it is not feasible that every child with cerebral palsy will have access to their autologous CB, this study will assess efficacy of two allogeneic sources of cells that can be available to all patients in need. The major goal of this study is to investigate change in motor function 12 months after treatment with two allogeneic cell sources, allogeneic CB and hCT-MSCs. This study will generate important data regarding the effect size of change in motor function of these two cell sources and a natural history cohort to aid in the planning of future trials. The rationale for the study and for the potential benefit of cell therapy in cerebral palsy is based upon the following hypotheses:

- We have demonstrated safety and preliminary evidence of potential dose-dependent efficacy of autologous CB infusions in children with cerebral palsy.
- It is possible that different cell types, e.g. cord blood mononuclear cells versus cord tissue MSCs, may influence brain connectivity by different mechanisms.
- Multiple doses of cells may be superior to a single dose of cells.
- The developing brain exhibits remarkable plasticity, making young children ideal candidates for deriving maximal therapeutic benefit from restorative therapies, including CB.
- CB cells, acting through paracrine mechanisms, may facilitate endogenous repair mechanisms and promote formation of new neural connections the motor cortex resulting in significant clinical improvements.
- Brain connectivity plays an important role in the pathophysiology, and potentially mechanism of repair, of brain injury in children with cerebral palsy. Specifically, we hypothesize that (1) impairments in brain connectivity account for the motor deficits in children with cerebral palsy, (2) increases in brain connectivity have a direct impact on functional improvements, (3) children with cerebral palsy who receive CB infusions will exhibit greater increases in brain connectivity than children who receive placebo infusions, and (4) the severity of baseline brain connectivity abnormalities predict the potential for benefit of CB therapy.

2.11 Study Design

This study is a phase I/II, prospective, randomized, open-label trial designed to assess the effect size of change in GMFM-66 score in subjects treated with hCT-MSC or allogeneic CB and assess the safety of repeated doses of hCT-MSC in young children with cerebral palsy. Children ages 2-5 years with cerebral palsy due to hypoxic ischemic encephalopathy, stroke, or periventricular leukomalacia may be eligible to participate. All participants will ultimately be treated with an allogeneic cell product at some point during the study. Participants will be randomized to one of three arms: (1) the “AlloCB” arm will receive one allogeneic CB infusion at the baseline visit; (2) the “MSC” arm will receive three hCT-MSC infusions, one each at baseline, three months, and six months; (3) the “natural history” arm will not receive an infusion at baseline but will receive an allogeneic CB infusion at 12 months. All participants will have an initial clinical evaluation to verify and classify the diagnosis of cerebral palsy and determine eligibility. They will return for study visits an additional two (AlloCB and natural history arms) or three (MSC arm) times. Outcome measures, described in detail in section 7.6, will be assessed at baseline, six-months, and one-year time points. Additional safety endpoints will be assessed remotely for 12 months after the final in-person visit.

2.12 Risks and Benefits

CB cells will be thawed, washed, and infused using standard operating procedures that have been used in over 35,000 individuals worldwide. The potential risks associated with infusion of allogeneic CB cells include a hypersensitivity reaction to the product (rash, shortness of breath, wheezing, difficulty breathing, hypotension, swelling around the mouth, throat or eyes, tachycardia, diaphoresis) to the product or transmission of infection. Additional risks associated with infusion of allogeneic CB cells include hypertension, bradycardia, anaphylaxis, hematuria, acute hemolytic reaction, rejection of cells, immune dysregulation (develop of HLA directed antibodies), and development of graft-versus-host disease. All CB units are screened for the risk of transmitting infectious agents and must meet release criteria prior to infusion, as described below. Participants must have normal immune function and will not receive immunosuppressive therapy prior to or after infusion of CB or hCT-MSC.

Potential risks associated with MSC infusion include a reaction to the product (rash, shortness of breath, wheezing, difficulty breathing, hypotension, swelling of the mouth, throat or eyes, tachycardia, diaphoresis), infection transmission, and HLA sensitization. Theoretical risks that must be considered but have not been associated with MSC administration include the possibility of immune suppression and ectopic tissue formation. CB collected with the cord tissue used to manufacture hCT-MSC is screened for the risk of transmitting infectious agents, and the product must meet release criteria prior to infusion, as described below. Participants must have normal immune function and will not receive immunosuppressive therapy prior to or after infusion of hCT-MSC.

Participants who undergo a brain MRI may require moderate sedation or, rarely, general anesthesia, in order to complete the MRI and infusion. Risks associated with anesthesia include nausea, vomiting, blood vessel injury, nerve injury, lung injury, heart attack, allergy to medications, brain damage, respiratory insufficiency, hypoxia, hypotension, and anaphylaxis, and death. These associated risks are described in detail in the consent form. Risks associated with blood draws and IVs include momentary discomfort or pain, bruising, infection, bleeding, clotting, and fainting. Risks associated with genetic testing include medical, psychosocial, and economic risks, effects on insurability and employability, limits on educational options, and social stigma.

Potential benefits of this intervention include the possibility that the cells may, via direct or indirect mechanisms, induce changes *in vivo* that result in motor improvement.

3.0 STUDY OBJECTIVES

Primary Objective: To determine the effect size of change in GMFM-66 score in children with cerebral palsy treated with a single dose of approximately 10 $x10^7$ cells/kg of allogeneic CB or three doses of $2x10^6$ cells/kg of hCT-MSC.

Secondary Objective: To assess the safety of repeated doses of hCT-MSC in children with cerebral palsy.

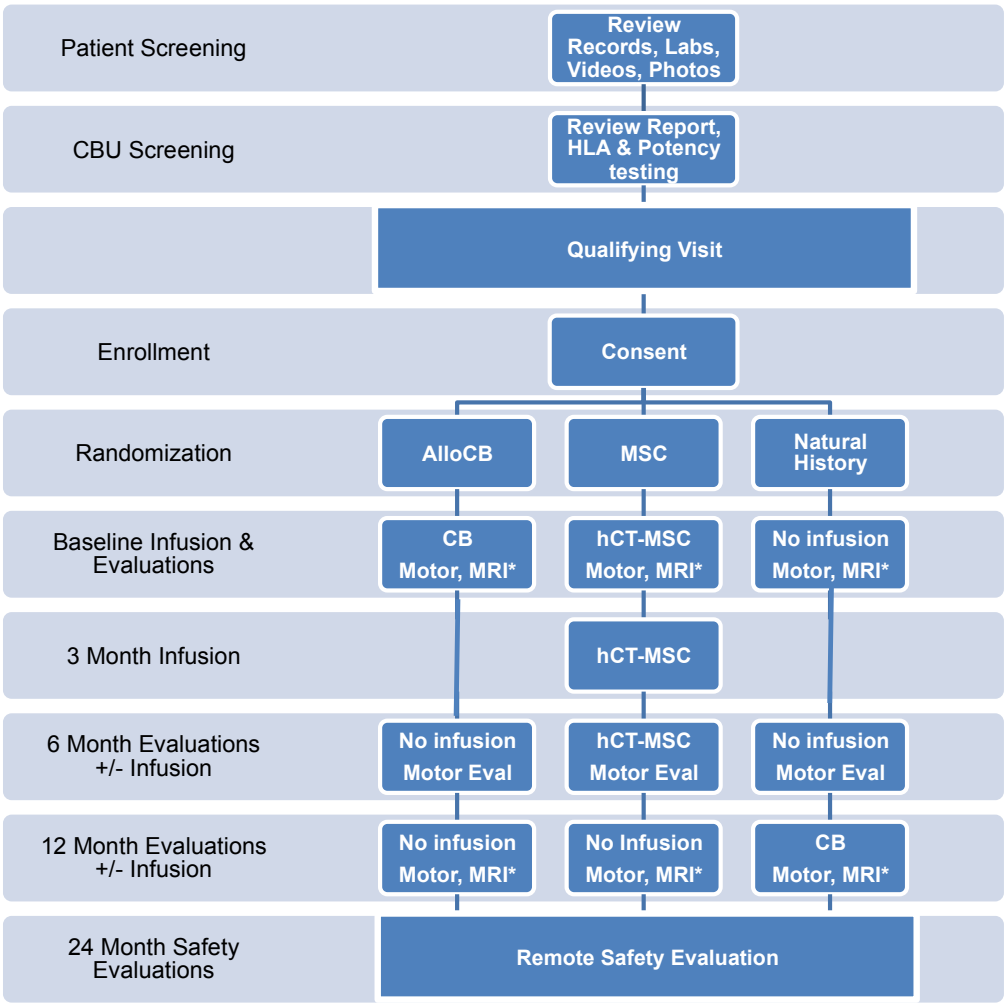
Exploratory Objectives: (1) To determine the change in the Peabody Developmental Motor Scale-2 (PDMS-2) score at 6 and 12 months in children treated with allogeneic CB or hCT-MSC. (2) To analyze the change in normalized total brain connectivity, as measured by brain MRI with DTI, from baseline to 12 months. (3) To assess changes functional and quality of life measures at 6 and 12 months.

4.0 STUDY DESIGN

4.1 General Design

This study is a phase I/II, prospective, randomized, open-label trial designed to determine the effect size of change in GMFM-66 score in subjects treated with hCT-MSC or allogeneic CB and assess the safety of repeated doses of hCT-MSC in children with cerebral palsy. Children ages 2-5 years with cerebral palsy due to hypoxic ischemic encephalopathy, stroke, or periventricular leukomalacia may be eligible to participate. All participants will ultimately be treated with an allogeneic cell product at some point during the study. Participants will be randomized to one of three arms: (1) the "AlloCB" arm will receive one allogeneic CB infusion at the baseline visit; (2) the "MSC" arm will receive three hCT-MSC infusions, one each at baseline, three months, and six months; (3) the "natural history" arm will not receive an infusion at baseline but will receive an allogeneic CB infusion at 12 months. Motor outcome measures will be assessed at baseline, six-months, and one-year time points. Safety will be evaluated at each infusion visit and remotely for an additional 12 months after the final visit. Duration of study participation will be 24 months from the time of baseline visit. Randomization to treatment arms will be stratified by GMFCS level at study entry and etiology of CP (Stroke vs. Other). .

