Phase 2 Study of Allogeneic Umbilical Cord Blood Infusion for Adults with Ischemic Stroke - CoBIS 2

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PROTOCOL SUMMARY

Title:	Phase 2 Study of Allogeneic Umbilical Cord Blood Infusion for Adults with Ischemic Stroke – CoBIS 2
Summary:	This is a multicenter, placebo controlled, randomized, double-blinded Phase 2 study in 100 subjects 18-90 years of age who have sustained a recent ischemic stroke. Potential subjects can be screened on the day of the primary stroke event, after confirmation of ischemic stroke, but neurological status must be confirmed within 24 hours prior to infusion. Consent may be obtained ≥ 24 hours following stroke onset (Day 1), but no later than 10 days after the index stroke event. Treatment with umbilical cord blood (UCB) cells or placebo will be administered intravenously as a single infusion as early as 3 days but no later than 10 days after the patient's stroke. Administration of UCB cells or placebo diluent will only be done after confirmation of eligibility criteria. UCB units will be selected from an accredited U.S. public cord bank based on blood type and a targeted cell dose ranging between 0.5 to 5 x 10^7 total nucleated cell count (TNCC)/kg. Study patients will not receive immunosuppressive or myeloablative medications prior to infusion of the cord blood or placebo. All subjects, families and medical staff will be blinded to treatment arm. When a subject is randomized to study drug at a clinical site without a cord blood bank, the selected cord blood units (CBU) will be shipped frozen overnight to the site. Once selected and available on site, each CBU will be thawed, washed, tested, released and infused intravenously using common standard operating procedures (SOPs) at all sites. For subjects randomized to placebo, a diluent with the same appearance and odor as a CBU will be prepared. Patients will have magnetic resonance imaging (MRI) at baseline and will be assessed at 1, 3, 6, and 12 months for functional outcomes. All patients will receive standard of care therapy while enrolled in this study and all subjects will be strongly encouraged to participate in rehabilitative therapy.
Objectives: **Primary**	To determine, in a randomized, placebo controlled trial, the efficacy of a single intravenous (IV) infusion of unrelated donor UCB for improving functional outcomes in patients with ischemic stroke

Secondary objectives	1. To describe the safety and tolerability of a single IV infusion of unrelated donor UCB in patients with ischemic stroke 2. To evaluate the efficacy of a single IV infusion of unrelated donor UCB for improvement of neurological symptoms following ischemic stroke 3. To evaluate the efficacy of a single IV infusion of unrelated donor UCB for improvement in quality of life and emotional and cognitive status in patients with ischemic stroke
Exploratory objectives: The following may be pursued if adequate data are available:	To determine whether biomarkers of brain inflammation, neuronal injury, and repair are differentially expressed as a function of UCB administration
Endpoints	
Primary endpoints:	The primary endpoint is the shift in modified Rankin Scale (mRS) from baseline to 3 months post infusion.
Secondary endpoints:	1. Safety and tolerability of donor UCB infusion in adults with ischemic stroke will be assessed by the following: a. Incidence and severity of infusion reactions b. Incidence and severity of product-related infections c. Evidence of alloimmunization via anti-HLA and anti-RBC antibodies and nonspecific markers of systemic inflammation (including erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]) d. Incidence and severity of graft vs. host disease e. Incidence and severity of unexpected adverse events (AEs), by relation to study product f. Mortality 2. Functional independence at 90 days defined as a 90-day mRS score of 0, 1, or 2 3. mRS shift score at 30 and 180 days post infusion 4. The National Institutes of Health Stroke Scale (NIHSS) score at 90 days 5. The Barthel Index (BI) at 90 days 6. The Stroke Impact Scale-16 at 90 days

