Autologous Cell Therapies for Cerebral Palsy-Chronic (ACT for CP)

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Phase II

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TABLE OF CONTENTS

1. STUDY OBJECTIVES

This clinical trial studies autologous cell therapies in subjects with cerebral palsy (CP). We aim to compare the effects of two specific autologous cell therapies - bone marrow derived mononuclear cells (BMMNCs) versus human umbilical cord blood cells (hUCBs). Although both cellular products have an established safety record in children in the setting of bone marrow transplantation as well as a more limited experience with traumatic brain injury, the safety of these cellular therapies in subjects with CP will be evaluated in this study. This study will examine the following broad aims:

1.1 Primary Objective

To determine if autologous cells using either BMMNCs or hUCBs are safe to administer in children with CP.
Primary safety outcome measures:

1. In-hospital infusion toxicity: pulmonary and hepatic function; new seizures, hemorrhagic lesions or ischemic lesions on imaging.
2. Long-term safety: development of new mass lesions or other pathological structural changes; worsening neurological status.

1.2 Secondary Objective

To determine if late functional outcomes are improved following the administration of autologous cells compared with subjects in the control group.
Efficacy outcome measures:

1. aMRI volumetric white matter, grey matter, and CSF space volumes at Baseline, 1 year and 2 years post-treatment. Detailed volumetric analysis of the corpus callosum and white matter tract such as the corticospinal tract will be measured (fractional anisotropy and mean diffusivity) as well. Specific white matter tract analysis will be identified at baseline MRI and correlated with motor function studies as the primary lesion of interest. Total volumes and specific tract lesions will be analyzed and correlated with functional outcomes.
2. The following functional outcomes studies will be performed at baseline, 6 months, 1 year after infusion, and 2 year after infusion.
    a. Gross Motor Function Measures
    b. Psychological Assessment Batteries

2. BACKGROUND

2.1 Rationale for Initiating Protocol in Children

Cerebral palsy (CP) is a worldwide major health problem, caused by brain damage during pregnancy, delivery or the immediate postnatal period. According to the Centers for Disease Control (2011), cerebral palsy is the most common motor disability in childhood, affecting approximately 1 in 303 8-year-old children in the US. It is estimated that the average lifetime costs associated with cerebral palsy are approximately $921,000 per person. There are no effective treatments for CP. Our stem cell research team in neurological disorders, in addition to other research centers, has shown that

various types of cell therapies are safe and may enhance recovery from brain injuries such as stroke and TBI. The mechanisms underlying how certain cell therapies exert beneficial effects are likely multi-factorial and include the ability to modulate the inflammatory response after brain injury and to release factors that stimulate intrinsic brain repair processes such as neurogenesis, angiogenesis, and synaptogenesis. In fact, stem cells may adapt the profile of different repair promoting factors they secrete depending on the timing, type, and chronicity of the brain injury.

2.2 Pre-Clinical Data

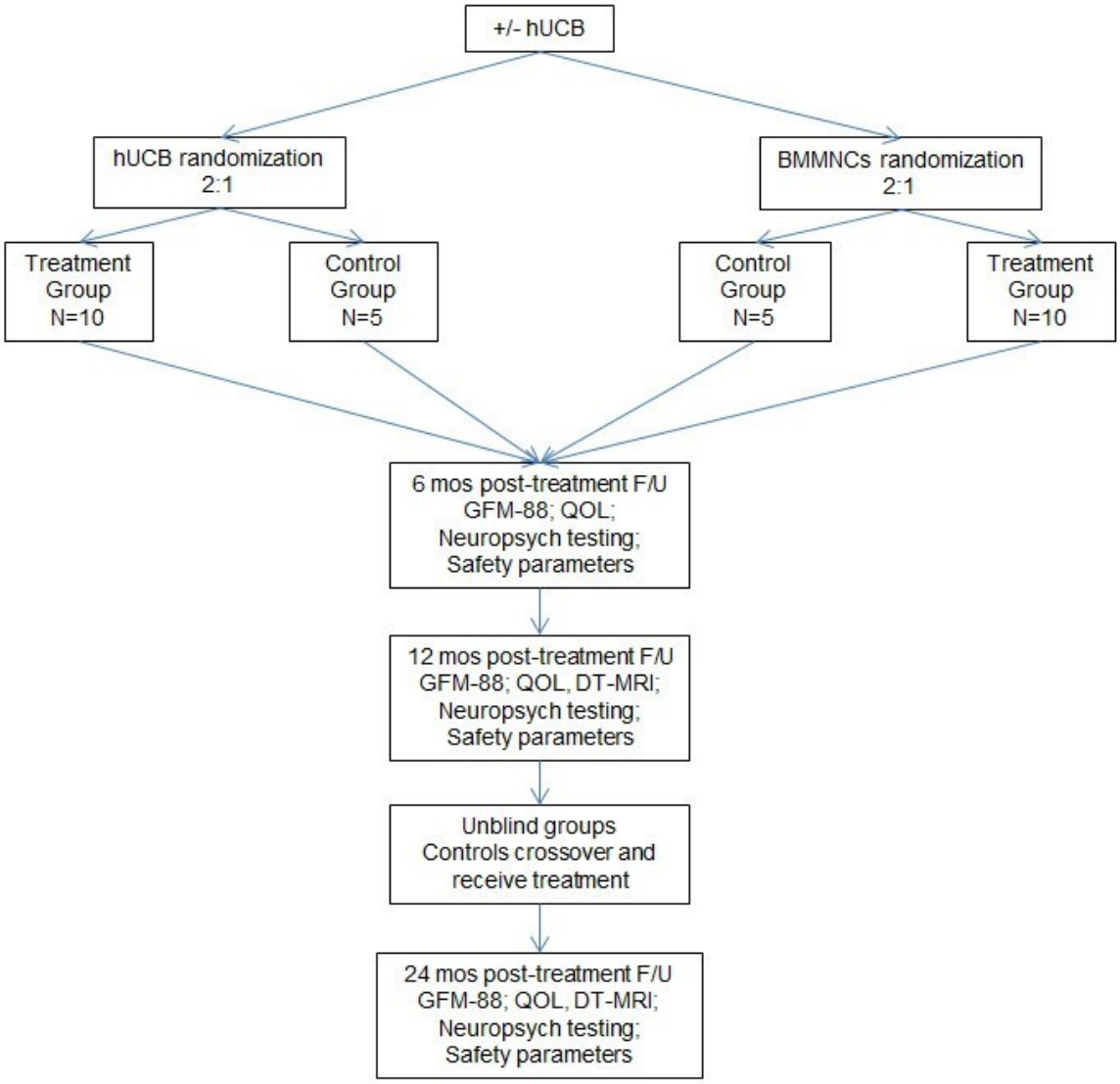
Cerebral Palsy and potential therapeutic time window: Stem cells and other types of cell therapies have been extensively studied in animal models of cerebral hypoxia-ischemia that simulate the conditions leading to cerebral palsy. Stem cells have been found in animal models of neonatal hypoxia to improve outcome when administered up to 10 days after injury and up to 30 days after stroke in young adult rats. One month in a rat would translate to several years in humans. Furthermore, one of the principal brain cell types that regulates inflammation after brain injury are microglia, which recent studies have shown remain activated for years after brain injury in patients (Ramlackhansingh, 2011). This chronic inflammatory response likely evolves over time and is associated with severe cognitive impairments. Our research has identified that some types of cell therapies target and modulate microglia to reduce ongoing damage after brain injury. Overall, these data raise the possibility that stem cells may carry therapeutic potential in children chronically disabled with cerebral palsy.

A large body of data indicates that both umbilical cord and bone marrow cells are attractive and highly promising sources of cell therapies for patients with brain injuries. The intravenous administration of mononuclear cells from umbilical cord and bone marrow has been shown in multiple animal models of cerebral ischemia to improve neurological outcome (Meier, 2006). A pilot study has already shown that autologous cord blood is safe to administer in infants with acquired neurological disorders (Sun, 2010). Pre-clinical and Phase 1 clinical studies have shown that BMMNC treatment for neurological injury reduces the neuro-inflammatory response and may preserve neural tissue. The precise mechanism of action and potential time-window of treatment has not been defined. Data in rodent models of stroke suggests that the 30-day time window may be the limit of potential effectiveness (de Vasconcelos dos Santos, 2010). In contrast, longitudinal imaging studies of patients after moderate TBI have shown progressive axonopathy, detectable by DTI in the regions of interest described above (callosal volumes, FA, MD) from the 2 week post-injury time point to 6 months post-injury (Kumar, 2009). This progressive, ongoing evolution of the imaging findings suggests progressive demyelination and gliosis (Kraus, 2007). Preservation of white matter volumes and callosal volumes correlate positively and significantly with neurocognitive outcome. Given the safety findings in our pediatric Phase 1 study, we have designed this study to use an imaging based/ structural outcome variable as our primary measure of efficacy.

3. STUDY DESIGN

This is a randomized, blinded, placebo-controlled, cross-over study designed to treat CP in pediatric subjects with an IV infusion of autologous bone marrow mononuclear cells or autologous umbilical cord blood cells. A total of 30 subjects (15/arm) will be enrolled into the study. Subjects will be randomized 2:1 (treatment: sham). All subjects will return for follow-up at 6 months, 12 months, and 24 months post-treatment. At the 12 months post-treatment visit, those subjects who originally received the sham will be offered the BMMNC or hUCB treatment (Figure 1).

Figure 1.



Thirty subjects will provide a preliminary assessment of potential SAEs with a 10 to 15% incidence that we would be able to detect with reasonable confidence. The sample size would also allow us to acquire point estimates of different events that we would use for a larger efficacy study. The sample size determination is based on the historical treatment effect size of 15% decrease in gray and white matter volumes. Preservation

of this volume is based on the Phase 1 pediatric acute treatment of severe TBI using autologous bone marrow derived mononuclear cells as compared to historical control volumetric analyses by Wilde et al. (Wilde, E, et. al. 2005; Cox, C et al., 2011.)

Drug/Biologic: A single dose of intravenously administered autologous BMMNC or hUCB. The BMMNC dose will be $6X10^6$ cells/kg body weight infused over 15 minutes at a 1 million cells/ml concentration. For the hUCB arm, the minimum acceptable dose will be $2X10^6$cells/kg body weight based on rodent data (Lu, 2001), and the maximum allowable dose will be $10X10^6$cells/kg. The target dose will be the maximum available dose within this range. The bone marrow harvest, the hUCB reanimation, cell processing and product infusion will occur at Children's Memorial Hermann Hospital and the Griffin Stem Cell Laboratory.

Placebo: 0.9% saline will be infused in an identical syringe over the same rate as if receiving a cell infusion, (See "Blinding of Placebo Infusion").