4.2 Study Flow Chart



*MRI will be performed in a subset of participants

4.3 Study Endpoints

Primary Endpoint:
The primary endpoint is the difference between a participant's observed and expected changes in GMFM-66 score 12 months after the initial study infusion. Interval estimates will be reported separately for the hCT-MSC, AlloCB, and Natural History arms. Expected GMFM-66 scores at 12 months will be calculated based on the participant's baseline age, GMFCS level, and GMFM-66 score at study entry using published reference percentiles.[64]

Secondary Endpoints:
The secondary endpoint is the number of adverse events occurring over the 12-month period post-infusion with hCT-MSC or AlloCB.

Exploratory Analyses:

- Observed GMFM-66 score at baseline, 6, and 12 months
- Change in the Peabody Developmental Motor Scale-2 (PDMS-2) score at 6 and 12 months.
- Change in normalized total brain connectivity, as measured by brain MRI with DTI, from baseline to 12 months.
- Change in functional and quality of life measures at 6 and 12 months.

5.0 RESEARCH PARTICIPANT SELECTION AND WITHDRAWAL

5.1 Study Population

Ninety evaluable children ages 2-5 years with hypertonic cerebral palsy.

5.2 Inclusion Criteria

1. Age ≥24 months and ≤60 months adjusted age at the time of enrollment.
2. Diagnosis: Unilateral or bilateral hypertonic cerebral palsy secondary to in utero or perinatal stroke/hemorrhage, hypoxic ischemic encephalopathy (including, but not limited to, birth asphyxia), and/or periventricular leukomalacia.
3. Performance status: Gross Motor Function Classification Score levels I – IV
4. Review of brain imaging (obtained as standard of care prior to study entry) does not suggest a genetic condition or brain malformation.
5. Legal authorized representative consent.

5.3 Exclusion Criteria

1. Available qualified autologous cord blood unit.
2. Hypotonic or ataxic cerebral palsy without spasticity.
3. Autism and autistic spectrum disorders.
4. Hypsarrhythmia.
5. Legally blind.
6. Intractable seizures causing epileptic encephalopathy.
7. Evidence of a progressive neurologic disease.
8. Has an active, uncontrolled systemic infection or documentation of HIV+ status.
9. Known genetic disease or phenotypic evidence of a genetic disease on physical exam.
10. Concurrent genetic or acquired disease or comorbidity(ies) that could require a future allogeneic stem cell transplant.

11. Requires ventilatory support, including home ventilator, CPAP, BiPAP, or supplemental oxygen.
12. Impaired renal or liver function as determined by serum creatinine >1.5mg/dL and/or total bilirubin >1.3mg/dL except in patients with known Gilbert's disease.
13. Possible immunosuppression, defined as WBC <3,000 cells/mL or absolute lymphocyte count (ALC) <1500 with abnormal T-cell subsets.
14. Patient's medical condition does not permit safe travel.
15. Previously received any form of cellular therapy.

5.4 Research Participant Recruitment and Screening

Patients may be recruited through IRB-approved advertising for the study on the websites of CB banks, parent sponsored websites, the NMDP website, selected cerebral palsy societies, local medical providers, and through a record of inquiries for previous studies (brain injury database. Separate IRB approval will be obtained for any advertisements.

Screening for this study is conducted under a separate, IRB-approved screening protocol (Pro00063563). Under this protocol, after written informed consent is obtained from a parent/guardian, the patient's medical records, videos, and results of brain imaging are obtained and reviewed. The medical review is conducted by a team of pediatric nurses, nurse practitioners, and physicians to identify the presence of any exclusion criteria. If no exclusion criteria are identified, screening labs are performed and a search may be conducted to identify a suitably matched CB unit (see section 6.1).

5.5 Early Withdrawal of Research Participants

Criteria for Removal from Protocol Therapy:

1. Diagnosis of a genetic disease while under evaluation or on study.
2. Change in medical condition that precludes study participation.

Patients who are off protocol therapy are to be followed until they meet off-study criteria (see below). Follow-up data will be obtained on off-protocol participants unless consent is withdrawn. Participants that are taken off study prior to infusion of the CB will be considered not evaluable and can be replaced with another participant.

Off-Study Criteria:

1. Death.
2. Lost to follow-up.
3. Withdrawal of consent for any further data collection.
4. Completion of the final study visit.

6.0 STUDY PRODUCTS

6.1 Allogeneic Umbilical Cord Blood

Allogeneic unrelated donor CB units utilized for this trial will be obtained from the Carolinas Cord Blood Bank, an FDA licensed Public Cord Blood Bank at Duke University Medical Center. However, if a qualifying CB unit cannot be found from the Carolinas Cord Blood Bank, another accredited bank may be used to locate a matching CB unit with the matching criteria. CB donors must be eligible for donation to a public cord blood bank for allogeneic use. Donor eligibility screening via questionnaires is performed in accordance with CFR 1271.75 and infectious disease testing is performed in accordance

with CFR 1271.80 and 1271.85. The unit must also have an appropriate degree of HLA matching and meet product specifications as detailed below.

HLA Matching
All potential study participants will undergo high resolution HLA typing at HLA-A, B, and HLA-DRB1 via blood or buccal swab. Patients receiving allogeneic CB will have HLA typing performed on two separate samples for confirmation. Allogeneic units that are potential matches will initially be identified from a search of the Carolinas Cord Blood Bank, or another accredited bank The best available HLA-matched (≥4/6), using intermediate level matching at HLA Class I A and B and high resolution-allele level matching at HLA Class II, DRB1, CB unit with a pre-cryopreservation nucleated cell dose ≥12 x 10^7 cells/kg will be selected (we target CB units that are at least 12 x 10^7/kg, however, we will accept units that are ≥10 x 10^7/kg.) Once a unit is selected, HLA typing will be used to confirm the original HLA typing and to select the best matching unit. When possible, at least 1 match at each HLA loci will be prioritized. A CB unit must be at least 4/6 HLA-matched with the patient.
ABO/Rh Compatibility
Recipients' ABO/Rh blood typing will be obtained. CB units will not be selected based on ABO typing. However, an Rh negative CB unit will be selected for Rh negative female participants to avoid Rh sensitization in young females.

CB Unit Characterization
Results of initial testing at the cord blood bank must include a pre-cryopreservation TNCC, viability and sterility culture. Pre-cryopreservation TNCC must be ≥12x10^7/kg (we will accept units that are ≥10 x 10^7/kg) to target administration of 10x10^7 cells/kg post thaw, sterility cultures must have been negative, total viability must have been ≥85%, and CD34+ cell viability (if performed) must have been ≥70%.

A test vial or segment must be available from each CB unit for potency testing and confirmatory HLA typing. The segment will be detached from the candidate unit and tested for potency and identity (HLA-confirmatory typing) per Standard Operating Procedures in the CCBB at Duke. Units will be deemed acceptable for the trial if viability of the CD45 cell population is ≥40% and viability of the CD34 cell population is ≥70%. CFU growth, expression of aldehydehydrogenase and CD34 will be described but will not be a specification for study enrollment.

Prior to the patients' arrival, their designated CB unit will be transferred from the Carolinas Cord Blood Bank (or other accredited bank) to the Duke STCL, located in the same building, where it will be stored in a liquid nitrogen freezer until the day of infusion. On the infusion day, the CB will be thawed and washed in dextran/albumin and resuspended in an appropriate volume based on recipient weight for administration to the patient the standard fashion[115] per SOP STCL-PROC-036. At the time of thawing, standard studies listed (see table) will be performed. Only TNCC is utilized for release. A maximum dose of 10x10^7 TNC/kg will be prepared for infusion in a syringe or bag and infused over 2-25 minutes.

Post-Thaw Cord Blood Unit Testing

Test	Specifications
Total Nucleated Cell Count (TNCC)	Report; used to calculate final dose
Viability	Report
Viability of the CD34+ population*	≥70%
Viability of the CD45+ population	≥40%
Sterility**	No Growth
Colony Forming Unit (CFU) growth	Report
$ALDH^{br}$ as a percentage of CD45+ cells	Report

*Viability of the CD34+ cells post-thaw was previously tested on a segment and required to meet the specification of ≥70%. Therefore, for the clinical product, we will report but not use the post-thaw viability as a release criteria.
**If a positive culture is obtained after product administration, a plan is put into effect to notify the clinical and study teams and treat the patient if indicated.

6.2 Human Umbilical Cord Tissue-derived Mesenchymal Stromal Cells (hCT-MSC)

hCT-MSCs are a product of allogeneic cells manufactured from digested umbilical cord tissue that is expanded in culture, cryopreserved and banked (complete composition and manufacturing details available in IND 17313). hCT-MSCs are manufactured in the Duke CT2 GMP cell manufacturing lab from umbilical cord tissue donated to the Carolinas Cord Blood Bank, an FDA-licensed, FACT-accredited, public cord blood bank at Duke University Medical Center, after written informed consent from the donor baby's mother. Cord tissue is harvested from the placentas of male babies delivered by elective C-section after a normal, full-term pregnancy. Donor screening questionnaires are completed by the maternal donor, and maternal blood is tested for communicable diseases by the CLIA-certified donor screening laboratory in Charlotte, NC. Donors must be eligible for donation to a public cord blood bank for allogeneic use. After delivery of the placenta and cord, the cord blood is aseptically drained from the placenta. Then the cord is dried and cleaned with chloropreps, separated from the base of the placenta, placed in a sterile bottle containing Plasmalyte A, and transported to the Robertson CT2 GMP cell processing laboratory at room temperature in a validated container.