	7. The European Quality of Life (EQ-5D-3L) survey at 90 days 8. Patient Health Questionnaire Scale (PHQ-8) at 90 days 9. Telephone Interview for Cognitive Status (TICS) total score at 30 days and 12 months 10. Stroke Inventory Test Battery scores at 90 days
Exploratory Endpoints	Inflammatory cytokines and biomarkers of brain injury and repair (for example, S100 calcium-binding protein B [S100B], TNFα, IL 1β, IL-4 and IL-10, VEGF, BDNF, MMP9) in peripheral blood at baseline, within 6-24 hours after UCB infusion, and 90 days (Research Blood Samples)
Population:	The target population is 100 patients ages 18-90 years with recent, acute, cortical, hemispheric, ischemic stroke in the middle cerebral artery (MCA) distribution without a clinically significant midline shift as detected by MRI as a DWI abnormality. Eligible patients will have National Institutes of Health Stroke Scale (NIHSS) scores of 6-15 (R) and 6-18 (L) at the time of informed consent. Subjects with >4 point increase of NIHSS from time of consent (worsening of score) will not be eligible for infusion. Patients must have a platelet count >100,000/uL, hemoglobin >8gm/dL, absolute lymphocyte count (ALC) ≥ 1200 for African American patients and ≥1500 for all other racial-ethnic groups, and white blood cells (WBC) >2,500/uL ***OR*** Historical pre-stroke value of ALC ≥ 1200 for African American and ≥1500 for all other racial-ethnic groups within 6 months of stroke **And** a post stroke ALC value of ≥ 1000, platelet count >100,000/uL, hemoglobin >8gm/dL and WBC >2,500/uL. Patients who have received tissue plasminogen activator (tPA) or undergone endovascular reperfusion may be included in the study if they are otherwise eligible.
Phase:	2

Number of centers enrolling participants:	up to 6
Description of Study Agent:	ABO/Rh matched CBU(s) with a minimum of 0.5×10^7 TNCC/kg based on the pre-cryopreservation TNCC
Study Duration:	Approximately 36 months: 20-24 months of accrual to enroll 100 patients, plus 12 additional months to the last patient's final follow-up assessment.
Participant Duration:	The duration of the study for the patient will be 12 months from the initial infusion. Patients will have a baseline MRI and will be assessed at 1, 3, 6, and 12 months for functional outcomes.

Summary of Amendment 19, February 2018

The protocol was amended as follows:

- Co-investigators:
  - Doctors Nagaraja, Vela Duarte and Belagaje from the University of Florida, University of Colorado, and Emory, respectively, have been added as co-investigators
- Protocol summary, population:
  - Revised "time of enrollment" to "time of informed consent" to add clarity surrounding the timing for the NIHSS used to confirm eligibility.
  - Revised language to clarify timing of screening and consent.
  - Revised language to clarify that subjects with >4-point increase of NIHSS score from time of consent will not be eligible for infusion.
  - Revised ALC language.
- Section 2.5.2.6 Stroke Impact Scale-16 (SIS-16):
  - Time to administer SIS-16 was included.
- Section 2.5.2.7 Telephone Interview for Cognitive Status (TICS)
  - Added guidance for administration of TICS with an aphasic patient.
- Section 3.1 Study Design Overview and Duration:
  - Revised language to provide clarity surrounding the timing for the NIHSS to be used for confirming eligibility.
- Figure 3:
  - Revised footnote to include additional detail and clarity surrounding the timing for screening and consent.
- Section 3.2.2 Inclusion Criteria:
  - ALC language has been updated as described in the protocol summary revision above.
  - Revised NIHSS inclusion criteria #3 to clarify timing of assessment.
  - Revised NIHSS language to clarify that subjects with >4-point increase of NIHSS from time of consent will not be eligible for infusion.
- Section 3.2.3.2 Prohibited Concomitant or Prior Therapies:
  - #6 – revised to add clarity regarding timing.
  - #8 – revised to add clarity regarding timing.
- Section 4.7 Care During Unexpected Events:
  - Revised language describing care during onset of a fever event so it reflects maximum temperature in degrees Celsius and Fahrenheit.
  - Included guidance on how to handle a fever on the day of infusion and that the infusion should be postponed if fever >38.1° C (100.5°F). If the temperature drops and patient stabilizes to 38.1° C (100.5°F) and remains no higher than this for 24 hours, then the infusion may proceed as planned.
- Table 5:
  - Revised column header from "UCB or Placebo Infusion" to "Day of Infusion"
  - Added guidance regarding the timing for pre-medications on the day of infusion.
  - Footnote a – revised to add clarity surrounding the timing for screening and consent.