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

1. Children with diagnosis of Cerebral Palsy (spastic CP due to periventricular white matter damage or neonatal brain injury from perinatal stroke or intra-ventricular hemorrhage)
2. Gross Motor Function Classification Score level II-V
3. Ages 24 months to 10 years
4. English speaking, if verbal
5. Ability to travel to Houston for treatment and follow-up

4.2 Exclusion Criteria

1. Known history of:
    a. Intractable seizures
    b. Traumatic brain injury
    c. Genetic disorder (as demonstrated by newborn screening or genetic diagnostic testing)
    d. Recently treated or current infection
    e. Renal insufficiency or altered renal function (as defined by serum creatinine > 1.5 mg/dl at screening)
    f. hepatic disease or altered liver function (as defined by SGPT > 150 U/L [non-contusion related], and/or T. Bilirubin >1.3 mg/dL at screening)
    g. HIV+ (as demonstrated by positive blood test)
    h. Immunosuppression (as defined by WBC <3,000 cells/ml at screening)
    i. Infectious related neurological injury
    j. Sensitivity to Ethylene Oxide (EtO) [found in fumigants and disinfectants]
2. If Athetoid CP diagnosis, other etiologies such as degenerative, mitochondrial, and metabolic disorders must be excluded, and the outcome assessments must be able to be conducted to assess for potential treatment effects

3. Normal brain MRI
4. Evidence of acute illness at the time of infusion, such as, but not limited to, fever (temperature > 37.5 C), vomiting, diarrhea, wheezing or crackles
5. Progressing neurological disease (as defined by Batten Disease, Leukodystrophies, Metabolic disorders, Mitochondrial disorders, Neurotransmitter disorders)
6. Microcephaly, macrocephaly, cortical malformations, genetic disorders of dysgenesis brain malformations due to infection or metabolic disorders
7. Pulmonary disease requiring ventilator support
8. If hUCB candidate, banked cord cells totaling <10 million/kg
9. If hUCB candidate, any positive maternal infectious disease test (Hepatitis A/B, HIV 1, HIV 2, HTLV 1, HTLV 2, and Syphilis), (r*efer to Appendix D*)
10. If hUCB candidate, cord blood sample contamination
11. Participation in a concurrent interventional research study
12. Unwillingness to return for follow-up visits
13. Contraindications to brain MRI (metal implants, dental braces, etc.)
14. Any subject that the investigators, feel in their opinion the study intervention is unlikely to benefit the subject will be a screen failure
15. Previous participating in other stem cell treatment or research studies

5. STUDY INTERVENTIONS

5.1 Bone Marrow Arm

5.1.1 Subject Recruitment

Fifteen pediatric subjects without banked umbilical cord blood meeting eligibility criteria will be enrolled into the bone marrow arm of the study. The parent(s)/LAR of potential subjects will contact the research team at The University of Texas Health Science Center at Houston. During this telephone call, an overview of the study and basic eligibility requirements will be reviewed. If eligibility requirements are met and the parent(s)/LAR willing to participate, instructions will be provided on forwarding medical records to the research team for review and a consent and authorization for release of information (for contact with the subjects primary care provider [PCP]) will be sent to the parent(s)/LAR. After receipt of medical records and contact with the PCP (if indicated) a telephone conference call will be scheduled with the parent(s)/LAR. During the call, study procedures and the informed consent document will be reviewed. Time will be given to answer questions. The parent(s)/LAR will not be pressured to make a decision on participation during the call. Parent(s)/LAR choosing to participate in the study will be asked to return the signed informed consent in the postage paid envelope provided.

After receipt of the signed informed consent document, the parent(s)/LAR will be contacted by the research team to schedule the baseline/treatment visit. The parent(s)/LAR will be responsible for making all travel arrangements.

5.1.2 Randomization

The randomization sequence will be computer-generated. The subject will be assigned to the treatment or control arm in a 2:1 ratio using sequentially numbered, opaque, sealed envelopes prepared by a member of the Center for Surgical Trials and Evidence-based Practice (C-STEP) not involved in the clinical trial.

5.1.3 Baseline Visit and Bone Marrow (Placebo) Harvest/Infusion

The outpatient baseline visit will be conducted in the Pediatric Outpatient Surgery Clinic. At the baseline outpatient visit, the signed informed consent document will be reviewed with the parent(s)/LAR and assent obtained from the subjects if they are able to do so. During this 1 day outpatient visit, subjects will undergo baseline assessments including physical and neurological exams, laboratory tests, chest radiograph, oxygen saturation monitoring, psychological testing, and quality of life measurements. The neuroexam will be videotaped for blinded assessments of dystonia using standardized scales validated for use on children with CP. The research team will review information from the baseline assessments to ensure the subject remains eligible for participation and that there are no unanticipated reasons to exclude the subject, (such as recent infection). After completing the outpatient baseline visit assessments, eligible subjects will be admitted to a Children's Memorial Hermann Hospital pediatric unit.

The bone marrow harvest will be performed under sedation (Propofol) and local anesthetic before or immediately after the baseline DTMRI. The subject may be log-rolled to a 45 degree angle for access to the posterior iliac crest or remain supine to access anterior iliac crest. The bone marrow harvest will be performed under aseptic conditions. The bone marrow harvest is performed using either an 11-gauge needle or 15-gauge needle inserted into the iliac crest(s). Approximately 2 – 3 mL (< 40 kg body weight) to 5 – 6 mL (> 40 kg body weight) of bone marrow aspirate will be obtained and transferred in as sterile manner in a validated Coleman® cooler at ambient temperature to the Griffin Stem Cell Laboratory (GSCL). An additional blood sample will be drawn for infectious disease testing that includes HIV 1 and II, Hepatitis B and C, HTLV 1 and II, Syphilis, CMV, West Nile Virus, and ABO blood type. Transportation, processing, assessing and quality assurance at GSCL are described in the Chemistry & Manufacturing section of the IND. Approximately 6 – 7 hours after bone marrow aspiration, subjects will receive the BMMNC via peripheral or central intravenous infusion in a time not to exceed 30 minutes. The product will be infused within 30 minutes of receipt at Children's Memorial Hermann Hospital, and will remain in the cooler until ready for infusion.

5.1.4 Sham Harvest Procedure

A 3mL sample of peripheral blood will be collected immediately prior to the harvest sham procedure. The sham harvest will be performed under sedation (Propofol) with a local anesthetic used at the site. The subject may be log-rolled to a 45 degree angle for access to the posterior iliac crest or remain supine to access the anterior iliac crest. An 11-gauge or 15-gauge needle will be used to puncture through the skin, but will not be inserted into the iliac bone. The puncture site will then be steri-stripped closed and covered with an identical external bandage. No bone marrow aspiration or harvest will

take place. The clinical care providers will not be allowed to observe the harvest and will not be informed of the assigned group.

Approximately 6 – 7 hours after the sham bone marrow aspiration, subjects will receive a placebo saline infusion via peripheral or central intravenous infusion in a time not to exceed 30 minutes. Identical to the BMMNC infusion, the placebo will be infused within 30 minutes of receipt at Children's Memorial Hermann Hospital, and will remain in the cooler until ready for infusion.

5.1.5 Schedule of Vital Signs during Harvest & Infusion

1. Immediately prior to bone marrow or sham harvest
2. During harvest: vital signs continually monitored & recorded every 5 minutes
3. Post-harvest: q 15 minutes x 4 (1st hour), q 30 minutes x 4 (next 2 hours), and then every hour until the infusion
4. Immediately prior to infusion
5. During infusion: vital signs continually monitored & recorded every 5 minutes
6. Post Infusion: q 15 minutes x 4 (1st hour), q 30 minutes x 4 (next 2 hours), q 1 hour x 4 (next 4 hours), and then every 4 hours thereafter.

5.1.6 BMMNC Dosing

Our target dose is 6×10^6 BMMNC/Kg body weight. The target dose is based on pre-clinical animal data where cells were administered locally (intramyocardial) in a clinical protocol for patients with congestive heart failure (cell harvest of > 30×10^6 cells or approximately 0.4×10^6 cells/Kg body weight from a total of 50 mL of bone marrow harvested) (Perin *et al.*, 2003), and our pediatric Phase I clinical trial using a similar protocol (Cox *et al*., 2011). In other studies, rat BMMNCs were isolated and 2×10^6 cells/350gm were administered intravenously (Lu *et al.*, 2001).

GSCL estimated that over 1×10^8 cells could be obtained from the proposed bone marrow harvest, and this was confirmed in our Phase I Trial. The cells will then be re-suspended to a 1 mL/Kg final volume. Below is a sample dosing calculation:

70Kg subject at the dose target:
Total Dose= Subject weight (Kg) X 6×10^6 cells
Dose required= 70Kg X 6×10^6 cells = 420×10^6
Yield expected= 350 mL bone marrow to yield 7×10^8 cells or 700×10^6 cells.

Smaller harvest yields may necessitate reducing our dose target. If the yield is below the target, then the upper doses may be impractical and we will use/infuse the maximum obtainable dose for each subject. Data will be analyzed as such. It will not be a protocol deviation if the dose target is not reached.

5.1.7 Post-Infusion Period

The day following infusion will be considered Post-Infusion Day 1, regardless of what time of the day the infusion was given. Post-infusion monitoring will include hemodynamics, infusion related toxicities (pulmonary, hepatic, and renal lab indices),

and neurologic complications. Laboratory tests will include a CBC with differential, chemistry panel, hepatic function panel, and coagulation panel. As part of the pulmonary monitoring a chest x-ray will be performed and blood-oxygen saturation will be monitored by finger oximeter. In the event of pulmonary dysfunction, standard supportive therapy will be given. The research team will review the first 24 hours of post-infusion monitoring for AE/SAE's and determine if the subject is eligible for discharge home. Any serious adverse event as described in the Statistical Considerations section will be reported immediately and referred to the DSMB and evaluated for potential relationship to the protocol.

Prior to discharge, the parent(s)/LAR will be given detailed instructions regarding signs and symptoms of infection such as fever, malaise, and chills and told to check the subject's body temperature twice daily for three weeks after discharge (through post-infusion day 21). Parents(s)/LAR will be instructed to immediately report neurological changes or seizure activity to the research team. A CBC and basic chemistry panel will be obtained 21 days post-infusion (+/- 7 days). Parent(s)/LAR will be given the option of having the lab tests performed by their PCP or, for local families, here at UTHealth. The research team will have daily contact with the parent(s)/LAR through post-infusion day 21. Monitoring information will be recorded on the case report form and any reports of abnormalities will be relayed to the study PI/Co PI immediately. If indicated, the parent(s)/LAR will be instructed to have the subject seen be their PCP for further evaluation. Local families may opt to be seen by the research team for adverse events evaluation.

After discharge, the subject's PCP will be given discharge summary information. The PCP will be asked to consult with the study PI on any AE/SAE's post-infusion to ensure that appropriate therapy/treatment is given to the subject.

5.1.8 Cross-Over and Follow-Up Visits

As part of the trial, the subjects will return to The University of Texas Health Science Center at Houston for 3 follow-up visits: 6 months (+/- 14 days), 1 year post-treatment (+/- 21 days), and 2 years post-treatment (+/- 21 days). Each visit will take place over 1-2 days in which the subject will undergo outpatient assessments that include physical and videotaped neurological exams, laboratory tests, chest radiograph, oxygen saturation monitoring, psychological testing, and quality of life measures. DTMRI will be performed at the 1 and 2 year post-treatment visits.

Prior to the 1 year follow-up visit, the treatment blind will be broken and the parent(s)/LAR notified. At this time, the parent(s) of children in the placebo group may choose to have their child admitted to the Children's Memorial Hermann Hospital pediatric unit for bone marrow harvest and infusion. All procedures will be followed in the same manner as was done for the Treatment Group.

5.1.9 Blinding of Placebo Infusion

To prevent distinguishing between placebo and cellular products, a 3 mL sample of peripheral blood will be collected immediately prior to sham harvest in addition to the

standard blood samples collected for QA and infectious disease testing. This blood will be anti-coagulated with acid citrate dextrose and delivered to the cell processing laboratory. The 3 mL blood sample will be added to 30 mL of 0.9% saline containing 5% volume/volume human serum albumin and loaded in a sterile syringe identical to the one used for the BMMNC cellular product. Sterility QC assays will be performed including gram stain, 14 day aerobic and anaerobic cultures and 28 day fungal cultures. A negative gram stain result will be required for release of the placebo. The final product will be labeled identically to the cellular product. The placebo will then be transported to the site of administration in the same manner as the cellular therapy product and infused at the same rate used to infuse cellular product.

5.2 Cord Blood Arm

5.2.1 hUCB Collection, Processing and Storage

We will only recruit pediatric subjects who have their hUCB stored with Cord Blood Registry® (CBR) (CBR Systems, Inc., San Bruno, CA) and meet the study entry criteria. CBR is a private cord blood bank accredited by the American Association of Blood Banks (AABB) with an inventory of over 400,000 banked hUCB samples. Their stringent laboratory processes, record keeping and quality control are designed to meet all federal (FDA) and state guidelines and regulations. CBR is licensed by the states of New York, Maryland, New Jersey, California, and Illinois who have their own set of strict regulations for stem cell banks. The CBR laboratory is also CLIA certified.

5.2.2 CBR hUCB Sample Quality

CBR collection methods are virtually free of microbial and fungal contamination as 98% of units are sterile upon testing (Harris, 2008) CBR has extensive experience with Ficoll-Hypaque and AXP processing and is able to attain high stem cell recovery rates as assessed by MNC and TNC recovery rates following processing. Ficoll-Hypaque processing recovers 98% of the MNC fraction (Harris, 2002), while AXP processing recovers 98.7% of the MNC fraction and 96.2% of the TNC fraction (Rosenthal, 2008). CBR has provided 86 hUCB samples for use in transplant to 26 different transplant centers with an average post-thaw viability of >90% based on measures conducted at the transplant centers and all hUCB units released have tested viable (Harris, 2008).