In the clean room manufacturing suite, in a biosafety cabinet, the cord tissue is removed from the media, placed in sterile dishes, cut into small pieces and then minced and digested in the Miltenyi Biotec GentleMacs Octo Dissociator with GMP-grade enzymes: hyaluronidase, DNase, collagenase, papain. The resultant cell suspension is placed in culture in Prime XV MSC Expansion XSFM (Irvine Scientific) media with 1% platelet lysate and grown to confluence (~7-14 days) to establish the P0 culture. To establish the master cell bank, P0 is harvested and cryopreserved in cryovials with Cryostor 10 media (BioLife), and stored in the vapor phase of liquid nitrogen. P1 and P2 cultures are grown under similar conditions, in HYPERFlasks or HYPERStacks without platelet lysate, as needed to create the working cell bank and product for administration, respectively. Cells from P1 and P2 are removed from plastic cultureware using TrypLE (Gibco). The final product is derived from the P2 cultures which are harvested into

plasmalyte with 5% human serum albumin, washed and cryopreserved in compartment cryobags containing 50-100 million cells in a final concentration of 10% DMSO with dextran (Akron Scientific). On the day of administration, one compartment is thawed, diluted in 10-40 mLs of plasmalyte IV solution, placed in a syringe or bag and transported to the bedside for administration over 30-60 minutes.

At each passage, the cell product is characterized by assessing cell surface phenotype by flow cytometry and functional assays via T-cell proliferation and organotypic models of microglial activation. Each lot, prior to cryopreservation of P2, will also be tested for sterility, endotoxin and mycoplasma and these tests must meet specifications. For dosing, release testing after thaw and dilution will include TNCC and viability via cellometer. Patients will be dosed with $2x10^6$ hCT-MSCs/kg based on the post thaw count.

Process and Final Formulation
hCT-MSC is manufactured from a single umbilical cord tissue in a series of three steps that generate a master cell bank, a working cell bank, and the study product. The product for each step is cryopreserved in a controlled rate freezer and stored in the vapor phase of liquid nitrogen. At P2, a representative cryobag is thawed and qualified prior to the treatment of any patients with that lot of product. Testing for product release includes total nucleated cell count, viability, phenotype, functional assays, endotoxin, mycoplasma, gram stain and sterility. Each lot of cells is also tested for adventitial viruses prior to cryopreservation.

On the day of treatment, cells are thawed per SOP STCLAOP-028 JA2 and then diluted in 10-40 mLs of plasmalyte A + 5% human serum albumin (HSA). An aliquot is removed for cell count, viability, and sterility culture. If the cells are ≥70% viable, the final product volume is adjusted to deliver $2x10^6$ cells/kg to the study subject. The cells are delivered to the bedside in a syringe containing plasmalyte A, 5% HSA, and residual DMSO. Any removed cell suspension is inoculated into aerobic and anaerobic culture bottles for sterility testing. The cells have a four-hour expiry at room temperature post thaw.

The hCT-MSC final product will be released conditionally for administration to the patient after testing a post thaw cell count and viability. Final release will occur after the 14-day sterility culture period for the study product. In the event that a sterility culture turns positive after administration of the product, the organism will be identified and antibiotic sensitivities performed. The patient's family will be contacted to determine if they are symptomatic (i.e. fever or other signs of infection). Asymptomatic patients will be observed but will not be treated with antibiotics. Symptomatic patients will be evaluated and treated accordingly, with blood cultures and antibiotics as appropriate. All patients receiving a product with subsequent positive sterility test will be followed with daily contact by a study nurse for 14 days after the positive sterility test is noted.

6.3 Donor Screening for CB and hCT-MSC

Donor screening and testing is performed per Carolinas Cord Blood Bank standard operating procedures to meet all requirements in 21CFR Part 1271. The screening and testing is current with recommendations and is approved by the FDA under biological license number 1870. Maternal donors of umbilical cord blood are screened and tested for HIV-1, HIV-2, HIV-O, hepatitis B virus (HBV, surface antigen and core antibody), hepatitis C virus (HCV) antibody, Treponema pallidum (syphilis), Creutzfelds-Jakob Disease (CJD, screening only), Chagas Disease, human T-lymphotropic virus types 1

and 2 (HTLV-1, HTLV-2) and total antibodies against CMV. Nucleic acid testing for HIV-1/2/O, HBV, West Nile Virus and HCV are also performed on maternal blood. Screening for Zika virus may also be performed.

Because the cord tissue used for this study will be obtained from donors consented for cord blood donation to the Carolinas Cord Blood Bank, they will undergo donor screening and infectious disease testing per Carolinas Cord Blood Bank standard operating procedures. The cord blood-associated maternal samples and cord tissue MSC samples will be retained as reference samples for future testing as part of this study.

6.4 Packaging of Study Products

All cellular products receive a unique identification number (ISBT Demand 128 bar code) to ensure product integrity and maintain chain of custody. The clinical site or cord blood bank assigns an ISBT Demand 128 bar code label to the CB unit or hCT-MSC product, which is placed on the product bag/syringe directly or via tie tag. Products are transported from the STCL to the infusion site in a validated cooler by a trained courier.

6.5 Administration of Study Product

Patients will be arrive in clinic on the morning of their scheduled infusion. A peripheral IV will be placed either by an anesthesiologist, clinical staff or study staff and premedication with Benadryl 0.5mg/kg/dose IV and Solumedrol 0.5-1mg/kg IV will be administered. Allogeneic CB products will be administered intravenously over 5 to 25 minutes under direct physician supervision. hCT-MSC products will be administered intravenously over 30-60 minutes under direct supervision. Vital signs (heart rate, blood pressure, temperature, respiratory rate) will be checked upon arrival to the clinic and as clinically indicated. Pulse oximetry will be monitored continuously throughout the infusion and for at least 5 minutes post infusion. Patients will be hydrated with standard intravenous fluids as tolerated and observed for at least one hour post infusion.

6.6 Safety Follow-up

On Day 1 following the infusion, the participant will be seen by study staff to assess for any infusion related adverse reactions or complications. At ~2 weeks post-infusion, a member of the study team will contact the parent or guardian via phone or email to assess patient status and any adverse events. In-person safety assessments will occur at each study visit. In addition, a questionnaire (see Appendix 1) will be administered at 6, 12, and 24 months post baseline visit to assess for serious adverse events.

7.0 STUDY PLAN

7.1 Overview

Parents/Guardians who have previously contacted our program and have a child who may meet eligibility criteria for this study will be notified that this study is available. After initial contact, parents/guardians of potential research participants will have an initial phone interview with study personnel to describe the study, verify basic eligibility criteria, and confirm their interest in participation. The participant's eligibility will then be screened through review of medical records, video, laboratory testing, and imaging under a separate screening protocol.

Once all screening is complete and the patient is likely to meet study criteria, a suitable

unrelated donor CB unit will be identified at the Carolinas Cord Blood Bank, or other accredited cord blood bank. The CB unit will be screened as described in section 6. Participants will then travel to Duke for their first visit. On day 1, written informed consent will be obtained. Patient eligibility will be confirmed by a physical observation and verification of cerebral palsy diagnosis and GMFCS level. If no exclusion criteria are realized, the participant will be randomized to a treatment arm. During their first visit, all participants will have physical therapy evaluations, and a subset of patients will undergo brain MRI. Participants will have study infusions as determined by their assigned treatment arm (at baseline only for AlloCB; at 12-months only for Natural History; at baseline, 3-, and 6-months for MSCs). Participants will be evaluated the day after each infusion, and parents will be contacted for phone or email follow-up ~2 weeks after each infusion. All participants will return to Duke six (motor assessments) and 12 months (motor assessments and brain MRI) after the baseline visit. Participants on the MSC arm will also return at three months for an hCT-MSC infusion. A remote safety assessment will be performed via phone or email at 24 months post-infusion.

7.2 Patient Screening

Initial patient screening will be conducted with informed consent under a separate protocol and will include a review of medical records, videos, and initial laboratory testing. If no exclusion criteria are identified, informed consent will be obtained, and the patient will be randomized to a treatment arm. If indicated (AlloCB and Natural History arms), an unrelated donor CB unit will be identified at the Carolinas Cord Blood Bank, or other accredited cord blood bank. Participants will travel to Duke for initial evaluation. Evaluations and treatments will be conducted in the outpatient setting. A physical exam and baseline GMFCS assessment will be conducted to confirm eligibility, and the participant undergo the remainder of the study evaluations.

7.3 CB Unit Selection

For participants randomized to the AlloCB and Natural History arms, an allogeneic unrelated donor CB unit will be identified at the Carolinas Cord Blood Bank, or other accredited cord blood bank. HLA typing will be obtained on the patient, and the best available HLA-matched CB unit with a pre-cryopreservation nucleated cell dose ≥$12x10^7$ cells/kg will be chosen. We target CB units that are at least $12x10^7$ cells/kg, however, we will accept units that are at least $10x10^7$ cells/kg. When possible, at least 1 match at each HLA loci will be prioritized. An Rh negative CB unit will be selected for Rh negative female participants to avoid Rh sensitization in young females.

Once a suitable allogeneic CB unit has been deemed an acceptable match, a sample of the CB unit will be tested for potency in the Duke STCL. If results of these tests are satisfactory, the CB unit will be delivered to the Duke STCL in the frozen state.