  - Foot note b – revised wording to increase clarity from "pre- and post-infusion for day of infusion" to "pre- and post-infusion on the day of infusion".
  - Footnote f – Added "(voluntary)" after (APOE) genotyping to indicate that this is optional and patients may opt out of this assessment.
- Section 5.4.1 Screening and Consent (Day 1-10)
  - Revised the first bullet to add additional clarity surrounding the timing for screening and consent.
  - Added "(Optional)" in front of "Buccal swab" to clarify that this analysis is voluntary and not required for participation.
- Section 5.4.2 Day of Infusion (Day 3-10), assessments
  - Added "(Optional)" to clarify that collection of research blood samples for biomarkers/immune-panel analysis is in-fact, voluntary.
  - Revised language surrounding NIHSS to clarify that the assessment should be performed prior to the start of the infusion and administration of pre-meds.
  - Added language to clarify that the neurological and physical exams should be performed before pre-meds and infusion and post infusion.
  - Added language to ensure that the safety questionnaire was also performed during the infusion visit
  - Added guidance to clarify that if on the day of infusion the patients NIHSS is <6, they will remain eligible for infusion.
  - Added guidance to clarify that subjects with >4 point increase of NIHSS from time of consent (worsening of score) will not be eligible for infusion.
- Section 5.4.3 Follow-up phone call or visit 24-hour post infusion
  - Added "(Optional)" to clarify that collection of research blood samples for biomarkers/immune-panel analysis is in-fact, voluntary.

Summary of Amendment 05, September 2017

The protocol was amended as follows:

- Protocol summary (summary):
  - Removal of race as criteria for selection of UCB unit.
  - Removal of "baseline" from "Patients will have *baseline* magnetic resonance imaging (MRI) at baseline and will be assessed at 1, 3, 6, and 12 months for functional outcomes."
- Protocol summary (population) – Revised population summary (page 4) – added "and ALC ≥ 1200 for African American patients and ≥1500 for all other racial-ethnic groups."
- Protocol summary (exploratory endpoints) - Included "Research Blood Samples" to further define 90-day exploratory endpoints.
- Protocol summary (number of centers enrolling) – Expanded from "4 centers" to "4 up to 6 centers".
- Section 1.3.2 – Added a brief description of the aims for the spleen sub-study.
- Section 2.0 – Removed race from selection criteria for cord blood units.
- Section 3.1 – Removed race as selection criteria for CBUs.
- Section 3.2.2:

  - Modified inclusion criteria to reduce ALC for African Americans to ≥1200 from ≥1500.
  - Modified inclusion criteria by removing race from selection criteria for CBUs.
- Section 3.2.3.1 – Amended exclusionary medical conditions so that pregnancy can be documented by blood test or lactating
- Section 3.2.3.3 – Removed section. The exclusion for pregnant or lactating women is documented in section 3.2.3.1 (#18). The exclusion of those unable or unwilling to be evaluated for follow-up visits is covered in section 3.2.2 (#9).
- Section 4.1 – Removed race from selection criteria for cord blood units.
- Section 4.2 – Removed race from selection criteria for cord blood units.
- Section 4.5 – Updated text to reflect that the placebo, along with the previously mentioned cells, expire after 6 hours at room temperature.
- Section 4.6:
  - Updated when vital signs should be checked to "before the infusion, every 5 minutes during the infusion, at the end of the infusion, then as scheduled in MOP-01 until 4 hours post infusion."
  - Replaced vital signs "oxygen saturation" to "pulse oximetry".
- Table 5:
  - Replaced "safety follow-up" with "safety questionnaire"
  - "Physical" and "Neurological" exams have been merged.
  - "Biomarker panel" is now referred to as "Research Blood Samples".
  - Updated foot note references.
  - (d)Added comment in footer regarding tube type and volume for HLA typing being sent to MD Anderson.
  - (d and e) Included mention of ESR and CRP tests in footnotes.
  - (f) Added "or at 90-day visit" to "Apolipoprotein E (APOE) genotyping will be performed on a buccal swab sample collected during screening or at any time prior to hospital discharge *or at 90 day visit".*
  - (g) revised "biomarker/immune panel" to "research". Also included information about tube types and volumes.
  - (h) Revised to "before administration of premeds"; previously "post-infusion".
- Section 5.4.1:
  - Added ESR and CRP tests to screening visit.
  - Revised order of assessments/procedures to be consistent with Table 5.
  - The following laboratory tests were excluded from the restriction of collection within 48 hours of screening: the ABO/Rh typing, lipid profile, coagulation panel, CBC and differential, and troponin. If these were performed as standard of care upon admission, the results can be used for screening, even if they are greater than 48 hours old.
  - Added "or at 90-day visit" to "Buccal swab collected for APOE genotyping at screening, prior to hospital discharge *or at 90 day visit*."
- Section 5.4.2:
  - Updated when vital signs should be checked to "before the infusion, every 5 minutes during the infusion, at the end of the infusion, then as scheduled in MOP-01 until 4 hours post infusion."
  - Removal of post infusion scan of spleen