5.2.3 Subject Recruitment

Fifteen pediatric subjects who have their umbilical cord blood banked with CBR and meet study criteria will be enrolled into the cord blood study arm. The parent(s)/LAR of potential subjects will contact CBR. After receiving permission to release the subject's contact information, CBR will then relay the contact information to the research team, who will then contact the parent(s). During this telephone call, an overview of the study and basic eligibility requirements will be reviewed. If eligibility requirements are met and the parent(s)/LAR willing to participate, instructions will be provided on forwarding medical records to the research team for review and a consent and authorization for release of information (for contact with the subjects primary care provider [PCP]) will be sent to the parent(s)/LAR. After receipt of medical records and contact with the PCP (if

indicated) a telephone conference call will be scheduled with the parent(s)/LAR. During the call, study procedures and the informed consent document will be reviewed. Time will be given to answer questions. The parent(s)/LAR will not be pressured to make a decision on participation during the call. Families choosing to participate in the study will be asked to return the signed informed consent in the postage paid envelope provided.

After receipt of the signed informed consent document, CBR will be notified to release cord blood information to the research team. The parent(s)/LAR will be contacted by the research team to schedule the baseline/treatment visit. The parent(s)/LAR will be responsible for making their travel arrangements.

Approximately 4 – 6 weeks prior to the scheduled procedure date, the subject will have the infectious disease testing for HIV 1 and II, Hepatitis B and C, HTLV 1 and II, Syphilis, CMV, and West Nile Virus. An additional 8 – 10 mL of blood will be collected at this time and shipped overnight to a Cell and Gene Therapy (CAGT) lab in Houston for HLA testing. All lab results will be sent to the research team for review prior to the baseline/treatment visit. If the subject has a positive HIV, Hepatitis B, or C test; they will be excluded from the study.

5.2.4 Maternal Infectious Disease Testing

CBR routinely collects a maternal blood sample at the time of delivery for infectious disease marker (IDM) testing. The maternal IDM results (if available) will be released to the research team along with the cord blood sterility and cell count information after informed consent has been obtained and CBR has obtained permission from the parent(s)(s). The infectious disease tests include: Human Immunodeficiency Virus (HIV) type 1, HIV type 2, Hepatitis B virus, Hepatitis C virus, Human T-cell lymphotrophic virus (HTLV) type I, HTLV type II, and Syphilis. If any maternal infectious disease markers were positive at the time of the collection the cord blood will not be used, and the subject will not be eligible, (*refer to Appendix D*). Per CBR, less than 10% of cord blood collections do not have the maternal IDM testing performed. In the event that a subject meets criteria for study entry, but CBR does not have the maternal IDM results, the parent(s) can submit IDM results that were performed during the pregnancy, for instance as part of the prenatal labs or performed during the hospital admission for delivery. If the IDM tests were not completed during the pregnancy, the mother will be required to have IDM testing completed at her expense and lab results submitted to the PI. If any of the results of the maternal IDM tests are positive, the subject will be ineligible. There is a chance the mother may not have been positive during the pregnancy and the time of delivery; however, we have chosen to err on the side of caution. It is also important to note, that maternal testing is not required under federal regulations for autologous cord blood infusions {CFR 21.1271.90}; however, we have chosen to require maternal infectious disease testing in this protocol.

5.2.5 Randomization

Subjects in the hUCB group will be randomized after receipt of subject and maternal infectious disease results and CBR cord blood information on viability and cell count.

Subjects found ineligible for cord blood infusion due to low or non-viable cell count or contamination will be offered the opportunity to enroll in the other treatment arm of the study if the enrollment limit for the BMMNC group has not already been met.
The randomization sequence will be computer-generated. The subject will be assigned to the treatment or control arm in a 2:1 ratio using sequentially numbered, opaque, sealed envelopes prepared by a member of the Center for Surgical Trials and Evidence-based Practice (C-STEP) not involved in the clinical trial.

5.2.6 Shipping of Cord Blood

For subjects randomized to receive hUCB at the baseline visit, the GSCL will request a segment of the subject's banked cord blood sample from CBR. The subject's HLA lab results will be compared to the cord blood sample for HLA type (6/6 match). Additionally, the cord blood segment will be tested for viability and cell count. Results from the HLA typing will be faxed to CBR to confirm the subject's identity. CBR will ship the frozen cord blood to the GSCL 7 – 14 days prior to the scheduled procedure, where it will be kept frozen until the morning of infusion. It is important to note that once cord blood has been shipped from CBR it cannot be returned. If the subject is randomized to the placebo group, cord blood unit will NOT be shipped. All HLA lab results will remain on record for reference at the 1 year cross-over visit (if indicated).

5.2.7 Baseline Visit and Cord Blood (Placebo) Infusion

The outpatient baseline visit will be conducted in the Pediatric Outpatient Surgery Clinic. At the baseline outpatient visit, the signed informed consent document will be reviewed with the parent(s)/LAR and assent obtained from the subjects if they are able to do so. During this 1 day outpatient visit, subjects will undergo baseline assessments including physical and neurological exams, laboratory tests, chest radiograph, oxygen saturation monitoring, psychological testing, and quality of life measurements. The neuroexam will be videotaped for blinded assessments of dystonia using standardized scales validated for use on children with CP. The research team will review information from the baseline assessments to ensure the subject remains eligible for participation and that there are no unanticipated reasons to exclude the subject, (such as recent infection). After completing the outpatient baseline visit assessments, eligible subjects will be admitted to a Children's Memorial Hermann Hospital pediatric unit.

The baseline DTMRI will be performed as soon as possible following early AM admission with the cord blood/placebo infusion immediately before or after the DTMRI. For subjects receiving cord blood, the GSCL will thaw, process, and transport the cord blood prior to infusion. For subjects randomized to the placebo group, the GSCL will prepare a placebo infusion according to the procedure described previously for the bone marrow placebo arm, with the exception that it will be loaded in a sterile transfer pack identical to the one used for hUCB cellular product, and the infusion medium will be Dextran 40 containing 5% volume/volume human serum albumin. The pack will be labeled in the manner described in the standard operating procedure (identically to the labeling used for the cellular product). The placebo will be transported to the site of administration in the same manner as the cellular therapy product. The infusion rate will be approximately 5cc/minute for both groups.

5.2.8 Schedule of Vital Signs during and Post-Infusion

1. Immediately prior to infusion
2. During infusion: vital signs continually monitored & recorded every 5 minutes
3. Post Infusion: q 15 minutes x 4 (1st hour), q 30 minutes x 4 (next 2 hours), q 1 hour x 4 (next 4 hours), and then every 4 hours thereafter.

5.2.9 Post-Infusion Period

The day following infusion will be considered Post-Infusion Day 1, regardless of what time of the day the infusion was given. Post-infusion monitoring will include hemodynamics, infusion related toxicities (pulmonary, hepatic, and renal lab indices), and neurologic complications. Laboratory tests will include a CBC with differential, chemistry panel, hepatic function panel, and coagulation panel. As part of the pulmonary monitoring a chest x-ray will be performed and blood-oxygen saturation will be monitored by finger oximeter. In the event of pulmonary dysfunction, standard supportive therapy will be given. The research team will review the first 24 hours of post-infusion monitoring for AE/SAE's and determine if the subject is eligible for discharge home. Any serious adverse event as described in the Statistical Considerations section will be reported immediately and referred to the DSMB and evaluated for potential relationship to the protocol.

Prior to discharge, the parent(s)/LAR will be given detailed instructions regarding signs and symptoms of infection such as fever, malaise, and chills and told to check the subject's body temperature twice daily for three weeks after discharge (through post-infusion day 21). Parents(s)/LAR will be instructed to immediately report neurological changes or seizure activity to the research team. A CBC and basic chemistry panel will be obtained 21 days post-infusion (+/- 7 days). Parent(s)/LAR will be given the option of having the lab tests performed by their PCP or, for local families, here at UTHealth. The research team will have daily contact with the parent(s)/LAR through post-infusion day 21. Monitoring information will be recorded on the case report form and any reports of abnormalities will be relayed to the study PI/Co PI immediately. If indicated, the parent(s)/LAR will be instructed to have the subject seen be their PCP for further evaluation. Local families may opt to be seen by the research team for adverse events evaluation.

After discharge, the subject's PCP will be given discharge summary information. The PCP will be asked to consult with the study PI on any AE/SAE's post-infusion to ensure that appropriate therapy/treatment is given to the subject.

5.2.10 Cross-Over and Follow-Up Visits

As part of the trial, the subjects will return to The University of Texas Health Science Center at Houston for 3 follow-up visits: 6 months (+/- 14 days), 1 year post-treatment (+/- 21 days), and 2 years post-treatment (+/- 21 days). Each visit will take place over 1-2 days, and the subject will undergo outpatient assessments which include physical and videotaped neurological exams, laboratory tests, chest radiograph, oxygen

saturation monitoring, psychological testing, and quality of life measures. DTMRI will be performed at the 1 and 2 year post-treatment visits.

Prior to scheduling the 1 year follow-up visit, the treatment blind will be broken and the parent(s) notified. At this time, the parent(s) of children in the placebo group may choose to have their child admitted to the Children's Memorial Hermann Hospital Pediatric ICU/MICU for cord blood cell infusion. If the subject's parent(s) agrees to the cord blood infusion, the study coordinator will notify the Director of GSCL of the scheduled visit date. The Director will then request that the cord blood be shipped from CBR. The frozen cord blood unit will be shipped overnight to the GSCL 7 – 14 days prior to the scheduled procedure. The cord blood will be kept frozen until the morning of the infusion. All procedures will be followed in the same manner as was done for the Treatment Group.

5.3 Handling of Cord Blood/BMMNC Interventions

Transportation, processing, assessing and quality assurance at GSCL are described in the Chemistry & Manufacturing section of the IND [redacted].

5.4 Pre-Infusion Medications (All Groups)

Thirty minutes prior to the infusion, all subjects (regardless of treatment assignment) will receive intravenous Benadryl 0.5mg/Kg and Solu-Medrol 0.5mg/Kg. Additionally, the following medications will be ordered prior to the infusion and kept at the subjects' bedside: Benadryl 50 mg for injection, Epinephrine 1:10,000 – 10 mL for injection and Hydrocortisone 100 mg for injection (or Solu-Medrol 0.5mg/Kg).

5.5 Prohibited Interventions

Subjects may not be enrolled in other interventional research protocols while participating in this study.

6. CLINICAL AND LABORARTORY EVALUATIONS

6.1 Schedule of Events (See Appendix A)

6.2 Infusion Toxicity Complications

6.2.1 Hemodynamic Instability

Subjects will be admitted to the pediatric at Children's Memorial Hermann Hospital following the baseline outpatient visit and will be monitored continuously until discharge as described in previous sections. A primary concern is the precipitation of hemodynamic instability associated with infusion-related toxicities of cellular product transplant. It has been reported that hypotension, hypertension and bradycardia can occur from transplantation of cellular product that has been cryopreserved in dimethylsulfoxide (DMSO) and RBC depletion. To minimize the effect of these, our hUCB product will be washed according to Standard Operating Procedures prior to

infusion. However, it is possible that some quantity of DMSO will remain. To minimize the risk of hemodynamic instability, maintenance + an additional 10 – 40mL/Kg intravenous 0.9% saline volume replacement will occur concomitantly with the cellular product infusion. Blood pressure elevation will be defined as an increment of more than 20% from the baseline value at the beginning of the infusion (Konuma, 2008). An adverse event will be defined as a sustained (> 10 minutes) >20% decrease in MAP. Transient decreases in the mean arterial pressure (MAP) that respond to fluid infusion or inotropes will not be considered adverse events. Prolongation of capillary refill greater than 2 seconds from baseline as well as a greater than 20% change in heart rate during the procedure will prompt an evaluation as to the etiology of the change in hemodynamic status. Bradycardia, defined by a sustained reduction in heart rate, less than 40beats/min, that is unresponsive to intravenous fluid or inotrope support will be considered an adverse event. Restoration of hemodynamics to baseline will allow resumption of infusion. Inability to transfuse will preclude further participation in the study, though the subject will be followed for data collection per protocol.