7.4 Study Product Infusion

On the day of infusion, CB cells or hCT-MSC product will be prepared by the STCL and provided for infusion of the patient in the outpatient clinic under the supervision of the study team and Pediatric Blood and Marrow Transplant Program staff. A peripheral IV will be placed by clinical staff, anesthesia or a member of the study team. Prior to the study infusion, premedications (Benadryl and Solumedrol) will be administered. CB cells will have a four-hour expiry at room temperature post-thaw.

Allo CB infusion will be given over approximately 5-25 minutes and hCT-MSC infusions over 30-60 minutes using standard practices. The child will receive 1-1.5x maintenance

IV fluids as described below and be observed in the clinic for a minimum of one hour after the infusion. Patients will be discharged from clinic after at least one hour providing all vital signs are at their baseline and they are awake and asymptomatic with no evidence of toxicity. Patients will be evaluated by study staff the day after the infusion to assess for any infusion-related adverse reactions or complications. A phone call to parents/guardians by study staff to assess safety of the infusion will be conducted two weeks after the infusion.

Maintenance IV Fluid Rate (Holliday-Segar Method from Harriet Lane Handbook)

Body weight:	mL/kg per day	
1st 10 kg	100	divided by 24hr/day
2nd 10 kg	50	divided by 24 hr/day
each add'l kg	20	divided by 24 hr/day

If a patient has evidence of illness on the day of planned infusion, including but not limited to fever >38.5° C, vomiting, diarrhea, or respiratory distress, the infusion will be postponed.

7.5 Care During Unexpected Events

In the event that a patient develops signs or symptoms of anaphylaxis including urticaria, difficulty breathing, cough, wheezing, or vomiting during their CB infusion, the infusion will be terminated and appropriate medical therapy initiated.

7.6 Motor Assessments

Gross Motor Function Measurement-66 (GMFM-66): The GMFM-66 is a standardized observational instrument designed and validated to measure change in gross motor function over time in children with cerebral palsy. Developmental curves of expected progression have been published for children ages 2 – 12 years,[64,116] allowing for the calculation of future expected scores based on the baseline age, GMFCS level, and GMFM-66 score. The GMFM-66 consists of 66 items, divided into five categories: lying and rolling, sitting, crawling and kneeling, standing, and walking, running, and jumping. Each item is scored on a four-point Likert scale. The GMFM-66 is a subset of the GMFM-88, which contains an additional 22 items, primarily in the lying and rolling category. Both measures have been validated in children with cerebral palsy from 5 months to 16 years of age. A 5-year old child without motor disabilities is able to reach the maximum score.[117] A computer program, the Gross Motor Ability Estimator, is used to calculate the GMFM-66 total scores. The primary endpoint of this study is the difference between a child's actual and expected changes in GMFM-66 score 12 months after the initial study infusion. Control (placebo) and treated patients will be compared. When possible, the entire GMFM-88 will be performed, and subsets may be analyzed as exploratory endpoints.

Peabody Developmental Motor Scales (PDMS-2): The PDMS-II is a standardized assessment of early childhood motor development that evaluates both gross and fine motor skills. It is designed for children from birth through 5 years of age. The assessment is composed of six subtests that measure interrelated motor abilities that develop early in life (i.e., reflexes, stationary, locomotion, object manipulation, grasping, and visual-motor integration). Gross Motor Quotient, Fine Motor Quotient, and Total Motor Quotient composite scores are obtained. For this study, the Gross Motor Quotient will be obtained and analyzed as a secondary endpoint.

7.7 Functional Assessment

Pediatric Evaluation of Disability Inventory-Computer Adaptive Test (PEDI-CAT): The PEDI-CAT measures abilities in three functional domains: Daily Activities, Mobility, and Social/Cognitive. The computerized adaptive version is intended to provide an accurate and precise assessment of a child's abilities while increasing efficiency and reducing respondent burden by utilizing item response theory statistical models to determent which items are assessed within each domain based on responses to prior items.

7.8 Imaging Assessments

Participants' brain imaging obtained previously as standard of care will be reviewed by a member of the Brain Imaging Analysis Center (BIAC) team to determine if accurate anatomical image parcellation would be likely on a brain MRI. Those participants for whom usable data is likely to be obtained (estimated as approximately two-thirds of eligible participants) will undergo brain MRI with diffusion tensor imaging (DTI). Diffusion weighted images will be acquired on a 3 Tesla GE scanner (Waukesha, WI). *T1*-weighted images will be obtained with an inversion-prepared 3D fast spoiled-gradient-recalled (FSPGR) pulse sequence. These images will be analyzed to obtain measures of whole brain connectivity.

7.9 Required Evaluations

	Time Points[#]					
	Screening	Baseline	3 months	6 months	12 months	24 months
All Participants						
CBCD*, CMP*, Type & Screen*, Patient HLA typing	X					
Patient DNA sample for chimerism		X				
CBCD*, CMP*, Type & Screen*, Direct Coombs, HLA Antibody Screen (PRA), Immune Reconstitution Panel, Humoral Immune Profile		X			X	
Motor Assessments (GMFM-66, PDMS-2)		X		X	X	
Brain MRI^		X			X	
Functional Assessment (PEDI-CAT)		X		X	X	
Safety Assessment – remote						X
AlloCB Participants						
CBU potency; HLA confirmatory typing	X					
History & Physical		X		X	X	
Donor Referral Panel		X				
CBU: TNCC, viable CD34^{+} count, CFU, sterility culture, donor DNA sample for chimerism		X				
Safety Assessment – in-person, day after infusion		X				
Natural History Participants						
CBU potency; HLA confirmatory typing	X					
History & Physical		X		X	X	
Donor Referral Panel					X	
CBU: TNCC, viable CD34^{+} count, CFU, sterility culture, donor DNA sample for chimerism					X	
Safety Assessment – in-person, day after infusion					X	
MSC Participants						
History & Physical		X	X	X	X	
Donor Referral Panel		X				
CBCD, CMP			X	X		
Safety Assessment – in-person, day after infusion		X	X	X		

**CBCD and CMP may be obtained at baseline or within 6 months prior to enrollment*
^Brain MRI will be performed on patients with eligible screening neuroimaging
[#]Safety and return assessments should be performed within a month of the indicated time point.

Abbreviations: CBCD-Complete Blood Count and Differential, CMP-Complete Metabolic Panel, HLA-Human Leukocyte Antigen, GMFM-66-Gross Motor Function Measure-66, PDMS-2-Peabody Developmental Motor Scale-2, MRI-Magnetic Resonance Imaging, CBU-Cord Blood Unit, TNCC-Total Nucleated Cell Count, CFU-Colony Forming Units

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design

This study is a phase I/II, prospective, randomized, open-label trial designed to provide interval estimates of the 12-month change in motor function after treatment with AlloCB and hCT-MSC, provide additional data to the clinical trials community on the natural history of the motor function in CP over short-term (less than 1 year) time periods relevant to conduct of clinical trials, and assess the safety of repeated doses of hCT-MSC and a single dose of AlloCB in children with cerebral palsy.

Children ages 2-5 years with cerebral palsy due to hypoxic ischemic encephalopathy, stroke, or periventricular leukomalacia will be eligible to participate. All participants will ultimately be treated with an allogeneic cell product at some point during the study. Participants will be randomized (1:1:1) to one of three arms: (1) the "AlloCB" arm will receive one allogeneic CB infusion at the baseline visit; (2) the "MSC" arm will receive three hCT-MSC infusions, one each at baseline, three months, and six months; the "natural history" arm will not receive an infusion at baseline but will receive an allogeneic CB infusion at 12 months. The occurrence of adverse events will be evaluated at 3, 6, 12, and 24 months post-randomization in all participants. Motor function outcome measures will be assessed at baseline, six-months, and one-year time points in all participants. Duration of study participation will be 24 months from the time of the baseline visit. Randomization will be stratified by GMFCS Level (I/II or III/IV) and etiology of CP (Stroke vs. Other).

8.2 Accrual

It is estimated that up to 8-12 research participants will be enrolled each month and that approximately 12-15 months of accrual will be necessary to enroll 90 evaluable participants.

8.3 Study Duration

Each subject's participation in the study will be 24 months, with clinic visits occurring during the first 12 months and a remote safety assessment at 24 months. Given that accrual will take up to 15 months it is estimated that the remote safety assessment will be conducted on that last patient 39 months (3.25 years) after the study opens.

8.4 Primary and Secondary Endpoints

The primary endpoint of this study is the difference between a child's observed and expected changes in GMFM-66 score 12 months after the initial study infusion. This study will provide separate interval estimates of the mean of this outcome measure in patients assigned to the hC-MSC, AlloCB, and Natural History arms at 12-months. The secondary endpoint of this study is the number of adverse events occurring over a 12-month period post-treatment with hCT-MSC or AlloCB.

8.5 Sample Size and Power Calculations

The sample size for this study was selected to provide a high level of precision for estimating the mean of the observed minus expected 12-month change on the GMFM-66 in each of the study arms, and to provide a high probability of detecting commonly occurring adverse events after infusion with AlloCB or hCT-MSC.

As shown in the table below, a sample size of 30 patients per group provides a 95.8% probability of detecting common adverse events that occur in 10% of infusions (with hCT-MSC or AlloCB). This sample size also provides a 78.5% probability of observing events that occur in 5% of infusions, and a 26.0% probability of observing rare events that occur in 1% of infusions.

Probability of Observing One or More Events with Various Sample Sizes*

	Probability (%)*			
	N=20	**N=30**	**N=40**	**N=50**
True Probability of an Event (%)				
1	18.2	26.0	33.1	39.5
5	64.2	78.5	87.1	92.3
10	87.8	95.8	98.5	99.5
20	98.8	99.9	100.0	100.0
50	100.0	100.0	100.0	100.0

*Binomial probability of 1 or more independent events.