  - Revised order of assessments/procedures to be consistent with Table 5.
  - Removal of "Neurological exam (post infusion)" from assessments.
  -
- Section 5.4.3:
  - Wording update - "Blood" samples for biomarkers/immune-panel analysis was updated to "research blood samples".
  - Revised order of assessments/procedures to be consistent with Table 5.
- Section 5.4.4:
  - Replaced "safety follow-up" with "safety questionnaire".
- Section 5.4.5:
  - Replaced "safety follow-up" with "safety questionnaire".
  - Removed "HLA typing" from laboratory tests.
  - Wording update - "Blood" samples for biomarkers/immune-panel analysis was updated to "research blood samples".
  - Removal of "Optional scan of spleen at selected sites".
  - Added ESR and CRP tests to 90-day post infusion visit.
  - Revised order of assessments/procedures to be consistent with Table 5.
- Section 5.4.6:
  - Replaced "safety follow-up" with "safety questionnaire".
  - Revised order of assessments/procedures to be consistent with Table 5.
- Section 6.1:
  - Increased the estimated number of research participants to be enrolled each month from "4" to "4-6".
  - Increased the number of centers from "4" to "4-6".
- Section 6.7 - Increased the number of centers from "4" to "4-6".

Summary of Amendment 1, May 2017

The protocol was amended in order to provide consistency between sections and clarification to study procedures, as well as to correct typographical errors. The following changes were made:

- Section 1.5.2:
  - Total number of AE's reported as of June 2016 was updated to 115 (previously 88).
  - The number of serious AE's was changed from 4 to 8, 6 of which occurred in one study patient and 2 in a second patient.
  - The SAE incidence rate, defined as the number of patients experiencing an event divided by the number receiving treatment, was revised to 20% (previously 10%)
- Table 3:
  - Corrected the age of patient number 6 to 51 years (previously 41).
- Added a new table:
  - "Table 4: CoBIS Phase 1 – Outcomes and Adverse Events"
- Section 4.3
  - Changed to "If able" ABO will be measured after thawing to cross-check pre-cryopreservation data prior to infusion.