6.2.2 Pulmonary Complications

A concern exists regarding the systemic infusion of leukocytes in a concentrated manner. Theoretically, activated monocytes could function to enhance polymorphonuclear leukocytes (PMN) migration into the lung, as the lung is the primary "first pass" filter for intravenous infusion of any cellular product. PMN mediated organ injury typically occurs over a 6 – 24 hour time frame. Based on this, chest radiographs will be performed and evaluated at Baseline and on Post-Infusion Day 1. Additionally, blood-oxygen saturation will be monitored by finger pulse oximetery (SpO_2). Moderate respiratory dysfunction within the first 24 hours post – infusion will be considered an adverse event but will not warrant stopping the trial unless recommended by the DSMB. In the event of pulmonary dysfunction, standard supportive therapy will be given. Pulmonary symptoms/events corresponding to the CTCAE v4.0 Grade 3 will trigger the stopping rules described in section 10.

6.2.3 Hepatic Injury

The reticuloendothelial system can sequester immature blood elements, theoretically resulting in hepatic injury. An acute elevation of the AST/ALT hepatic enzymes > 900 U/dL in the first 24 hours post – infusion will trigger the stopping rules. This level is corresponds to the CTCAE v4.0 Grade 4 adverse event. It is unlikely that "end vessel" microthrombosis would occur in the liver due to the dual blood supply of the liver and the lung is the first pass organ.

6.2.4 Renal Complications

DMSO induces hemolysis of contaminating red blood cells during the hUCB storage and may also induce in vivo hemolysis and ultimately pigment nephropathy (Smith, 1987). To minimize the effect of these, our hUCB product will be washed according to Standard Operating Procedures prior to infusion. Though unlikely, it is possible that some quantity of DMSO will remain which could cause toxicity. Renal function/events corresponding to the CTCAE v4.0 Grade 3 will trigger the stopping rules.

6.2.5 Neurologic Complications

The subject's acute neurologic status will be monitored hourly until discharged. Hourly GCS, pupillary size/reactivity, motor/sensory evaluation of extremities, and any seizure activity will be recorded from time of infusion to discharge. *Grade 3 – 5 central nervous* system event as defined in the NCI CTCAE v4.0 occurring within 12 hours of cellular product infusion will trigger the stopping rules. Other changes temporally related to cellular product infusion (those events occurring within 12 hours of infusion) will be considered associated with the protocol and recorded as an adverse event.

6.3 Psychological & Functional Outcome Assessment Tests

The following tests will be performed at baseline and all follow-up visits for children who have the visual, motor, and language abilities needed to complete the assessment tasks, (note table in Appendix B).

6.3.1 Motor Functioning

The Gross Motor Fine Motor - 88 Scale (GMFM-88) is an 88-item standardized, observation-based assessment of children's gross and fine motor abilities. It was designed for motor assessment of children with cerebral palsy. Administration time is 45-60 minutes.

6.3.2 Quality of Life

The parent(s) proxy version of the Cerebral Palsy Quality of Life Questionnaire (CP QOL) is measure of health related quality of life for children ages 4 through 12 years. The 66-item questionnaire assesses QOL across seven domains (1) social well-being and acceptance; (2) feelings about functioning; (3) participation and physical health; (4) emotional well-being; (5) access to services; (6) pain and feeling about disability; and (7) parent(s) health. Completion time is approximately 30-45 minutes. The questionnaire may be completed by telephone at a time convenient for Parent(s) prior to the study visit.

6.3.3 Adaptive Behavior Functioning

The Vineland Adaptive Behavior Scales – 2nd Edition (VABS-2), is a parent(s)/caregiver report measure that provides information regarding a child's adaptive behavior/daily living skills. The VABS-2 is appropriate for children from birth through age 10 and produces standard scores in the following domains: Communication, Daily Living Skills, Socialization, and Motor Skills. The measure takes between 25 and 90 minutes to complete.

The Pediatric Evaluation of Disability Inventory is a parent-report measure of adaptive functioning that is designed specifically for children with functional impairments and is sensitive to small changes in adaptive skills at the low end of the functioning spectrum.

6.3.4 Visual-Spatial Processing

The Motor Free Visual Perception Test- 3rd Edition (MVPT-3) will be administered to obtain information regarding visual perceptual abilities among children ages 4-10 years old across 5 domains: (1) spatial relationship, (2) visual closure, (3) visual discrimination, (4) visual memory, and (5) figure ground. Standard scores are produced and administration time is approximately 25 minutes.

6.3.5 Learning/Memory

The Narrative Memory subtest from the NEPSY-II, a developmental psychological battery for children, will be administered to obtain information regarding memory and learning ability: The Narrative Memory subtest can be administered to participants ages 3-10 years old and produces a standard score that indicates memory for organized verbal material under free recall, cued recall, and recognition conditions. Administration time will take approximately 10 minutes.

6.3.6 Expressive and Receptive Vocabulary

The Peabody Picture Vocabulary Test – 4th Edition (PPVT-4) produces a standard score indicating subjects' receptive one word vocabulary and will be administered to children ages 2.6-10 years old. Responses can be indicated either verbally or pointing to a picture and so is appropriate for all participants, regardless of motor abilities. Administration time is approximately 15 minutes. The PPVT-4 score is often considered an appropriate indication of general cognitive functioning. The Expressive Vocabulary Test - 2nd Edition (EVT-2) will provide a standard score indicating subjects' expressive one word vocabulary. Responses are provided verbally and so the test is appropriate for all children (ages 2.6-10 years old), regardless of motor abilities. Administration time is approximately 15 minutes.

6.4 MRI Evaluation

6.4.1 CP Neuroimaging

CP is a clinical diagnosis inclusive of a heterogeneous group of patients with differing patterns of neurological subtype (spastic -quadriplegic –hemiplegic –diplegic; ataxic/hypotonic; dyskinetic), severity (GMFCS level), type of lesion(s), location of lesion(s), and functional status (ambulatory/non-ambulatory). Although the pathogenesis of CP is still poorly understood, advanced neuroimaging studies have led investigators to the identification of some cerebral abnormalities frequently observed in young children with a diagnosis of CP (Towsley *et al*., 2011). Such cerebral anomalies include the following: (1) periventricular white matter damage, (2) diffuse gray matter damage, (3) cerebral vascular accident, and (4) malformation of cortical development. Due to heterogeneity in etiological bases of primary and secondary insults to the developing brain which manifest as different cerebral anomalies, new interdisciplinary studies of CP are needed to evaluate cerebral anomalies in relation to neurological subtype and identifiable lesion types (and locations). Such an interdisciplinary approach has high potential to identify quantitative measures of brain structure with

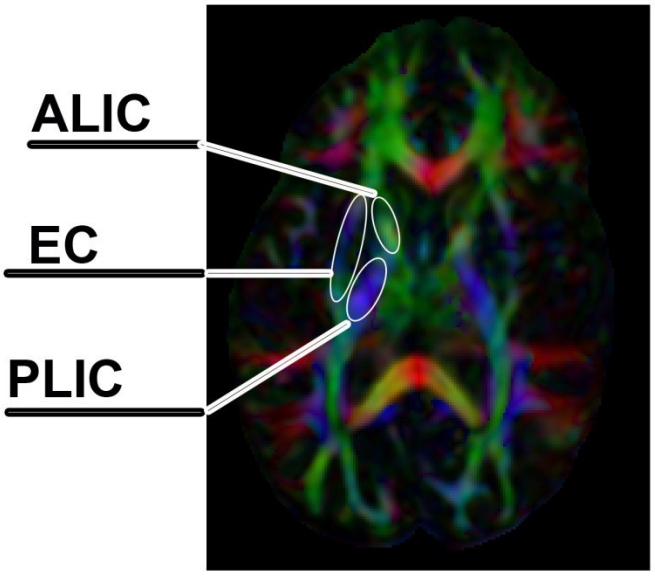
prognostic value as they explain neurological subtype, severity, and functional status. Such information is vital for the development of interventional strategies and predicting response to different types of interventions given the “profile” of each individual patient. Some neuroimaging studies have reported specific regions which are highly susceptible to being compromised in CP (Hoon *et al*., 2005; Hoon *et al*., 2009; Panigrahy *et al*.,2005; Son *et al*., 2007; Son *et al*.,2009; Thomas *et al*., 2005; Yoshida *et al*., 2010). By conducting an objective analysis which integrates quantitative measures of microstructural integrity (in white matter pathways) and quantitative measures of associated cortical/subcortical atrophy (in gray matter), the proposed study has the potential to identify neural systems compromised by CP and subsequently monitor response to treatment in a system-specific fashion.

Based on published literature (Towsley *et al*., 2011; Yoshida *et al*., 2011), spastic CP is expected to be predominately related to evidence of periventricular leukomalacia (PVL) or diffuse gray matter injury (GFCMS levels IV-V); or a cerebral vascular accident. In contrast, ataxic CP is expected to be related to white matter volume loss and increased mean diffusivity in deep gray matter structures (e.g. putamen, globus pallidum, and thalamus).

WM ROIs compromised in children with CP (Yoshida *et al*., 2011):

Anterior Limb of the Internal Capsule (ALIC) – dyskinetic = ↓ FA
Posterior Limb of the Internal Capsule (PLIC) – spastic & dyskinetic = ↓ FA
External Capsule (EC)Posterior thalamic radiation – spastic & dyskinetic = ↓ FA
CC_genu – dyskinetic = ↓ FA
CC_splenium – spastic & dyskinetic = ↓ FA
CC_body – spastic = ↓ FA
Cingulum Bundle – dyskinetic = ↓ FA
CST – spastic & dyskinetic = ↓ FA
SLF – spastic & dyskinetic = ↓ FA
ILF – spastic & dyskinetic = ↓ FA
Thalamocortical pathways – spastic = ↓ FA

Figure 3.



GM ROIs compromised in children with CP:

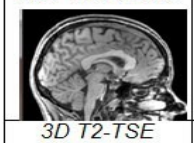
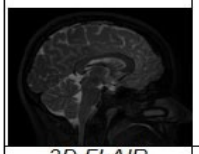
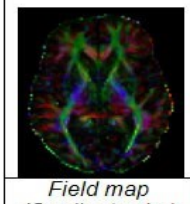
Basal ganglia = ↓ volume
Thalamus = ↓ volume
Precentral Gyrus = ↓ volume
Postcentral Gyrus = ↓ volume
Paracentral lobule = ↓ volume

6.4.2 MRI Acquisition and Analyses

The primary goal of acquiring whole brain MRI data is twofold: (1) to quantitatively assess macro- and micro-structural properties of GM and WM regions in two groups (+BMMNC / -BMMNC) using state-of-the-art neuroimaging acquisition/analysis methods and (2) to investigate macro- and micro-structural correlates of behavior in terms of cognitive performance and underlying neuropathology in a hypothesis – driven manner.

To accomplish these objectives, the MRI data needs to be collected using two different, yet complementary modalities of MRI: (1) high-resolution anatomical MRI (aMRI) and (2) diffusion tensor imaging (DTI). While data from both modalities is easily acquired in the same imaging session, the total scan time needs to be kept to the minimum necessary to achieve the specific aims within a timeframe generally well-tolerated by young children (e.g. ~30min). An earlier version of our proposed MRI protocol has been successfully completed by more than 400 children between the ages of 6 and 18 years old at our Imaging Research Facility during the course of ongoing NIH-supported studies involving diverse study populations including SBM, TBI, Learning Disabilities, and, typically-developing healthy controls. The following five pulse sequences constitute a complete imaging session: (1) conventional localizer/scout, (2) 3D isotropic T1-weighted MPRAGE, (3) 3D isotropic T2-weighted TSE, (4) 32-direction single-shot spin-echo diffusion sensitized echo-planar (DTI-32dir), and (5) 3D-FLAIR. Morphometric, diffusion tensor tractography, and DTI-derived metrics of microstructural properties of GM and WM (e.g. MD, FA) can all be performed in about 20 minutes. Parameters for each sequence are listed below in Figure 4.

Figure 4.