The sample size for this study must also support estimation of the mean observed-minus-expected GMFM-66 change score at 12 months post-intervention with MSC, AUCB, and in the Natural History arm. Thus, three interval estimates will be constructed using the t-distribution as follows.

$$\left(\bar{x} - t_{\alpha/2} * \frac{s}{\sqrt{n}}, \bar{x} + t_{\alpha/2} * \frac{s}{\sqrt{n}}\right)$$

The margin of error *E* is the confidence interval half-width:

$$E = t_{\alpha/2} * \frac{s}{\sqrt{n}}$$

The margin of error for this study was selected as 2 points with a confidence level of 95%. The following formula was solved iteratively to obtain the sample size for each treatment group.

$$N = \left(\frac{t_{\alpha/2} * s}{E}\right)^2$$

The standard deviation, *s*, was estimated using 36 participants in the CP-AC trial who met age and GMFCS inclusion criteria for the present study: 5.16 (95% CI: 4.18, 6.13).

Starting with a sample size of 20, and assuming a standard deviation of 5.16, a total of 3 iterations were required to reach a final group sample size of 28 as shown in the table below.

Iteration #	Starting N	Degrees of Freedom	$t_{\alpha/2}$	Ending N
1	20	19	2.093	29
2	29	28	2.048	28
3	28	27	2.052	28

Therefore, a group size of 28 patients allows for 95% confidence in the estimation of the mean 12-month observed-minus-expected GMFM-66 change score in one of the study arms (Natural History, MSC or AUCB) with a margin of error of no more than 2. This sample size is also concordant with what is required (N=30) for reasonable probability of detecting commonly occurring adverse events, as described above. Finally, if the standard deviation of the secondary outcome measure is as high as that indicated by the upper limit of the 95% confidence interval from the CP-AC study (6.13 points) then a sample of 126 patients allows for a margin of error no larger than ~2.5 points for each of the three interval estimates.

The total sample size for this study is therefore set at 90 evaluable patients (30 per group).

8.6 Analysis Plan

8.6.1 Analysis Populations

The following populations are defined to support analyses of the primary and secondary endpoints.

Intention to Treat Population

This population will include all enrolled and randomized participants according to their assigned treatment. The primary endpoint will be evaluated in this population.

Safety Population

The safety population defines the patients in whom the secondary endpoint will be evaluated and will include all subjects who received at least 1 infusion. Analyses of the Safety Population will be conducted using an as-treated approach, which considers each patient according the treatment actually received rather than the treatment they were assigned.

8.6.2 Timing of Analyses

The analysis of the primary and secondary outcome measures will be conducted when the last patient reaches their 12-month visit. An update will be made to the safety analyses when the last patient reaches their 24-month visit.

8.6.3 Demographics, Baseline Characteristics, and Disposition

Demographics and baseline characteristics will be summarized for all research participants and separately by randomized assignment. Characteristics to be examined include age, sex, race/ethnicity, baseline GMFM-66 score, GMFCS level, and etiology of CP. The number of participants entering and completing the study will be diagrammed using the CONSORT guidelines.

8.6.4 Analysis of the Primary and Secondary Endpoints

The occurrence of adverse events in the Safety Population will be summarized descriptively in tables and figures for all subjects and separately by treatment received. Estimates of the mean observed-minus-expected GMFM-66 change score at 12 months will be reported in the Intention to Treat Population along with 95% confidence intervals as described above.

9.0 SAFETY AND ADVERSE EVENT REPORTING

9.1 Definitions

Adverse Event (AE): An adverse event is any untoward medical occurrence associated with the use of the investigational product regardless of whether it is considered related to the investigational product.

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Grade/Severity: Grade/severity will be assessed according to CTCAE v4.0 guidelines.

Suspected Adverse Reaction: A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the investigational product and the adverse event.

Causality: The investigator will use the following question when assessing causality of an adverse event to the investigational product: "Is there a reasonable possibility that the investigational product caused the event?" An affirmative answer designates the event as a suspected adverse reaction.

9.2 Adverse Event Reporting

All adverse events reported or observed during the study beginning at the time of enrollment must be recorded. Information to be reported includes when the site became aware of the event, investigator-specified assessment of severity and relationship to study therapy, whether there is an alternative etiology, seriousness, as well as any required treatment or evaluations, and outcome. In general, investigators should report adverse events as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequelae.

Severe adverse infusion reactions (fatal, life-threatening or requiring hospitalization) will be reported within seven calendar days of receipt of the information. All fatal or life threatening SAEs will be reported by the investigator or its representatives to the FDA by telephone or fax within seven calendar days after receipt of the information, following FDA guidelines. All serious and unexpected AEs will be reported to the FDA via a written report within 15 days of receipt of the information (21 CFR 312.32). If the principal investigator assesses an event to be unrelated to the study, then the event will not require expedited reporting but will be included in the annual summary report.

The following events within 24 hours of study product infusion will be also be recorded in the e-CRF: allergic reaction/hypersensitivity, sinus bradycardia, sinus tachycardia,

hypertension, hypotension, fever in the absence of neutropenia, rigors/chills, nausea, vomiting, infection with unknown ANC, dyspnea, hypoxia, and hemoglobinuria.

9.3 Serious Adverse Event Reporting

The Principal Investigator or its representative will be responsible for telephone or fax reporting of any unexpected SAEs to the FDA. The Principal Investigator or its representative will notify the FDA by telephone or fax of any fatal or life threatening experience (expedited report) associated with the use of the study therapy as soon as possible but no later than seven calendar days after receipt of the information. Initial notification will be followed by a written report within 15 calendar days. For SAEs associated with the use of the study therapy, the Principal Investigator will notify the FDA as soon as possible, but no later than 15 days, of the initial receipt of the information. The Principal Investigator or Sub-Investigator is responsible for informing the Institutional Review Board (IRB) and DSMB of any study related and unexpected SAEs.

9.4 Eliciting Adverse Event Information

In addition to research participant observations, AEs will be documented from any data collected throughout the study including clinically significant laboratory values or physical exam findings.

9.5 Stopping Guidelines

The following stopping guidelines will be monitored during the duration of the study. The stopping guidelines will be monitored by the CRO, The EMMES Corporation, and are to be used to indicate boundaries requiring discussion by the investigators and DSMB. The study will be stopped for a safety review if:

- Any subject experiences a grade 4-5 infusion reactions within 24 hours of infusion;
  OR
- Two or more grade 4-5 adverse events determined to be temporally related by the medical safety monitor and/or the DSMB occur;
  OR
- Any subject experiences a blood stream infection within 6 months of infusion;
  OR
- Any death.

9.6 Subject Replacement

The sponsor may replace any subject who has not been dosed.

10.0 DATA SAFETY MONITORING BOARD (DSMB)

A DSMB will be formed and a charter established. Members of the DSMB will be independent of Duke University. The DSMB will be notified immediately for all SAEs directly related to the study product throughout the study. A total safety assessment will be prepared on an annual basis and forwarded to the DSMB for review as well. Policies of the DSMB will be described in the DSMB charter and signed by all members.

11.0 DATA HANDLING AND QUALITY ASSURANCE

11.1 Case Report Forms

As part of the responsibilities assumed by participating in the study, the Principal Investigator or Sub-Investigators agree to maintain adequate case histories of the

research participants treated as part of the research under this protocol. The Principal Investigator or Sub-Investigator agrees to maintain accurate CRFs and source documentation as part of the case histories.

EMMES will supply the CRF electronically (eCRF) through an electronic data entry system. All eCRFs should be completed by the clinical site. An authorized user at the clinical center completes the initial screening by entering in the research participant demographics and inclusion/exclusion criteria on the eligibility form. Upon enrollment, a form submission schedule is generated for each participant, and displayed as a grid of forms by study visit, that permits direct access to each eCRF for data entry. All CRF information is to be filled in. If an item is not available or is not applicable, an exception can be requested through the data system. Corrections to data fields can be made in the eCRF which maintains a data audit trail.

11.2 Video Recordings

Video recordings of potential subjects from parents and guardians may be submitted and used for determining study eligibility. Video recordings may also be obtained of portions of the motor evaluations if indicated with parental consent, and may include full facial features. The recordings will be used solely for analysis by the research team. They will be stored electronically on a password-protected server.

11.3 Inspection of Records

The Principal Investigator or Sub-Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the Principal Investigator or Sub-Investigator agrees to allow the Food and Drug Administration (FDA), or other regulatory agency access to all study records. The Principal Investigator or Sub-Investigators should promptly notify all relevant parties of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the both.

11.4 Study Record Retention

Study results will be retained in the patient's research record for six years after the study is completed or until the patient reaches the age of 21, whichever is longer. Essential documents should be retained until at least two years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements.

12.0 ADMINISTRATIVE ASPECTS

The following administrative items are meant to guide the Principal Investigator or Sub-Investigator in the conduct of the study but may be subject to change based on industry and government Standard Operating Procedures or Working Practice Documents or Guidelines.

12.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain research participant confidentiality. All records will be

kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the research participant's guardian except as necessary for monitoring and auditing.

The Principal Investigator or Sub-Investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study.

12.2 Institutional Review Board Approval

Federal regulations and the ICH guidelines require that approval be obtained from an IRB prior to participation of human research participants in research studies. Prior to the study onset, the protocol, informed consent, any advertisement used to recruit study patients, and any other written information regarding this study to be provided to the research participant or the research participant's legal guardian must be approved by the IRB.

All IRB approvals should be signed by the IRB Chairman or designee and must identify the IRB name and address, the clinical protocol by title and/or protocol number, and the date the approval and/or favorable opinion was granted.

The Principal Investigator or Sub-Investigator is responsible for obtaining continued review of the clinical research at intervals not exceeding one year or otherwise specified by the IRB. The Principal Investigator or Sub-Investigator must supply the Sponsor or its designee with written documentation of continued review of the clinical research.