- Section 4.6:
    - Added time requirement for measuring vital signs during the infusion – Now reads "Vital signs (heart rate, blood pressure, temperature, respiratory rate, oxygen saturation) will be checked before the infusion, every 5 minutes during the infusion, at the end of the infusion, every 15 minutes for 1 hour after the infusion and then hourly until 4 hours post infusion."
- Table 5:
    - Removed "Eligibility".
    - Separated "Physical Exam" and "Neurological Exams"
    - Added "Vital Signs"
    - Renamed "Clinical lab testing" to "Laboratory testing"
    - Renamed "Research lab" to Buccal swab"
    - Added "Biomarker Panel"
    - Added "Pre-medications", "Infusion of UCB or placebo", "IV hydration" and "TICS"
    - Removed "Safety questionnaire".
    - Updated the schedule for "Safety follow-up" and "Concomitant medications"
    - Updated performance criteria for Apolipoprotein E genotyping so that buccal swab sample collection may be collected during screening or "at any time prior to hospital discharge."
    - [h] now indicates "Post infusion"
    - Added a new note - [l] = NIHSS should be done 24 hours post infusion and daily post infusion for 7 days or until discharge
- Section 5.4.1:
    - Revised "History" to "Medical History"
    - Revised "Physical" to "Physical exam"
    - Revised "Clinical Laboratory Tests" to "Laboratory Tests"
    - Revised criteria for NIHSS performance to "should be performed daily beginning the day of consent through the day of infusion (prior to infusion of the UCB cells or placebo), within 24 hours of administration of post-infusion fluids, and for up to 7 days post-infusion or until discharge."
- Section 5.4.2:
    - Added a requirement for the review of pre-infusion labs by study staff which reads: "Results of labs related to the inclusion/exclusion criteria should be reviewed by the study staff to verify that the subject remains qualified for the study prior to infusion. Results for the HLA typing, infectious disease panel, direct and indirect Coombs, and HLA antibody screen do not need to be final prior to infusion. In addition, clinical labs should be reviewed by the PI or designee in order to establish that there are no contraindications to infusion of the cord blood."
    - Added criteria for neurological exam which now reads: "Neurological exam is conducted prior to administration of premeds and pre- and post-infusion for day of infusion. If subject is to be discharged less than 24 hours post infusion, the physical and neurological exam should be performed after administration of post-infusion fluids but prior to discharge."

  - Added criteria for administration of pre-meds which reads: "Administration of pre-meds 30-60 minutes prior to the start of the infusion. Premedication consists of diphenhydramine 0.5mg/kg/dose IV (maximum 50 mg), hydrocortisone 1mg/kg /dose IV (maximum 100 mg), and acetaminophen 10-15mg/kg (maximum 650 mg orally [PO] or per rectum [PR]). Antihypertensives may be administered if clinically indicated or should be made available if needed in a timely manner."
  - Revised post-infusion cardia-respiratory hemodynamic monitoring and vital signs to "15 minutes for 1 hour after the infusion and then hourly until 4 hours post infusion."
  - Revised NIHSS assessment criteria which now reads "within 24 hours after administration of post-infusion fluids"
- Section 5.4.3:
  - Added the following note "NIHSS should be done within 24 hours of administration of post-infusion fluids and daily for 7 days post-infusion or until discharge."

- Section 5.4.4:
  - Removed "Safety questionnaire"

- Section 5.4.6:
  - Removed "Safety questionnaire"

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INVESTIGATOR SIGNATURE PAGE

Phase 2 Study of Allogeneic Umbilical Cord Blood Infusion for Adults with Ischemic Stroke – CoBIS 2

Protocol Version 4.0, 19 February 2018

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

I agree to comply with all other requirements regarding the obligations of clinical investigators according to FDA regulations and guidance, and to conduct this study according to the principles of the International Council on Harmonisation E6 Guideline on GCP and the principles of the World Medical Association Declaration of Helsinki. I will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

I agree to ensure that all people assisting in the conduct of this study are informed in meeting the above commitments.

_____

(Investigator's printed name)

_____

(Investigator's signed name)

ABBREVIATIONS

Abbreviations:

ACTIsSIMA	Study of Modified Stem Cells (SB623) in Patients with Chronic Motor Deficit From Ischemic Stroke
ADL/ IADL	Activities of daily living/instrumental activities of daily living
AE	Adverse Event
ALC	Absolute Lymphocyte Count
ANC	Absolute Neutrophil Count
anti-HBc	Hepatitis B Core Antibody
APC	Antiphospholipid Syndrome
ApoE	Apolipoprotein E
BDNF	Brain Derived Neurotropic Factor
BI	Barthel Index
BMSC	Bone Marrow Stromal Cell
CBU	Cord Blood Unit
CCBB	Carolinas Cord Blood Bank
CDE	Common Data Elements
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CHI	Catholic Health Initiative
CIBMTR	Center for International Blood and Marrow Transplant Research
CLIA	Clinical Laboratory Improvement Amendments
COWAT	Controlled Oral Word Association Test
CMV	Cytomegalovirus
CNS	Central Nervous System
CoBIS 2	Phase 2 Study of Allogeneic Umbilical Cord Blood Infusion for Adults with Ischemic Stroke
CP	Cerebral Palsy
CRF	Case Report Form
CRP	C-Reactive Protein
CT	Computed Tomography