Sequence	Plane	Voxdim	Matrix	FoV	Flip (α)	TR (ms)	TE (ms)	TI (ms)	SENSE	Time
Scout	Sag									0'30"
3D T1-MPRAGE 	Sag	1x1x1	256x256	256x256	8°	6.7	3.1	842	2	2'45"
3D T2-TSE 	Sag	1x1x1	256x256	256x256	90°	2500	363		2	2'45"
3D FLAIR 	Sag	2x2x2	256x256	256x256	50°	8000	330	2400	2	1'12"
32 dir DTI (single shot EPI) 	Ax b=1000	2x2x2	128x128	256x256	90°	7000	74		2	5'11"
Field map (Gradient echo)	Ax	2x2x2	128x128	256x256	90°	250	3.3		2	2'52"

6.4.3 Anatomical MRI Analysis

All scans will be analyzed blind to diagnosis, age, and gender. Datasets with significant motion artifact during acquisition will have been re-acquired in the same imaging session based on real-time evaluation of imaging data and decisions made by Dr.

Juranek. See figures 5a/b for example of significant motion artifact subject to re-acquisition.

Figure 5.

A B

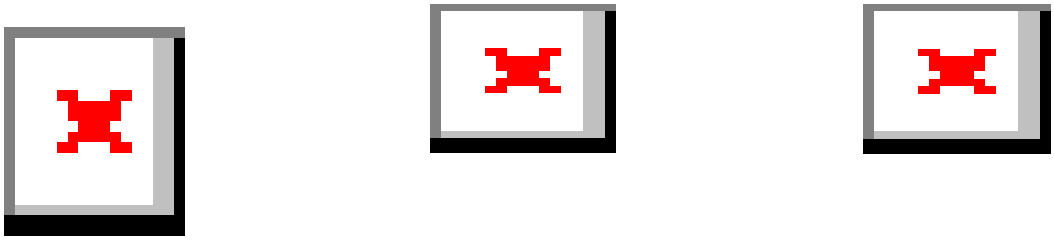
Figure 5A/B: Examples of T1-weighted image acquisition data from children without head injury. Panel on the left is an example of good quality T1-weighted data. Panel on the right is an example of poor quality T1-weighted data requiring re-acquisition in the same imaging session due to signs of motion artifact (e.g. ringing).

6.4.4 Freesurfer Analyses of Cortical and Subcortical Volume

For each participant, the 3D isotropic MPRAGE data is processed via the Freesurfer v5.0.0 (www.surfer.nmr.mgh.harvard.edu) image processing stream (recon-all –all). Each brain is skull-stripped and segmented into 3 classes of voxels: gray matter, white matter, and cerebrospinal fluid (Dale & Sereno, 1993; Dale *et al*., 1999). Subsequently, the cortex is parcellated for individual gyri yielding morphometric measures of volume within each gyrus (Fischl & Dale, 2000). Once data sets have completed the Freesurfer pipeline, the color-coded segmentation maps are visually reviewed using Freesurfer'sTkmedit viewer (Figure 6a). Inaccurate boundary delineations of non-cortical structures and white matter are manually edited and the data re-processed via the second and third stages of the Freesurfer pipeline. Color-coded cortical parcellation maps (Figures 6b and 6c) will also be reviewed via Freesurfer's Tksurfer viewer for any inaccuracies that require editing. Quantitative non-cortical volumes are extracted from the aseg.stats file; surface-based cortical data (e.g. volume) are extracted from the ?h.aparc.annot files.

Figure 6.

A B C



Figures 6 A/B/C: Example segmentation of subcortical structures and parcellation of cortical gyri based on delimiting sulci. Panel on the level demonstrates volume-based subcortical segmentation of subcortical GM structures. Panels on the right demonstrate surface-based color-coded parcellation of cortical gyri from lateral and medial views of the left hemisphere.

6.4.5 DTI Processing and Analysis

DTI data will be reviewed for image quality during acquisition and re-acquired in the same imaging session based on real-time evaluation of imaging data and decisions made by Dr. Juranek. The isotropic 32-direction DTI dataset will be processed and analyzed using FSL v4.1.7 (FMRIB's Software Library, www.fmrib/ox.ac.uk/fsl). Minor head motion and eddy currents will be corrected with the Eddy Current Correction tool in FSL's Diffusion Toolbox v2.0. FSL's FUGUE module will be used to unwarp geometric distortions in the EPI-based DTI dataset with the gradient echo field map, correcting for EPI-related distortions in the DTI dataset (particularly fronto-orbital susceptibility regions) to improve co-registration with T1 structural images. Skull-stripping and removal of non-brain tissue is performed using BET v2.1. Diffusion tensors are reconstructed using FSL's DTIFIT tool within the Diffusion Toolbox.

6.4.6 DTI Co-Registration with Same Subject's aMRI

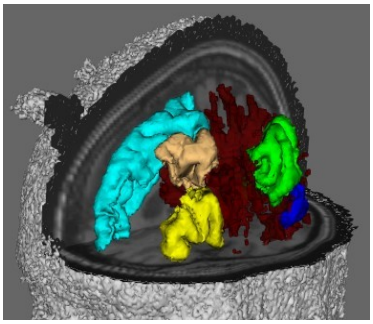
FMRIB's Linear Image Registration Tool v5.5 (FLIRT) is used to perform co-registration between each subject's T1-weighted series and corresponding non-diffusion-weighted series from the DTI dataset. The resultant transformation matrix, and the calculation of its inverse, provides the basis for co-registering the tractography results (performed in diffusion space) and volumetric results (performed in T1 space). For group-level analyses, a study-specific template will be constructed within FSL by pooling and aligning the FA maps of all study participants using a non-linear registration algorithm included with FSL (FNIRT). Although FSL includes FA templates, these were generated from adult subjects and may introduce some error in our analyses of pediatric data.

6.4.7 Region of Interest and Fiber Tracking Analyses for DTI Data

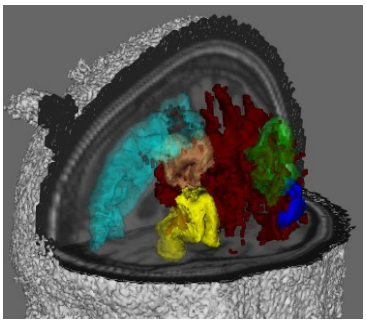
Using FSL's ProbtrackX tool, probabilistic tractography methods are used for fiber tracking of major WM bundles. Combined use of color-coded tensor maps (Douek, Turner et al. 1991) generated by FSL's DTIFIT tool co-registered with the high resolution anatomy available in the T1-weighted series will facilitate tractography by utilizing the seed-to-mask feature in ProbtrackX where one mask is used as the seed mask and one or more masks are used as waypoints/targets. Such "masked-based" tractography reliably generates well-characterized tracts across study participants while preserving individual topology. In the ROI-based analyses FA values will be computed for major white matter bundles including three branches of the superior longitudinal fasciculus (SLF I, SLF II, SLF III) from the arcuate fasciculus [AF]), the corticospinal tract (CST), and the corpus callosum (CC). Using methods of tract delineations described by Catani (2008), Makris (2005), and Glasser (2008), Figures 7 A/B illustrates the AF with 3 branches of the SLF.

Figure 7

A



B



Figures 7 A/B: Integration of multi-modal imaging data. Cortical GM masks are displayed in a 3D viewer with results of mask-based tractography of the arcuatefasiculus. Separate branches of the SLF (e.g. I,II,III) are evident in distinct terminations in superior frontal (SLF I), middlefrontal (SLF II), and inferior frontal (SLF III) cortices. Panel on the right represents the same data with the cortical GM more transparent(s) to view the underlying tractography results. AF: lateral to cingulum bundle connecting posterior temporal (dark blue=bank of the superior temporal sulcus) with parietal (green=supramarginal) and frontal cortices; SLF I: branches into superior frontal (light blue); SLF II: branches into middle frontal (tan); SLF III: branches into inferior frontal (yellow).

For analysis of inter-hemispheric connectivity by the corpus callosum, Freesurfer-generated volumes as well as DTI measures of FA and MD from five subdivisions of the corpus callosum (e.g. anterior=pink, mid-anterior=green, central=yellow, mid-posterior=blue, and posterior=red) will be related to neurobehavioral assessments of function and outcome.

Figure 8

A

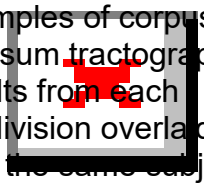
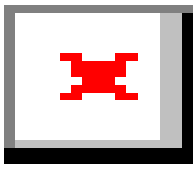


Figure 8, A/B: Examples of corpus callosum tractography results from each subdivision overlaid onto the same subject's T1-weighted volume.

B

Figures 8



For investigating **motor networks**, Freesurfer-generated volumes of midbrain subcortical regions (e.g., thalamus and basal ganglia) will be related to measures of motor performance and processing speed.

Figure 9

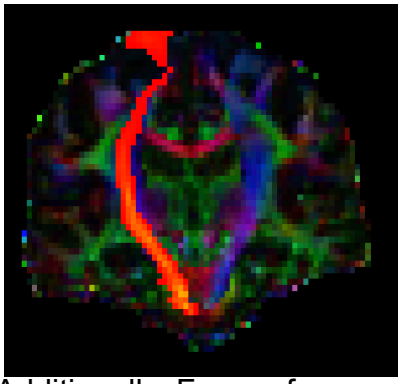
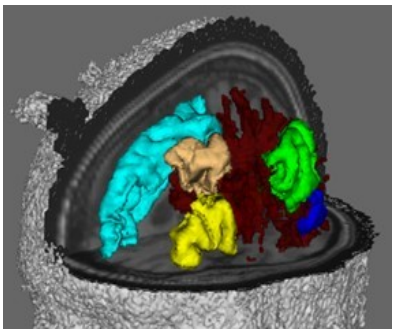


Figure 9: Reconstruction of right cst using FSL's probabilistictractography methodology (red/yellow probability map) overlaid onto color-coded reconstruction of diffusion tensors (red=interhemispheric; blue=superior-inferior; green=anterior-posterior).

Additionally, Freesurfer-generated volumetric measures from primary motor cortex and DTI measures of FA and MD from the corticospinal tract (CST) will be related to neurobehavioral assessments of function and outcome.

To study language networks, Freesurfer-generated volumetric measures from inferior frontal regions (e.g. pars opercularis and pars triangularis in yellow), temporal regions (superior- and middle-temporal not shown), and parietal regions (e.g. supramarginal and angular in green) will be related to language measures. Furthermore, DTI measures of FA and MD from the same GM regions and the AF/SL (in red) will be related to neurobehavioral assessments of function and outcome (Figure 10).

Figure 10

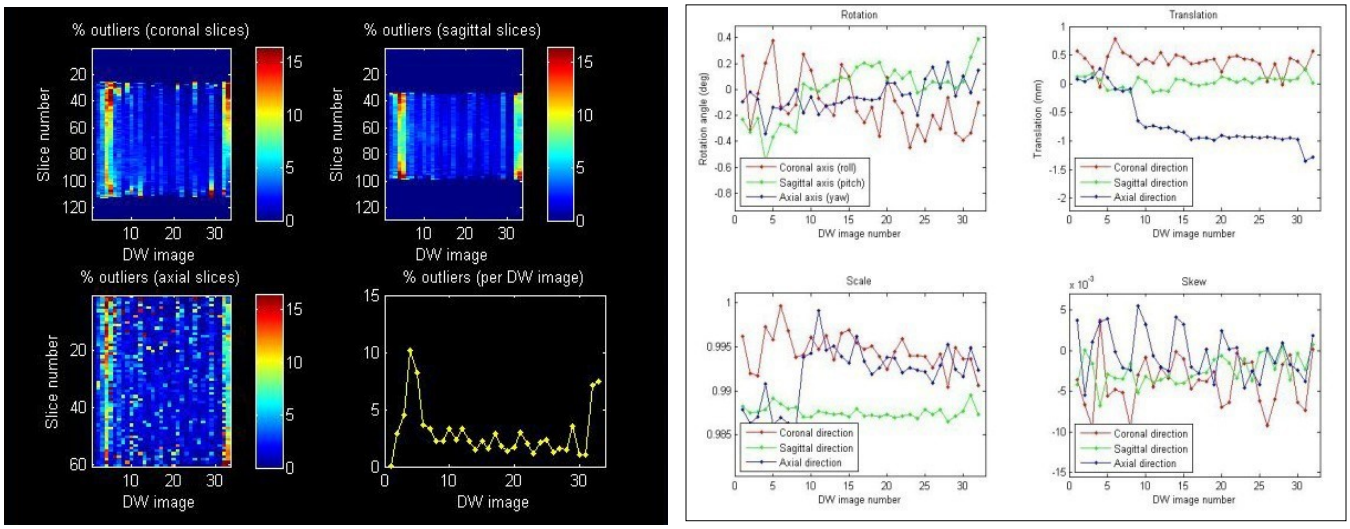


6.4.8 MRI Quality Control Procedures

At the time of acquisition, we have procedures to check the aMRI and DTI data for outliers and confounding artifacts due to acquisition (e.g. image geometric distortions, field in homogeneity, signal-to-noise-ratio) and motion. If problems occur during scan acquisition, the sequence will be repeated to optimize image quality within the same imaging session based on real-time evaluation of imaging data and decisions made by Dr. Juranek. All post-processing procedures of aMRI and DTI data include highly interactive protocols (i.e., not fully automated) requiring visual inspection of the data at each processing stage, which provides the best assurance of image quality. A standard water phantom will document and monitor scan quality at each imaging session. Post-processing procedures include quantitative evaluation of eddy currents and head movement using ExploreDTI v4.8.1 software provided by Dr. Leemans. Evaluation of DTI data acquired from our living phantom in Houston is presented below in Figures 11 and 12. Our criteria for disqualifying DTI datasets and re-acquiring DTI data within the

same imaging session are translations greater than one voxel (e.g. 2 mm) or rotations greater than 1˚.