12.3 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the research participant, must be reviewed and approved by the IRB.

12.4 Informed Consent

A written informed consent in compliance with Part 50 of Title 21 of the Code of Federal Regulations (CFR) and Institutional IRB shall be obtained from each research participant prior to entering the study or performing any unusual or non-routine procedure that involves risk to the research participant.

Before enrollment, each prospective research participant and/or his/her legal guardian will be given a full explanation of the study and be allowed to read the approved informed consent form. Once the Principal Investigator or Sub-Investigator is assured that the research participant/legal guardian understands the implications of participating in the study, the research participant/legal guardian will be asked to give consent to participate in the study by signing the informed consent form.

The Principal Investigator or Sub-Investigator shall provide a signed/dated copy of the signed informed consent to the research participant and/or legal guardian.

12.5 Protocol Violations and Deviations

The Principal Investigator or Sub-Investigator or designee must document and explain in the research participant's source documentation any deviation from the approved protocol. The Principal Investigator or Sub-Investigator may implement a deviation from,

or a change of, the protocol to eliminate an immediate hazard to study research participants without prior IRB approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendment(s) should be submitted to the IRB for review and approval, to the Sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the Sponsor and the IRB and agreed to by the Principal Investigator or Sub-Investigator. Deviations usually have an impact on individual research participants or a small group of research participants and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when there is nonadherence to the protocol that results in a significant, additional risk to the research participant, when the research participant or Principal Investigator or Sub-Investigator has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the research participant was enrolled without prior Sponsor approval, or when there is nonadherence to FDA regulations and/or ICH GCP guidelines.

The IRB should be notified of all protocol violations and deviations in a timely manner as required by the site's IRB.

12.6 Study Reporting Requirements

By participating in the study, the Principal Investigator or Sub-Investigator agrees to submit reports of serious adverse events according to the timeline and method outlined in the protocol. In addition, the Principal Investigator or Sub-Investigator agrees to submit annual reports to his/her IRB as appropriate. The Principal Investigator or Sub-Investigator also agrees to provide the Sponsor with an adequate report shortly after completion of the Principal Investigator's or Sub-Investigator's participation in the study.

12.7 Study Conduct

The Principal Investigator agrees that the study will be conducted according to the principles of the ICH E6 Guideline on GCP and the principles of the World Medical Association Declaration of Helsinki. The Principal Investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

12.8 Publications

Following completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, Duke University will be responsible to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues.

13.0 REFERENCES

1. Bax M, Goldstein M, Rosenbaum P, et al. Proposed definition and classification of cerebral palsy, April 2005. *Dev Med Child Neurol.* 2005;47(8):571-576.
2. Clark SL, Hankins GD. Temporal and demographic trends in cerebral palsy--fact and fiction. *Am J Obstet Gynecol.* 2003;188(3):628-633.
3. Jones MW, Morgan E, Shelton JE, Thorogood C. Cerebral palsy: introduction and diagnosis (part I). *J Pediatr Health Care.* 2007;21(3):146-152.
4. Kancherla V, Amendah DD, Grosse SD, Yeargin-Allsopp M, Van Naarden Braun K. Medical expenditures attributable to cerebral palsy and intellectual disability among Medicaid-enrolled children. *Res Dev Disabil.* 2012;33(3):832-840.
5. Koman LA, Paterson Smith B, Balkrishnan R. Spasticity associated with cerebral palsy in children: guidelines for the use of botulinum A toxin. *Paediatr Drugs.* 2003;5(1):11-23.
6. Mikkola K, Ritari N, Tommiska V, et al. Neurodevelopmental outcome at 5 years of age of a national cohort of extremely low birth weight infants who were born in 1996-1997. *Pediatrics.* 2005;116(6):1391-1400.
7. Bliss T, Guzman R, Daadi M, Steinberg GK. Cell transplantation therapy for stroke. *Stroke.* 2007;38(2 Suppl):817-826.
8. Wagenaar N, Nijboer CH, van Bel F. Repair of neonatal brain injury: bringing stem cell-based therapy into clinical practice. *Developmental medicine and child neurology.* 2017;59(10):997-1003.
9. Llado J, Haenggeli C, Maragakis NJ, Snyder EY, Rothstein JD. Neural stem cells protect against glutamate-induced excitotoxicity and promote survival of injured motor neurons through the secretion of neurotrophic factors. *Mol Cell Neurosci.* 2004;27(3):322-331.
10. Vendrame M, Gemma C, de Mesquita D, et al. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev.* 2005;14(5):595-604.
11. 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(10):2385-2389.
12. Arien-Zakay H, Lecht S, Bercu MM, et al. Neuroprotection by cord blood neural progenitors involves antioxidants, neurotrophic and angiogenic factors. *Exp Neurol.* 2009;216(1):83-94.
13. Carmichael ST. Plasticity of cortical projections after stroke. *Neuroscientist.* 2003;9(1):64-75.
14. Chen J, Zhang ZG, Li Y, et al. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. *Circ Res.* 2003;92(6):692-699.
15. Taguchi A, Soma T, Tanaka H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest.* 2004;114(3):330-338.
16. Staudt M, Grodd W, Gerloff C, Erb M, Stitz J, Krageloh-Mann I. Two types of ipsilateral reorganization in congenital hemiparesis: a TMS and fMRI study. *Brain.* 2002;125(Pt 10):2222-2237.
17. Carr LJ, Harrison LM, Evans AL, Stephens JA. Patterns of central motor reorganization in hemiplegic cerebral palsy. *Brain.* 1993;116 (Pt 5):1223-1247.
18. Guzzetta A, Bonanni P, Biagi L, et al. Reorganisation of the somatosensory system after early brain damage. *Clin Neurophysiol.* 2007;118(5):1110-1121.
19. You SH, Jang SH, Kim YH, Kwon YH, Barrow I, Hallett M. Cortical reorganization induced by virtual reality therapy in a child with hemiparetic cerebral palsy. *Dev Med Child Neurol.* 2005;47(9):628-635.

20. Sutcliffe TL, Gaetz WC, Logan WJ, Cheyne DO, Fehlings DL. Cortical reorganization after modified constraint-induced movement therapy in pediatric hemiplegic cerebral palsy. *J Child Neurol.* 2007;22(11):1281-1287.
21. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood.* 2013;122(4):491-498.
22. Dori I, Petrakis S, Giannakopoulou A, et al. Seven days post-injury fate and effects of genetically labelled adipose-derived mesenchymal cells on a rat traumatic brain injury experimental model. *Histol Histopathol.* 2017;32(10):1041-1055.
23. Mueller M, Oppliger B, Joerger-Messerli M, et al. Wharton's Jelly Mesenchymal Stem Cells Protect the Immature Brain in Rats and Modulate Cell Fate. *Stem Cells Dev.* 2017;26(4):239-248.
24. Pischiutta F, Brunelli L, Romele P, et al. Protection of Brain Injury by Amniotic Mesenchymal Stromal Cell-Secreted Metabolites. *Crit Care Med.* 2016;44(11):e1118-e1131.
25. Xie J, Wang B, Wang L, Dong F, Bai G, Liu Y. Intracerebral and Intravenous Transplantation Represents a Favorable Approach for Application of Human Umbilical Cord Mesenchymal Stromal Cells in Intracerebral Hemorrhage Rats. *Med Sci Monit.* 2016;22:3552-3561.
26. Cameron SH, Alwakeel AJ, Goddard L, et al. Delayed post-treatment with bone marrow-derived mesenchymal stem cells is neurorestorative of striatal medium-spiny projection neurons and improves motor function after neonatal rat hypoxia-ischemia. *Mol Cell Neurosci.* 2015;68:56-72.
27. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One.* 2012;7(10):e47559.
28. Chen S, Liu Z, Tian N, et al. Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery. *J Invasive Cardiol.* 2006;18(11):552-556.
29. Kurtzberg J, Prockop S, Prasad V, et al. Effect of Human Mesenchymal Stem Cells (Remestemcel-L) on Clinical Response and Survival Confirmed in a Large Cohort of Pediatric Patients with Severe High-Risk Steroid-Refractory Acute Graft Versus Host Disease. *Biology of Blood and Marrow Transplantation.* 2016;22(3):S59.
30. Kuci Z, Bonig H, Kreyenberg H, et al. Mesenchymal stromal cells from pooled mononuclear cells of multiple bone marrow donors as rescue therapy in pediatric severe steroid-refractory graft-versus-host disease: a multicenter survey. *Haematologica.* 2016;101(8):985-994.
31. Rupp S, Jux C, Bonig H, et al. Intracoronary bone marrow cell application for terminal heart failure in children. *Cardiol Young.* 2012;22(5):558-563.
32. Zeinaloo A, Zanjani KS, Bagheri MM, Mohyeddin-Bonab M, Monajemzadeh M, Arjmandnia MH. Intracoronary administration of autologous mesenchymal stem cells in a critically ill patient with dilated cardiomyopathy. *Pediatr Transplant.* 2011;15(8):E183-186.
33. Zeinaloo A, Zanjani KS, Khosroshahi AG. Further follow up of the cardiomyopathic patient treated by intracoronary administration of autologous mesenchymal stem cells. *Pediatr Transplant.* 2011;15(4):442.
34. Cai J, Wu Z, Xu X, et al. Umbilical Cord Mesenchymal Stromal Cell With Autologous Bone Marrow Cell Transplantation in Established Type 1 Diabetes: A Pilot Randomized Controlled Open-Label Clinical Study to Assess Safety and Impact on Insulin Secretion. *Diabetes Care.* 2016;39(1):149-157.