CTCAE	Common Terminology Criteria for Adverse Events
CTX	CTX0E03 human neural stem cell product (ReNeuron; Surrey, England)
DMSO	Dimethyl Sulfoxide
DSMB	Data Safety Monitoring Board
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
EBST	Elevated Body-Swing Test
ESR	Erythrocyte Sedimentation Rate
EQ-5D	European Quality of Life
FDA	Food and Drug Administration
FDR	False Discovery Rate
FGF	Fibroblast Growth Factor
F-MA	Fugl-Meyer Assessment
FSPGR	Fast Spoiled Gradient Echo
GCP	Good Clinical Practice
GvHD	Graft versus Host Disease
H0	Null hypothesis
HA	Alternative hypothesis
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIE	Hypoxic Ischemic Encephalopathy
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HTLV I/II	Human T cell Lymphotropic Virus-1 and -2
HUCB	Human Umbilical Cord Blood
HVLT-R	Hopkins Verbal Learning Test-Revised
ICH	Intracerebral Hemorrhage
IL-1-ß	Interleukin-1-beta
IL-2	Interleukin-2
IRB	Internal Review Board
ISBT	International Society of Blood Transfusion
IV	Intravenous

LAR	Legally Authorized Representative
LUQ	Left Upper Quadrant
MCA	Middle Cerebral Artery
MCAO	Middle Cerebral Artery Occlusion
MMP9	Matrix Metallopeptidase 9
MMSE	Mini-mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
MSC	Mesenchymal Stem Cells
NAT	Nucleic Acid Testing
NCT	National Clinical Trial [identifier number]
NIH	National Institute of Health
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NM	Neuromuscular
NSS	NeuroSeverity Score
OR	Odds Ratio
PHQ-8	Patient Health Questionnaire 8
PI	Principal Investigator
PISCES	Pilot Investigation of Stem Cells in Stroke
PR	Per Rectum
PRA	Panel Reactive Antibody
RR	Rotarod
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SB623	Modified Allogeneic Mesenchymal Stem Cells Derived from Bone Marrow of Healthy Human Adult Donors (SanBio, Inc; Mountain View, CA)
SDMT	Symbol Digit Modalities Test
SIS-16	Stroke Impact Scale-16
SITB	Stroke Inventory Test Battery
SNR	Signal to Noise Ratio
SOP	Standard Operating Procedure

SUSAR	Suspected Unexpected Serious Adverse Reaction
TC-199	Tissue Culture Medium 199 (pink)
TICS	Telephone Interview for Cognitive Status
TNCC	Total Nucleated Cell Count
TNFα	Tumor Necrosis Factor-alpha
tPA	Tissue Plasminogen Activator
UCB	Umbilical Cord Blood
UCMSC	Umbilical cord mesenchymal stem cell
ULN	Upper Limits of Normal
VEGF	Vascular Endothelial Growth Factor

1 BACKGROUND AND HYPOTHESIS

1.1 Overview

Stroke is a major cause of death and long-term disability, affecting one in every six people worldwide. Approximately 795,000 Americans suffer a stroke each year, 140,000 of which result in fatality, making stroke the third leading cause of death in the US.[2-4] Although stroke can occur at any age, the majority (~75%) occur among individuals over the age of 65, and risk of stroke more than doubles each decade after the age of 55. The costs associated with stroke in the US, including health care services, medication, follow-up and missed work days, total approximately $36.5 billion each year.