Figure 11



Figures 11: Quantitative evaluation of DTI data quality using Explore DTI v 4.8.1. On the left, the percentage of outlier voxels is plotted as a function of diffusion-weighted gradient direction number and slice number. On the right, rotations, translations, scale, and skew are plotted for each cardinal plane as a function of diffusion-weighted direction number.

6.4.9 Secondary Imaging Outcome Measures & Correlation with Functional Outcome

As noted in the section, “CP Neuroimaging”, cerebral palsy is a clinical diagnosis comprising a heterogeneous group of patients. This heterogeneity is manifest both in the dominant clinical symptom complex, but also in the imaging correlates of the symptoms. To that end, prior to treatment this study will quantify the degree of functional impairment (GFCMS level), and correlate it with the CP subtype and imaging abnormality. The imaging ROIs will be prospectively identified pre-treatment. Then, according to the qualitative and quantitative metrics, the imaging data will be recorded. Post-treatment, these same ROIs will be compared to the baseline data, and correlated with any changes in functional outcomes as measured by specific tasks on the GFCMS. Ultimately, the changes in WM and GM ROIs that are associated with CP will be compared with and without treatment using autologous bone marrow derived mononuclear cells or autologous cord blood mononuclear cells. These data will provide a structural metric around which future studies could be powered. Further, these data may provide potential mechanistic targets in terms of structural preservation and restoration in patients with CP.

7. MANAGEMENT OF ADVERSE EXPERIENCES

There are currently no neuroprotective/restorative treatments for CP in adults or children. Any potential risks must be weighed against the lack of treatment alternatives.

The majority of the risk related to the study is associated with the bone marrow harvest and infusion periods and are discussed in section 6.2.

7.1 Infection

There have been studies on complications of marrow harvest, and these studies note a 0.1-0.4% serious complication rate including: cellulitis at aspiration site, pneumonia and bacterial sepsis (Cairo, 1989; Jin, 1985; Kessinger, 1987). It is anticipated that the infectious risk will be lower than that reported in the oncology/BMT literature due to the relative immunocompetence of the CP population. We had no infections in our Phase I Trial. Parent(s)/LAR will receive detailed instructions at discharge regarding monitoring for signs of infection. There will be daily contact with the parent(s)/LAR for the first 21 days post-infusion for safety monitoring.

7.2 Other Harvest Related Issues

Bone marrow aspiration may cause discomfort. Hemorrhage, nerve injury and bone fracture are rare but reported complications that will monitored. Because the autologous BMMNCs used, are intravenously infused we do not anticipate any adverse reaction to them as may be occur in allogenic bone marrow transplants. Because this type of treatment has only been reported in head injured children in our Phase I trial, we may encounter other unexpected and unforeseen complications related to this treatment.

7.3 Psychological and Functional Outcome Testing

Subjects enrolled in the trial will be asked to take tests of memory, attention and motor skills. Parent(s)/LAR will be asked to fill out questionnaires that ask about the child's learning and behavior. Measures will be taken to minimize any stress participants may experience with testing procedures. All tests will be conducted in a private room located in the Outpatient Pediatric Surgery Clinic. Ample time will be given for completion of the tests.

7.4 Loss of Confidentiality

There is a potential risk of loss of confidentiality of subject information and data. All study related records will be stored in locked office and cabinets within the Dept. of Pediatric Surgery. Access to study records will be restricted to research team members, the study monitor, and UT IRB representatives. Case report forms will contain only the subject's study ID. The study database will reside within UTHealth Medical School, firewall protected, zone 100 servers. Assess to the study database will be limited to research team members.

8. CRITERIA FOR INTERVENTION DISCONTINUATION

8.1 Description of Subject Completion

Subjects are considered to have completed the study if they have undergone the BMMNC/hUCB infusion at either the baseline or the 1 year cross-over visit and have completed the 2 year end of study visit.

8.2 Withdrawal of Individual Subjects

The Principal Investigator will make every reasonable effort to keep each subject in the study through the 2 year visit. If a subject withdraws from the study, the reason for withdrawal must be documented in the study chart. Possible reasons for withdrawal include: adverse experiences, protocol deviation, including non-compliance, lost to follow-up, discretion of investigator or DSMB, or other regulatory agency/body. Subjects will be withdrawn from the study immediately if any of the following occur:

a. The subject or their parent(s)/LAR requests withdrawal from the study.
b. The investigator believes it is in the best interest of the subject.
c. There is clinically significant deterioration of the subject's medical status that warrants termination from the study.
d. There are clinically significant abnormal laboratory results that warrant termination from the study.

8.3 Withdrawal of Subject from the Study Following Adverse Events

The investigator must apply his clinical judgment to determine if an adverse event (AE) is of sufficient severity to require that the subject should immediately be withdrawn from the study. If the withdrawal from the study is due to an AE, the subject should be given appropriate care under medical supervision until the symptoms of the AE resolve or his/her condition becomes stable. Subsequent review by the MSM, DSMB, or IRB may also result in the suspension of further trial enrollment (see Stopping Rules).
A subject (or Parent/LAR) may also voluntarily withdrawal from treatment due to what he/she perceives as an intolerable AE, or for any other reason. If voluntary withdraw is requested, or if withdrawal occurs for any reason, the subject should be asked to continue (at least limited) scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable.

8.4 Procedures for Handling Withdrawals

In case of early withdrawal, subjects will be asked to continue (at least limited) scheduled evaluations and to complete an end-of-study evaluation which includes all scheduled exams, procedures, and laboratory tests as if it is the 12 Month visit; and should be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable. If the subject is unwilling or unable to complete visits, he/she will be asked to return for the end-of-study visit and then should be followed through telephone calls by the coordinator. Subjects who do not complete study treatment or all follow-up will be asked to provide the same data as subjects who withdraw.

8.5 Subject Replacement

Subjects will not be replaced once randomized.

9. STATISTICAL CONSIDERATIONS

9.1 Outcomes

9.1.1 Primary Safety Objective

Baseline characteristics of participants will be summarized using descriptive statistics and tabulations. Evaluation of data will be guided by exploratory analyses. All results will be reported as point estimates (percentages for binary outcomes and means $\pm$ SD and median (range) for continuous outcomes) with corresponding interval estimates (two-sided 95% confidence intervals). No adjustments for multiple comparisons will be made and a p-value of 0.05 will be considered statistically significant.

These data are collected over time and will be longitudinal in nature with several pre-defined points of follow-up. We will use analytic strategies and methods applicable to such desig ns. For each participant in the cohort we have a set of "k" independent variables $\mathbf{x} = (x_1, x_2, ..., x_k)$, of which some may be time varying (e.g., blood pressure) and others do not change over time (e.g., gender). In addition, the outcome variables of interest are also measured repeatedly over time (e.g., temperature, signs of infection, neurological exams, CBC, chem-20). We plan to explore any changes over time between hospital discharge and long-term follow-up at 6 months, 1 year, and 2 years after cell treatment with repeated measure model analyses. In this study, we plan to explore and identify potential factors associated with time to SAEs, AEs, death *(refer to section 6.2)*, loss to follow-up (short term and long term), and improvements in functional outcomes *(refer to section 6.3)*. We plan to explore the factors associated with the time to an event using survival analysis methods which incorporate information about the timing of withdrawals, deaths, and functional improvements (if any) of subjects during the follow up period. We plan to first use descriptive statistics to explore the characteristics of the subjects among events and then will use the Kaplan-Meier method to estimate the time to an event. Combined, these secondary analyses may provide useful preliminary estimates for the design of future cell studies.

9.1.2 Secondary Objectives/Outcomes

The models proposed will be tested using general linear models (GLM). We will examine all variables to determine that they are relatively symmetric and unimodal, examine group variances for obvious deviations, and examine the residuals from each model to ensure reasonable adherence to the assumptions of normality and independence. If any variables violate the assumptions, we might transform skewed variables. We will evaluate the comparability of our groups on key demographic variables and will covary variables if necessary (e.g., socioeconomic status).
For Efficacy Outcome Measure 1 *(refer to section 1.2.1)*, we will use repeated measures ANOVA or ANCOVA (including intracranial volume as a covariate for macrostructure analyses) with Group (Treatment, Control) as the between-subjects factor and Time of Assessment (baseline, 1 year, and 2 years) and Age as the within-subjects factors. We

will examine the Age x Group x Time interaction and two-way interactions for each analysis and will trim non-significant terms from each model. If GM and WM volumes or DTI metrics vary across time, we will complete trend analysis to identify whether a linear or quadratic function best fits the data. Dependent variables will be evaluated in separate analyses. Dependent variables will be 1) the volume of whole brain gray matter, white matter, and CSF; 2) FA of core white matter pathways (corpus callosum, arcuate/superior longitudinal fasciculus), and 3) MD of gray matter in frontal and temporal cortical regions.

To examine brain-behavior relations *(Efficacy Outcome Measure 2, section 1.2.2)*, we will first examine the distribution of variables to determine whether assumptions are met for Pearson or Spearman correlation approaches. To evaluate strength of brain-behavior relations, outcome scores will be correlated with specific macrostructural and microstructural metrics from the DTI. For global outcome, the GOS-EC score will be correlated with gray and white matter volumes and corpus callosum FA. For neurocognitive outcomes, the accompanying table shows the expected relations between outcomes and gray and white matter microstructural metrics. We expect positive correlation of dependent variables with FA and negative correlation of dependent variables with MD. Similar to our recent approach (Juranek *et al*., 2012), brain-behavior analysis will also be completed using individual outcomes as dependent variables with group (Treatment, Control) and MRI/DTI variables as independent variables (e.g., examine the effect of group and FA of the corticospinal tract on fine motor scores; examine group and MD of dorsolateral frontal cortex on working memory scores).

Expected Relations of Neurocognitive Outcomes with Gray and White Matter Microstructure from Diffusion Tensor Imaging.		
Neurocognitive Outcome	Microstructure: Gray Matter MD	Microstructure: White Matter FA
IQ		Corpus callosum
Motor GMFM-88		Corticospinal
Verbal Fluency PPVT4/EVT2		Arcuate/superior longitudinal fasciculus
Learning/Memory NEPSY-II	Temporal cortex, hippocampus	

9.2 Sample Size and Accrual

As this is primarily a safety study, we should be able to ascertain serious AEs with 15patients/group. We have already identified approximately 15 patients who meet screening criteria for enrollment, thus accrual should not be problematic.

10. DATA COLLECTION, SAFETY MONITORING, ADVERSE EVENTS REPORTING

10.1 Records Retention

The period of paper and electronic record retention will be consistent with the record retention policies of the University of Texas Health Science at Houston and the applicable regulatory agencies for the trial, including the FDA and OHRP. However, in certain instances, documents should be retained for a longer period if required by the applicable regulatory agency.

10.2 Quality Assurance

The study will be conducted according to Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, US 21 Code of Federal Regulations (CFR) Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards.

All pediatric research at the University of Texas Health Science Center at Houston is subject to random audits throughout the year by a monitor from the institution's IRB as well.