35. Hu J, Yu X, Wang Z, et al. Long term effects of the implantation of Wharton's jelly-derived mesenchymal stem cells from the umbilical cord for newly-onset type 1 diabetes mellitus. *Endocr J.* 2013;60(3):347-357.
36. Chang YS, Ahn SY, Yoo HS, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. *J Pediatr.* 2014;164(5):966-972 e966.
37. Vendrame M, Cassady J, Newcomb J, 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(10):2390-2395.
38. Chen J, Sanberg PR, Li Y, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke.* 2001;32(11):2682-2688.
39. Meier C, Middelanis J, Wasielewski B, et al. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. *Pediatr Res.* 2006;59(2):244-249.
40. Nan Z, Grande A, Sanberg CD, Sanberg PR, Low WC. Infusion of human umbilical cord blood ameliorates neurologic deficits in rats with hemorrhagic brain injury. *Ann N Y Acad Sci.* 2005;1049:84-96.
41. Taguchi A, Soma T, Tanaka H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest.* 2004;114(3):330-338.
42. Derrick M, Drobyshevsky A, Ji X, Tan S. A model of cerebral palsy from fetal hypoxia-ischemia. *Stroke.* 2007;38(2 Suppl):731-735.
43. Drobyshevsky A, Cotten CM, Shi Z, et al. Human Umbilical Cord Blood Cells Ameliorate Motor Deficits in Rabbits in a Cerebral Palsy Model. *Developmental neuroscience.* 2015.
44. 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(3):387-393.
45. Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology.* 2001;56(12):1666-1672.
46. Shen LH, Li Y, Chen J, et al. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. *J Cereb Blood Flow Metab.* 2007;27(1):6-13.
47. Willing AE, Lixian J, Milliken M, et al. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *J Neurosci Res.* 2003;73(3):296-307.
48. Yang B, Migliati E, Parsha K, et al. Intra-arterial delivery is not superior to intravenous delivery of autologous bone marrow mononuclear cells in acute ischemic stroke. *Stroke.* 2013;44(12):3463-3472.
49. Donega V, van Velthoven CT, Nijboer CH, et al. Intranasal mesenchymal stem cell treatment for neonatal brain damage: long-term cognitive and sensorimotor improvement. *PLoS One.* 2013;8(1):e51253.
50. Chua CO, Chahboune H, Braun A, et al. Consequences of intraventricular hemorrhage in a rabbit pup model. *Stroke.* 2009;40(10):3369-3377.
51. Mukai T, Mori Y, Shimazu T, et al. Intravenous injection of umbilical cord-derived mesenchymal stromal cells attenuates reactive gliosis and hypomyelination in a neonatal intraventricular hemorrhage model. *Neuroscience.* 2017;355:175-187.
52. Beam D, Poe MD, Provenzale JM, et al. Outcomes of unrelated umbilical cord blood transplantation for X-linked adrenoleukodystrophy. *Biol Blood Marrow Transplant.* 2007;13(6):665-674.
53. Prasad VK, Mendizabal A, Parikh SH, 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(7):2979-2989.

54. Boelens JJ. Trends in haematopoietic cell transplantation for inborn errors of metabolism. *J Inherit Metab Dis.* 2006;29(2-3):413-420.
55. Staba SL, Escolar ML, Poe M, et al. Cord-blood transplants from unrelated donors in patients with Hurler's syndrome. *N Engl J Med.* 2004;350(19):1960-1969.
56. Provenzale JM, Escolar M, Kurtzberg J. Quantitative analysis of diffusion tensor imaging data in serial assessment of Krabbe disease. *Ann N Y Acad Sci.* 2005;1064:220-229.
57. Martin PL, Carter SL, Kernan NA, et al. Results of the cord blood transplantation study (COBLT): outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with lysosomal and peroxisomal storage diseases. *Biol Blood Marrow Transplant.* 2006;12(2):184-194.
58. Escolar ML, Poe MD, Provenzale JM, et al. Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. *N Engl J Med.* 2005;352(20):2069-2081.
59. Kurtzberg J KB, Stephens C, Snyder EY. Umbilical cord blood cells engraft and differentiate in neural tissues after human transplantation. *Biol Blood Marrow Transplant.* 2003(9):128a.
60. Hoogerbrugge PM, Suzuki K, Poorthuis BJ, Kobayashi T, Wagemaker G, van Bekkum DW. Donor-derived cells in the central nervous system of twitcher mice after bone marrow transplantation. *Science.* 1988;239(4843):1035-1038.
61. Sun J, Allison J, McLaughlin C, et al. Differences in quality between privately and publicly banked umbilical cord blood units: a pilot study of autologous cord blood infusion in children with acquired neurologic disorders. *Transfusion.* 2010;50(9):1980-1987.
62. Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of Autologous Cord Blood Cells for Infants with Hypoxic-Ischemic Encephalopathy. *The Journal of Pediatrics.* 2013.
63. Sun JM, Song AW, Case LE, et al. Effect of Autologous Cord Blood Infusion on Motor Function and Brain Connectivity in Young Children with Cerebral Palsy: A Randomized, Placebo-Controlled Trial. *Stem cells translational medicine.* 2017;6(12):2071-2078.
64. Hanna SE, Bartlett DJ, Rivard LM, Russell DJ. Reference curves for the Gross Motor Function Measure: percentiles for clinical description and tracking over time among children with cerebral palsy. *Phys Ther.* 2008;88(5):596-607.
65. Englander ZA, Sun J, Laura C, Mikati MA, Kurtzberg J, Song AW. Brain structural connectivity increases concurrent with functional improvement: evidence from diffusion tensor MRI in children with cerebral palsy during therapy. *Neuroimage Clin.* 2015;7:315-324.
66. Min K, Song J, Kang JY, et al. Umbilical cord blood therapy potentiated with erythropoietin for children with cerebral palsy: a double-blind, randomized, placebo-controlled trial. *Stem Cells.* 2013;31(3):581-591.
67. Kang M, Min K, Jang J, et al. Involvement of Immune Responses in the Efficacy of Cord Blood Cell Therapy for Cerebral Palsy. *Stem Cells Dev.* 2015;24(19):2259-2268.
68. Romanov YA, Tarakanov OP, Radaev SM, et al. Human allogeneic AB0/Rh-identical umbilical cord blood cells in the treatment of juvenile patients with cerebral palsy. *Cytotherapy.* 2015.
69. Wang L, Ji H, Zhou J, et al. Therapeutic potential of umbilical cord mesenchymal stromal cells transplantation for cerebral palsy: a case report. *Case Rep Transplant.* 2013;2013:146347.
70. Wang X, Cheng H, Hua R, et al. Effects of bone marrow mesenchymal stromal cells on gross motor function measure scores of children with cerebral palsy: a preliminary clinical study. *Cytotherapy.* 2013;15(12):1549-1562.
71. Wang X, Hu H, Hua R, et al. Effect of umbilical cord mesenchymal stromal cells on motor functions of identical twins with cerebral palsy: pilot study on the correlation of efficacy and hereditary factors. *Cytotherapy.* 2015;17(2):224-231.

72. Dulamea AO. The potential use of mesenchymal stem cells in stroke therapy--From bench to bedside. *J Neurol Sci.* 2015;352(1-2):1-11.
73. Mays R, Deans R. Adult adherent cell therapy for ischemic stroke: clinical results and development experience using MultiStem. *Transfusion.* 2016;56(4):6S-8S.
74. Lv YT, Zhang Y, Liu M, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *J Transl Med.* 2013;11:196.
75. Bonab MM, Sahraian MA, Aghsaie A, et al. Autologous mesenchymal stem cell therapy in progressive multiple sclerosis: an open label study. *Curr Stem Cell Res Ther.* 2012;7(6):407-414.
76. Karussis D, Karageorgiou C, Vaknin-Dembinsky A, et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. *Arch Neurol.* 2010;67(10):1187-1194.
77. Yamout B, Hourani R, Salti H, et al. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. *J Neuroimmunol.* 2010;227(1-2):185-189.
78. Connick P, Kolappan M, Crawley C, et al. Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. *Lancet Neurol.* 2012;11(2):150-156.
79. Venkataramana NK, Kumar SK, Balaraju S, et al. Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. *Transl Res.* 2010;155(2):62-70.
80. Drela K, Lech W, Figiel-Dabrowska A, et al. Enhanced neuro-therapeutic potential of Wharton's Jelly-derived mesenchymal stem cells in comparison with bone marrow mesenchymal stem cells culture. *Cytotherapy.* 2016;18(4):497-509.
81. Park SJ, Kim HJ, Kim W, et al. Tumorigenicity Evaluation of Umbilical Cord Blood-derived Mesenchymal Stem Cells. *Toxicol Res.* 2016;32(3):251-258.
82. Li Q, Chen CF, Wang DY, et al. Changes in growth factor levels in the cerebrospinal fluid of autism patients after transplantation of human umbilical cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells. *Genet Mol Res.* 2016;15(2).
83. Xie B, Gu P, Wang W, et al. Therapeutic effects of human umbilical cord mesenchymal stem cells transplantation on hypoxic ischemic encephalopathy. *Am J Transl Res.* 2016;8(7):3241-3250.
84. Wang L, Zhang Y, Li H, et al. Clinical Observation of Employment of Umbilical Cord Derived Mesenchymal Stem Cell for Juvenile Idiopathic Arthritis Therapy. *Stem Cells Int.* 2016;2016:9165267.
85. Chen H, Niu JW, Ning HM, et al. Treatment of Psoriasis with Mesenchymal Stem Cells. *Am J Med.* 2016;129(3):e13-14.
86. Hu J, Wang Y, Gong H, et al. Long term effect and safety of Wharton's jelly-derived mesenchymal stem cells on type 2 diabetes. *Exp Ther Med.* 2016;12(3):1857-1866.
87. Hua R, Li P, Wang X, et al. Evaluation of Somatosensory Evoked Potential and Pain Rating Index in a Patient with Spinal Cord Injury Accepted Cell Therapy. *Pain Physician.* 2016;19(4):E659-666.
88. Kim HS, Lee JH, Roh KH, Jun HJ, Kang KS, Kim TY. Clinical Trial of Human Umbilical Cord Blood-derived Stem Cells for the Treatment of Moderate-to-Severe Atopic Dermatitis: Phase I/IIa Studies. *Stem Cells.* 2016.
89. Qin HL, Zhu XH, Zhang B, Zhou L, Wang WY. Clinical Evaluation of Human Umbilical Cord Mesenchymal Stem Cell Transplantation After Angioplasty for Diabetic Foot. *Exp Clin Endocrinol Diabetes.* 2016;124(8):497-503.