10.3 Independent Medical Monitor (IMM)

A monitor from Juno Research Inc. will serve as the IMM. Juno Research has provided this service for previous TBI trials at UT Health and Children's Memorial Hermann Hospital. The IMM will provide oversight of the protocol and ongoing study activities with an emphasis on data integrity and quality assurance, protocol adherence, study participant safety issues, and in particular the review of AEs and SAEs. In addition to data quality, the IMM will review consent forms and regulatory documents as part of the monitoring plan.

The IMM will make recommendations to the PI regarding continuation, modification or conclusion of the trial, while protecting the confidentiality of the trial data. In the unlikely event of unexpected or unduly high rate of SAEs, the IMM will notify the MSM, the DSMB, and the PI of these findings. The IMM will review the medical record and data collected following post-infusion Day 21 of the first subject. Thereafter, they will review after subject 5, and each subsequent fifth subject, unless another visit is recommended by the MSM, DSMB, FDA or IRB.

10.4 Medical Safety Monitor (MSM)

George Carrum, MD will serve as the MSM. Dr. Carrum is faculty for the Department of Hematology/Oncology-Bone Marrow Transplantation at The Methodist Hospital and the Center for Cell and Gene Therapy in Houston, Texas. Dr. Carrum is experienced in the field of cellular therapies, including bone marrow harvest and transplantation. In addition, he is experienced in the release criteria of cellular products and potential AEs related to infusion toxicity. He has served on previous DSMBs, including having served as the DSMB Chair of the Phase 1 Pediatric TBI stem cell trial. Dr. Carrum's primary responsibility will be to review SAEs in real time to ensure good clinical practice and to quickly identify safety concerns. He may suggest protocol modifications to prevent the occurrence of particular adverse events. He will remain blinded to the treatment assignment, unless the DSMB approves unblinding, and will review SAE reports following post-infusion Day 21 of each subject (if applicable) for submission to the PI,

DSMB, FDA and IRB, and prior to each DSMB meeting. Dr. Carrum is not affiliated with UT Health in any manner, nor does he practice within the Memorial Hermann Hospital System. The MSM will work in conjunction with the IMM and the DSMB to ensure the safety of the intervention in this trial.

All reports of hospitalization due to infection or neurological events, and all subject deaths will be reported to the MSM.

10.5 Data Safety and Monitoring Board (DSMB)

Although this is a single site, relatively small sample size study, we plan to use a DSMB instead of a SMC-monitored approach. DSMB membership, responsibilities, meetings, and DSMB reports are described in the DSMB Charter (Appendix C).

10.6 Adverse Experience Reporting

10.6.1 Overview

All safety data will be reviewed by the Medical Safety Monitor (MSM) in real time, as well as, the Independent Medical Monitor (IMM) at regular monitoring visits according to the monitoring plan. Based on the data, the MSM may suggest protocol modifications to prevent the occurrence of particular adverse events. The data will also be reviewed by the DSMB at bi-annual meetings, or on an as-needed basis if required. The DSMB can recommend protocol modifications and/or study termination because of safety findings. All FDA, OHRP and the University of Texas Health Science Center at Houston IRB requirements for reporting adverse experiences will be followed.

10.6.2 Definitions [21CFR310.305]

10.6.2.1 Adverse Experience

Any adverse event associated with the use of a drug or biologic in humans, whether or not considered drug related. For this protocol, temporally association with the use of the cellular product will be considered.

10.6.2.2 Disability

A substantial disruption of a person's ability to conduct normal life functions.

10.6.2.3 Life-Threatening Adverse Experience

Any adverse experience that places the patient, in the view of the initial reporter, at *immediate* risk of death from the adverse experience as it occurred, *i.e.*, it does not include an adverse experience that, had it occurred in a more severe form, might have caused death.

10.6.2.4 Serious Adverse Experience

Any adverse experience that results in any of the following outcomes: Death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience 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.

10.6.2.5 Unexpected Adverse Experience

Any adverse experience not listed in the current labeling for the drug or biologic product. This includes events that may be symptomatically and pathophysiologically related to an event listed in the labeling, but differ from the event because of greater severity or specificity. “Unexpected," as used in this definition, refers to an adverse experience that has not been previously observed.

10.7 Recording Adverse Experiences

Safety data will be recorded on CRFs designed for this purpose. Adverse experiences will be collected from the time of randomization until completion of study visits or until 30 days after premature withdraw from the study. If an abnormal value or result is determined by the investigator to be clinically significant and/or temporally related, the adverse experience will be recorded on the appropriate CRF.

10.7.1 Grading Criteria

The severity of adverse events experienced by the subjects will be graded according to the criteria set forth in the National Cancer Institute’s Common Toxicity Criteria for Adverse Events Version 4.0. This document (CTCAE v4.0) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.
Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE:

Grade 1 = mild adverse event
Grade 2 = moderate adverse event
Grade 3 = severe and undesirable adverse event
Grade 4 = life-threatening or disabling adverse event
Grade 5 = death

10.7.2 Attribution Definitions

The relation, or attribution, of an adverse event or experience to the investigation product will be determined by the principal investigator and will be recorded on the CRF. The relation of an adverse event to the treatment will be determined according the definitions in the NCI-CTCAE.

10.8 Termination of the Cellular Product Infusion

If at any point during the cellular product infusion (BMMNCs or hUCB) the subject experiences symptoms associated with anaphylaxis (such as wheezing, blood pressure changes, rash, vomiting), the infusion will be stopped. The subject will continue to be enrolled and followed in the study through 2 years.

10.8.1 Stopping Rules

1. ANY death that is deemed by the DSMB to be related or possibly related to the study treatment.
2. Both P:F ratio < 70 AND PaC02 > 90 within the first 48 hours post BM infusion occurring in a single subject.
3. AST/ALT > 900 U/dl in the first 24 hours post infusion occurring in a single subject.
4. Grade 4 – 5 CNS cerebrovascular ischemia event or Grade 4 – 5 seizure event as defined in the NCI CTCAE v4.0 occurring within 12 hours of BMMNCs or Cord blood Cell re-infusion.
5. Any Grade 4 – 5 Adverse Event as defined in the NCI CTCAE v4.0 and determined to be temporally-related by the Medical Safety Monitor and/or DSMB.

10.9 Communication and Reporting Serious Adverse Events (SAEs)

Any grade 4 – 5 adverse event (CTCAE v4.0) deemed by the MSM and/or the DSMB to be temporally associated with the treatment will prompt cessation and trigger the stopping rules. Triggering the stopping rules will prompt notification of the FDA and IRB. If a patient that receives study treatment dies, and the DSMB determines that it may be related to the treatment, then the study will be terminated and a comprehensive safety review undertaken.

10.9.1 Timeline for Reporting SAE's

1. The PI will notify the MSM and DSMB of any communication from the FDA concerning the trial within 72 hours of notification.
2. MSM and DSMB written reports regarding SAEs will be submitted to the IRB within five working days.
3. Subject deaths will be reported to the MSM and IRB by telephone within 24 hours of discovery, and entered into IRB electronic database (iRIS) within five working days.
4. SAE's directly related to the cellular infusion will be reported to the MSM and IRB within five days of discovery and entered into the IRB electronic database (iRIS).
5. Other AEs do not need to be reported if the PI determines the AE is expected or due to the natural progression of the subject's underlying disease, however an internal log/record will be kept of these for tracking potential risks and reporting purposes.

11. HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects (CPHS), also known as the IRB. A signed consent form will be obtained from the parent(s) or Legally Authorized Representative (LAR) after a thorough explanation of the purpose of the study, the study procedures (including the bone marrow harvest/sham procedure, infusion, MRI, and assessment procedures), follow-up visits, potential risks/benefits, the time commitment involved, and measures to protect confidentiality. Adequate time will be given for questions. A copy of the signed consent form will be given to the parent(s)/LAR. In addition, a written note will be placed in the patient's medical chart documenting the communication between investigator and the parent(s)/LAR about the research.

11.2 Subject Confidentiality

To protect against loss of confidentiality, subjects' names will be replaced by a study identification number on case report forms, reports and on computer entry into the database. No identifying information will be mentioned in any presentations or publications. Strict security will be maintained, with limited access only to the password protected database, and all personnel involved will be trained to be aware of the importance of maintaining privacy. Clinical information will not be released without written permission of the subject (or parent(s)/LAR), except as necessary for monitoring by the DSMB, IRB, FDA, or other State or Federal regulatory agencies.

11.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the DSMB, IRB, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

12. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the Executive Committee. Any presentation, abstract or manuscript will make available for review by the sponsor prior to submission.

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14. APPENDIX A
Bone Marrow Group:

Study Procedures	Screening Period	Baseline Clinic Visit	Admission to MHH	Study Day 1 (Post-Infusion)	Study Day 2-21 (Post-Infusion)	* 6-Month Follow-Up Clinic Visit	* 1-Year Follow-Up Clinic Visit	* 1-Year Crossover Group Clinic Visit	Crossover Group Admission to MHH	Crossover Group Study Day 1 (Post-Infusion)	Crossover Group Study Days 2 - 21 (Post-Infusion)	* 2-Year End of Study Clinic Visit
Review of Medical Records	X	X						X				
Consent		[1]X						X				
Randomization		X										
History	X	X				X	X	X				X
Physical		X	X	X		X	X	X	X	X		X
Videotaped Motor/Cognitive Evaluation		X				X	X	X				X
Vital Signs		X	X	X	X	X	X	X	X	X	X	X
Infectious Disease Tests			[2]X									
Lab Evaluation		[3]X	[3]X	[3]X	[4]X	[3]X	[3]X	[3]X	[3]X	[3]X	[4]X	[3]X
Chest X-Ray			X	X		X	X		X	X		X
DT-MRI			X				X		X			X
Bone Marrow / Sham Harvest			X						[6]X			
Stem Cell or Placebo Infusion			X						[6]X			
Neuropsych Evaluation		X				X	X	X				X
Hospital Discharge				[5]X						[5]X		
Post-Infusion Safety Monitoring					X						X	
Evaluation for AE/SAEs		X	X	X	X	X	X	X	X	X	X	X

* The 6 month return visit window is +/- 14 days from infusion and +/- 21 days for the 1 and 2 year return visits.

[1] The informed consent document will be sent to the family/LAR during the screening period and reviewed by conference call before the baseline visit.

[2]. Infectious Disease Tests include HIV, Hepatitis, Syphilis, and West Nile Virus.

[3] Lab tests include CBC with Differential and Platelets, and Comprehensive Metabolic/Coagulation Panels.

[4] Post-infusion CBC and basic chemistry pannel on day 21 (+/- 7 days).

[5] Subjects will receive 24 hours of monitoring post-infusion before discharge.

[6]. Placebo Crossover to BMMNC harvest and stem cell infusion.

Cord Blood Group:

Study Procedures	Screening Period	6 - 8 Weeks Before Visit	Baseline Clinic Visit	Admission to MHH	Study Day 1 (Post-Infusion)	Study Days 2 - 21 (Post-Infusion)	* 6-Month Follow-Up Clinic Visit	* 1-Year Follow-Up Clinic Visit	* 1-Year Crossover Group Clinic Visit	Crossover Group Admission to MHH	Crossover Group Study Day 1 (Post-Infusion)	Crossover Group Study Days 2 - 21 (Post-Infusion)	* 2-Year End of Study Clinic Visit
Review of Medical Recods	X												
Consent			[1]X					X	X				X
History			X										
Physical			X	X	X		X	X	X	X	X		X
Infectious Disease and HLA Tests		[2]X							X				
CBR Release of Cord Blood Tests		[3]X							X				
Randomization		[4]X											
Shipment of Cord Blood		[5]X											
Videotaped Motor/Cognitive Evaluation			X				X	X	X				X
Vital Signs			X	X	X	X	X	X	X	X	X	X	X
Lab Evaluation			[6]X	[6]X	[6]X	[7]X	[6]X	[6]X	[6]X	[6]X	[6]X	[7]X	[6]X
Chest X-ray				X	X		X	X		X	X		X
DT-MRI				X				X		X			X
Stem Cell or Placebo Infusion				X						[8]X			
Neuropsych Evaluation			X				X	X	X				X
Hospital Discharge					[9]X						[9]X		
Post-Infusion Safety Monitoring						X						X	
Evaluate for AE/SAEs			X	X	X	X	X	X	X	X	X	X	X

***** The 6 month return visit window is +/- 14 days from infusion and +/- 21 days for the 1 and 2 year return visits.

1 The informed consent document will be sent to the family/LAR during the screening period and reviewed by conference call before the baseline visit.

2 Subject will have HLA & basic infectious disease tests prior to baseline visit. Maternal infectious disease tests will also be required if not avialable from CBR.

3 After the signed informed consent document is received, CBR will be contacted to release cord blood information (HLA Type, Cell Count, Contaminates).

4 Cord blood subjects will be randomized after comfirmation of viable cord blood stem cells, confirmation of HLA match, and neg. infectious disease test results.

5 CBR will ship the frozen hUCB to the GSCL 7 to 14 days before the visit. Umbilical cord blood for subjects assigned to the placebo group will not be released from CBR until the 1 year cross-over visit.

6 Lab tests include CBC with differential and platelets, comprehensive metabolic and coagulation panels, and hepatic function tests.

7 Post-infusion CBC and basic chemistry pannel on day 21 (+/- 7 days).

8 Cross-Over Group Stem Cell infusion.

9 Subjects will receive 24 hours of monitoring post-infusion before discharge.

15. Appendix B: Table of Psychological and Functional Outcome Tests

Age	Motor	Adaptive Behavior	Visual-Spatial Processing	Learning	Language
2-2.5	GMFM-88	VABS2	--	--	--
2.6-3	GMFM-88	VABS2	--	--	PPVT4/EVT2
3	GMFM-88	VABS2	--	NEPSY-II	PPVT4/EVT2
4	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2
5	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2
6	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2
7	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2
8	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2
9	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2
10	GMFM-88	VABS2	MVPT-3	NEPSY-II	PPVT4/EVT2

16. Appendix C: DSMB CHARTER

DATA AND SAFETY MONITORING BOARD CHARTER

Protocol Title:	Autologous Cell Therapies for Cerebral Palsy-Chronic (ACT for CP)
Principal Investigators:	Charles S. Cox, Jr., MD, Sean Savitz, MD
Co-Investigator:	Fabio Triolo, PhD, Maria Matuszczak, MD, Jenifer Juranek, PhD, Allison Dempsey, PhD, Claudia Pedroza, PhD
Study Staff:	Joiya Arrington, MSN, RN, Steven Kosmach, MSN, RN, CCRC
Protocol Number:	HSC-MS-12-0876
BB IND Number:	[redacted]
Sponsor:	CBR, Inc., TIRR Foundation, Let's Cure CP Foundation
Document Version Date:	18 OCT 2017

Purpose:
The purpose of this charter is to define the responsibilities of this Data and Safety Monitoring Board (DSMB), its membership, and timing of meetings. It will also provide the procedures used to carry out these responsibilities.

DSMB Responsibilities:
The DSMB has the following responsibilities:

- Review and sign this charter signifying understanding of responsibilities;
- Review the research protocol, informed consent documents and plans for data safety and monitoring;
- Evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial site, and other factors that can affect study outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- Make recommendations to the PI about continuation, termination, or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- If appropriate, conduct interim analysis of efficacy in accordance with stopping criteria which are clearly defined in advance of data analysis and have the approval of the DSMB;
- Ensure the confidentiality of the trial data and the results of monitoring; and,
- Recommend solutions to address problems with study conduct, enrollment, and sample size and/or data collection.

Membership:
The DSMB will consist of at least three ~~four~~ members. Three members will constitute a quorum. All members must be completely independent of the trial and have no financial, scientific, or other conflict of interest with the trial. Collaborators or associates of Charles Cox, MD, and Sean Savitz, MD, are not eligible to serve on the DSMB. The DSMB includes

experts in or representatives of the fields of relevant clinical expertise, clinical trial methodology, and biostatistics.

The Chair is responsible for overseeing the meetings, and developing the agenda in consultation with the Principal Investigator. The chair is the contact person for the DSMB. DSMB membership is for the duration of the clinical trial. If any members leave during the course of the trial, the Principal Investigator will appoint their replacement.

Names, Affiliations and Contact Information

DSMB Chair:	George Carrum, MD
Affiliation:	The Methodist Hospital, Dept. of Hematology/Oncology-Bone Marrow Transplantation
Phone:	713.441.1450
Mobile:	713.562.0307
Email:	gcarrum@tmhs.org
Voting Member:	Ian Butler, MD
Affiliation:	UTHealth, Professor of Pediatric Neurology
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Voting Member:	Amir Khan, MD
Affiliation:	Medical Director, Children's Memorial Hermann Neonatal ICU
Contact Information:	Heather Justice (Executive Assistant) 713.500.5733 / heather.r.justice@uth.tmc.edu
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DSMB Coordinator	Steven Kosmach, MSN, RN, CCRC
Affiliation:	UTHealth, Medical School, Dept. of Pediatric Surgery, Children's Program in Regenerative Medicine
Phone:	713.500.7329
Mobile:	713.505.6883
e-mail:	steven.kosmach@uth.tmc.edu

DSMB members will disclose any real or perceived conflict(s) of interest. The DSMB will then determine the appropriateness of the member continuing to serve on the Board.

Operational Plan:
This DSMB will abide by its charter. The charter may be amended by a quorum vote of the Board at a scheduled meeting. The proposed amendment must be provided to the membership a minimum of ten business days prior to the proposed decision date.

Estimated Time Commitment:
DSMB members acknowledge the *estimated* time commitment serving on the DSMB as listed below. The *estimate* of time may be greater or less, depending on enrollment numbers and adverse events reported.

- Treatment summary reviews may take approximately 30 minutes.
- Two or three subjects are projected to enroll each month and then enter the long-term follow-up phase of the study. Pending barriers to recruitment, it is anticipated that all baseline visits the 30 subjects will be completed by the end of 2014.
- The DSMB will meet every 6 months or as needed. The estimated duration of each meeting is 1 hour.

Protocol Information:
DSMB members will be provided a full copy of the protocol along with pertinent documents prior to the first meeting. Documents may be provided via secure email or in hard copy. Hard copies of any documents are considered confidential, and will be mailed via intra-office mail or certified carrier. Updated or new documents will be distributed by the DSMB coordinator.

Scheduled Reports:
The study team should provide reports at least a week prior to the date of the meeting. The study team must submit subject summary reports to the DSMB Chair and Medical Safety Monitor within 30 days of each completed baseline visit, and thereafter, prior to each DSMB meeting, or as requested by the DSMB..

DSMB Report Format:
DSMB reports will include the following:

1. Accrual/Recruitment Information – include number of patients screened, enrolled, completed, withdrawn and reasons for withdrawals if any. For multi-site studies include distribution by site.
2. Characteristics of Subjects Enrolled – include gender, ethnicity (Hispanic/ Non-Hispanic), race: White, Black/African American, etc.
3. Study information – table with age, gender, missed doses, extra doses if any, PTT, whether hypertensive or not, NIH stoke scale before and after intervention.
4. Adverse events – include description, grade, expectedness, relatedness.
5. Compliance with protocol – include protocol deviations.
6. Any other information as requested by the DSMB or Study P.I.

Safety Reports:

1. Serious Adverse Events (SAE) that are determined by the PI to be unexpected + related will be submitted to the DSMB within 7 calendar days of the determination by telephone or fax; Submit written report no later than 15 calendar days of the determination.
2. All other SAEs will be collected and submitted with the standard DSMB reports as described above.

Meetings:
The first meeting will be held at trial initiation and after the 5th subject has completed the baseline infusion visit. Subsequent meetings will be held after every 6 months, or as needed, at the discretion of the Chair to address safety concerns.

Meetings may be in person or via teleconference, depending upon the schedule of the members. If members of the DSMB cannot attend a scheduled meeting, the members present *may* decide the meeting will be cancelled and rescheduled to reconvene at the earliest possible time.

The Principal Investigator and research team will be notified of planned meetings no less than three weeks prior to the meeting date. The Principal Investigator will be expected to submit the required reports at least two weeks before to the scheduled meeting.

The Principal Investigator and/or study team members will attend regularly scheduled open DSMB meetings to provide specific clarification or respond to issues. The DSMB may invite guests to meetings for their expertise or for needed information. Discussion will focus on the conduct and progress of the study with special attention to the aggregate safety and efficacy data. The Principal Investigator may present summary statements of previously submitted updates, adverse events, etc. Review and discussion of unblinded primary outcome data will be limited to voting DSMB members during closed session meetings, or at the discretion of the DSMB Chair.

Recommendations:
Following a DSMB review, the board must submit a written report to the PI. The options available for the outcome of the review are:
1. Recommend continuation with no modification,
2. Recommend continuation with modification(s) to protocol,
3. Recommend suspension of enrollment pending additional information,
4. Recommend suspension of all trial activities pending additional information,
5. Recommend termination of trial.

Recommendations to the Principal Investigator must have majority approval by the Board members. Prior to dissemination, the Chair will review summary statements and recommendations from DSMB members.

Distribution of DSMB Report:
The DSMB Chair will formalize the recommendations in secure email or formal letter and forward to the DSMB Coordinator for forwarding to the PI. The DSMB coordinator should forward the DSMB's final recommendations to the Principal Investigator within 2 weeks of the meeting. The Principal Investigator is responsible for dissemination to the study team, IRB, FDA, and any other entity.

Confidentiality:
All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

DSMB Member Acknowledgement:
DSMB members will confirm their understanding of the DSMB Charter, roles, responsibilities and disclosure of conflict of interests by e-mail acknowledgement.

17. Appendix D: Maternal Infectious Disease Testing

FDA 21 CFR part 1271 requires donor eligibility determination, based on donor screening and testing for relevant communicable diseases and disease agents, for all human cells, tissues and cellular & tissue-based products (HCT/P) collected on or after May, 2005. However, 21 CFR part 1271.90 allows exceptions to this requisite and also elaborates on the labeling requirements for these special cases. The relevant exception to this protocol states that "You are not required to make a donor eligibility determination under 1271.50 or to perform donor screening or testing under 1271.75, 1271.80 and 1271.85 for: (1) Cells and tissues for autologous use".

Nevertheless, we have chosen to perform maternal infectious disease markers (IDM) testing in this protocol. The maternal IDM results (if available) will be released to the research team along with the cord blood sterility and cell count information after informed consent and permission for releasing the test results have been obtained from the parent(s). The IDM tests include: Human Immunodeficiency Virus (HIV) type 1, HIV type 2, Hepatitis B virus, Hepatitis C virus, Human T-cell lymphotrophic virus (HTLV) type 1, HTLV type 2, and Syphilis.

Again, donor eligibility will not be determined for autologous infusions but specific labeling rules will be applied. The subject will be excluded from the study if any IDM result is positive or reactive EXCEPT for the following cases:

- Donor is CMV positive. Since CMV infection is not a relevant communicable disease or disease agent, the product will not be excluded but will require specific labeling.
- If donor mother screens HBcAb reactive and HBsAg non-reactive, the product will not be excluded but will require specific labeling.
- For syphilis, if donor tests reactive on a NON-treponemal screening and non-reactive on a specific treponemal confirmatory test, the product will not be excluded but will require specific labeling.

Per CBR, less than 10% of cord blood collections do not have maternal IDM testing performed. In the event that a subject meets criteria for study entry, but CBR does not have the maternal IDM results, the parent(s) will be able to submit IDM results that were performed during the hospital admission for delivery. If the IDM tests were not completed during the pregnancy, the mother will be required to have IDM testing completed at her expense and the correspondent lab results submitted to the PI.

Whenever applicable, HCT/P must be prominently labeled per 21 CFR 1271.90 as follows:

Label Type	Usage
"FOR AUTOLOGOUS USE ONLY"	On all autologous products
"Warning: Advise Patient of Communicable Disease Risk"	If the result of any screening or testing performed indicate the presence of relevant communicable disease agent. For example, medical and/or family history puts donor at high risk for acquiring communicable disease agent.
"Warning: Reactive test results for (name of disease agent or disease)"	If the result of any screening or testing performed is positive or reactive
"Advise recipient that screening and testing of the donor is not performed at the time of cryopreservation of the cells or tissues but have been performed subsequently"	Screening or testing not performed at the time of cryopreservation but at later time

Refer to the Chemistry and Manufacturing Section of the IND for more detailed information.