90. Wang D, Li J, Zhang Y, et al. Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study. *Arthritis Res Ther.* 2014;16(2):R79.
91. Wang D, Niu L, Feng X, et al. Long-term safety of umbilical cord mesenchymal stem cells transplantation for systemic lupus erythematosus: a 6-year follow-up study. *Clin Exp Med.* 2016.
92. Zhao XF, Xu Y, Zhu ZY, Gao CY, Shi YN. Clinical observation of umbilical cord mesenchymal stem cell treatment of severe systolic heart failure. *Genet Mol Res.* 2015;14(2):3010-3017.
93. Liang J, Wang F, Wang D, et al. Transplantation of mesenchymal stem cells in a laryngeal carcinoma patient with radiation myelitis. *Stem Cell Res Ther.* 2015;6:213.
94. Li X, Hu YD, Guo Y, et al. Safety and efficacy of intracoronary human umbilical cord-derived mesenchymal stem cell treatment for very old patients with coronary chronic total occlusion. *Curr Pharm Des.* 2015;21(11):1426-1432.
95. Rajput BS, Chakrabarti SK, Dongare VS, Ramirez CM, Deb KD. Human Umbilical Cord Mesenchymal Stem Cells in the Treatment of Duchenne Muscular Dystrophy: Safety and Feasibility Study in India. *J Stem Cells.* 2015;10(2):141-156.
96. Gu F, Wang D, Zhang H, et al. Allogeneic mesenchymal stem cell transplantation for lupus nephritis patients refractory to conventional therapy. *Clin Rheumatol.* 2014;33(11):1611-1619.
97. Pan Y, Wang X, Wang C, et al. Extensive bone marrow necrosis resolved by allogeneic umbilical cord blood mesenchymal stem cell transplantation in a chronic myeloid leukemia patient. *Bone Marrow Transplant.* 2015;50(9):1265-1268.
98. Wu QL, Liu XY, Nie DM, et al. Umbilical cord blood-derived mesenchymal stem cells ameliorate graft-versus-host disease following allogeneic hematopoietic stem cell transplantation through multiple immunoregulations. *J Huazhong Univ Sci Technolog Med Sci.* 2015;35(4):477-484.
99. Zhu L, Wang Z, Zheng X, et al. Haploidentical hematopoietic stem cell transplant with umbilical cord-derived multipotent mesenchymal cell infusion for the treatment of high-risk acute leukemia in children. *Leuk Lymphoma.* 2015;56(5):1346-1352.
100. Miao X, Wu X, Shi W. Umbilical cord mesenchymal stem cells in neurological disorders: A clinical study. *Indian J Biochem Biophys.* 2015;52(2):140-146.
101. Li P, Cui K, Zhang B, et al. Transplantation of human umbilical cord-derived mesenchymal stems cells for the treatment of Becker muscular dystrophy in affected pedigree members. *Int J Mol Med.* 2015;35(4):1051-1057.
102. Wang Y, Chen F, Gu B, Chen G, Chang H, Wu D. Mesenchymal stromal cells as an adjuvant treatment for severe late-onset hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. *Acta Haematol.* 2015;133(1):72-77.
103. Cheng H, Liu X, Hua R, et al. Clinical observation of umbilical cord mesenchymal stem cell transplantation in treatment for sequelae of thoracolumbar spinal cord injury. *J Transl Med.* 2014;12:253.
104. Li JF, Zhang DJ, Geng T, et al. The potential of human umbilical cord-derived mesenchymal stem cells as a novel cellular therapy for multiple sclerosis. *Cell Transplant.* 2014;23 Suppl 1:S113-122.
105. Kong D, Zhuang X, Wang D, et al. Umbilical cord mesenchymal stem cell transfusion ameliorated hyperglycemia in patients with type 2 diabetes mellitus. *Clin Lab.* 2014;60(12):1969-1976.
106. Wang S, Cheng H, Dai G, et al. Umbilical cord mesenchymal stem cell transplantation significantly improves neurological function in patients with sequelae of traumatic brain injury. *Brain Res.* 2013;1532:76-84.

107. Wang L, Li J, Liu H, et al. Pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. *J Gastroenterol Hepatol.* 2013;28 Suppl 1:85-92.
108. Zhang Z, Fu J, Xu X, et al. Safety and immunological responses to human mesenchymal stem cell therapy in difficult-to-treat HIV-1-infected patients. *AIDS.* 2013;27(8):1283-1293.
109. Jin JL, Liu Z, Lu ZJ, et al. Safety and efficacy of umbilical cord mesenchymal stem cell therapy in hereditary spinocerebellar ataxia. *Curr Neurovasc Res.* 2013;10(1):11-20.
110. Wu KH, Tsai C, Wu HP, Sieber M, Peng CT, Chao YH. Human Application of Ex-Vivo Expanded Umbilical Cord-Derived Mesenchymal Stem Cells: Enhance Hematopoiesis after Cord Blood Transplantation. *Cell Transplant.* 2013.
111. Jiang Y, Zhu W, Zhu J, Wu L, Xu G, Liu X. Feasibility of delivering mesenchymal stem cells via catheter to the proximal end of the lesion artery in patients with stroke in the territory of the middle cerebral artery. *Cell Transplant.* 2013;22(12):2291-2298.
112. Shi M, Zhang Z, Xu R, et al. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. *Stem Cells Transl Med.* 2012;1(10):725-731.
113. Zhang Z, Lin H, Shi M, et al. Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. *J Gastroenterol Hepatol.* 2012;27 Suppl 2:112-120.
114. Qu Z, Mi S, Fang G. [Clinical study on treatment of bone nonunion with MSCs derived from human umbilical cord]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi.* 2009;23(3):345-347.
115. Rubinstein P, Dobrila L, Rosenfield RE, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci U S A.* 1995;92(22):10119-10122.
116. Rosenbaum PL, Walter SD, Hanna SE, et al. Prognosis for gross motor function in cerebral palsy: creation of motor development curves. *Jama.* 2002;288(11):1357-1363.
117. Russell DJ, Rosenbaum PL, Wright M, Avery LM. *Gross Motor Function Measure (GMFM-66 & GMFM-88) User's Manual.* London: Mac Keith Press; 2013.

14.0 APPENDICIES

Appendix 1: Safety Questionnaire

SAFETY QUESTIONNAIRE

Patient Name: _____

Date completed: __ __/__ __/__ __ __ __
m m d d y y y y

Please answer the following items regarding your child to the best of your ability. Some of the questions may be the same as the questions you answered previously.

Since your child's last study visit, did any of the following occur?

1. Was your child hospitalized?
   - θ Yes; if so, why? _____
   - θ No
2. Did your child develop a tumor?
   - θ Yes
   - θ No
3. Did your child develop abnormal skin lesions?
   - θ Yes
   - θ No
4. Was your child diagnosed with cancer?
   - θ Yes
   - θ No
5. Did your child have a serious infection?
   - θ Yes
   - θ No
6. Was your child diagnosed with an autoimmune disease?
   - θ Yes
   - θ No
7. Did your child require a blood transfusion?
   - θ Yes
   - θ No
8. Has your child developed any other health problems not described above?
   - θ Yes; if so, what? _____
   - θ No
9. Has your child developed any new onset seizures?
   - θ Yes
   - θ No
10. Has your child started any new medications?

θ Yes; if so, which ones? _____

θ No

11. Has your child's diet changed (i.e. started/stopped a special type of diet)?

θ Yes; if so, how? _____

θ No

12. Have you noticed an increase in any of the following in your child since their last visit? Select all that apply.

Appetite (increased or decreased)	θ Yes	θ No
Fatigue	θ Yes	θ No
Headache	θ Yes	θ No
Restlessness	θ Yes	θ No
Tremor	θ Yes	θ No

13. Has your child participated in any of the following:

Other cell therapy	θ Yes	θ No
Immune therapy (i.e. IVIG, steroids)	θ Yes	θ No
Other clinical trials	θ Yes	θ No

If yes, please describe: _____

Please describe any changes you have seen in your child since receiving their first infusion:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Please rate your child's development in the following domains since receiving their first infusion (Circle the number that corresponds best.):

1	2	3	4	5
Slowed down	*Stayed about the same*	*Improved a little more than expected*	*Improved more than expected*	*Improved much more than expected*

1. **Fine Motor** (use of hands/fingers for activities like holding toys, playing, drawing, eating, etc)

1	2	3	4	5

2. **Gross Motor** (use of big muscles for activities like head control, rolling, crawling, walking, running or climbing)

1	2	3	4	5

3. **Receptive Language** (language listening and understanding, following spoken requests, etc.)

1	2	3	4	5

4. **Expressive Language** (speaking skills: use of signs, words, sentences, etc.)

1	2	3	4	5

5. **Cognitive Skills** (thinking, learning, reasoning, and problem solving)

1	2	3	4	5

6. **Adaptive and Self Help Skills** (feeding, dressing, toileting, etc.)

1	2	3	4	5

7. **Social Skills and Behavior** (interpersonal skills, peer skills, emotions, behaviors)

1	2	3	4	5