About 88% of all strokes are ischemic strokes, which occur when blood flow to the brain is blocked by a clot or mechanical event. Following vascular occlusion, a complex chain of events occur at a molecular level leading to irreversible tissue injury, including failure of energy synthesis and loss of transmembrane ionic gradients dependent on active transport. In the region with severely reduced blood flow, processes result in rapid cell necrosis affecting all the cellular elements. Rapid restoration of perfusion or blood flow to the surrounding area is the most robust predictor of good clinical prognosis following ischemic stroke. Immediately after the stroke, edema, loss of sensory input and inflammation occur around the infarct.[5] Some early recovery is realized with resolution of edema and inflammation but immunomodulation, angiogenesis, endogenous neurogenesis, and altered gene expression may be required for long-term recovery.

To date, no approved pharmacological treatment to promote revascularization in ischemic stroke exists outside of intravenous (IV) tissue plasminogen activator (tPA). The most effective use of tPA is limited to treatment within 3.0 hours from documented symptom onset, with diminishing effectiveness after 4.5 hours.[6] Given the time limitations and increased risk of bleeding including symptomatic intracerebral hemorrhage (ICH), tPA is used in only about 20% of patients with ischemic stroke, and cannot be used in hemorrhagic stroke.[6,7] Recent data also suggest the utility of mechanical reperfusion via endovascular intervention; however, therapy is limited to patients with proximal occlusion, requires specialized facilities, and has greatest benefit when performed within 6 hours following stroke onset.[8-12] Thus, there remains a compelling unmet need for therapies to improve outcome after stroke.

1.2 Rationale for Cellular Therapy in Ischemic Stroke

There is recent evidence that cellular therapy using unrelated donor, banked umbilical cord blood (UCB) has potential to favorably alter the natural history of stroke in affected patients. If administered within the first few weeks following stroke, UCB cells are thought to modulate ischemic damage in part through paracrine signaling to decrease inflammation and promote neurogenesis, thereby improving functional outcomes in patients. When banked in an accredited public cell bank, UCB is a readily available, prequalified, cryopreserved product that can be

selected for the stroke patient and shipped overnight. Thus, allogeneic UCB cells have the potential to expand access to therapy to many more patients with stroke.

The CoBIS Phase 1 clinical study, initiated in June, 2015, demonstrated a favorable safety profile based on the treatment of 10 male patients, ages 45 to 79, with an IV infusion of 0.80-2.00 x 10^7 TNCC/kg UCB cells from Day 3 to Day 9 post ischemic stroke (**Section 1.5.2**). The results from this Phase 1 safety study indicate that a placebo controlled study is warranted.

We hypothesize that cell therapy using stem cells from UCB will provide benefit by reducing the area of permanent injury, promoting plasticity, and improving functional outcomes for these patients. Cell therapy may favorably alter the natural history of these processes through paracrine signaling that reduces inflammation, promotes angiogenesis, neurogenesis and recruitment of endogenous cell repair.

Human UCB has been widely used as a donor graft for allogeneic, unrelated donor hematopoietic stem cell transplantation for more than two decades,[13] predominantly to treat patients with refractory malignancies or potentially lethal genetic diseases. For those indications, the intent is for donor cells to effect permanent engraftment, providing life-long support of the hematopoietic and immune systems. When long-term engraftment is the goal, the patient must undergo immunosuppression and myeloablation prior to the infusion of donor cells.

In contrast, for the indication of ischemic stroke, the desired paracrine effects and recruitment of cells to the area of injury can be achieved with short-term presence of donor cells in the recipient, thus eliminating the need for immunosuppression. The proposed therapy of allogeneic UCB cells given to immunocompetent patients with no prior immunosuppression is based on the assumption that UCB cells will ultimately be rejected by an immunocompetent host, without sustained engraftment.

In animal models of brain injury from ischemic stroke, extensive data support the safety profile of the infusion of allogeneic cord blood cells, as well as improvements in functional recovery (**Section 1.3**). Recent, but limited, pilot trials in human stroke patients support the safety of infusion of expanded or non-expanded allogeneic cells from cord blood, umbilical cord, adipose tissue and bone marrow without immunosuppression and myeloablation. A recent search on ClinicalTrials.gov identified 11 current clinical trials of allogeneic stem cells for the treatment of patients with acute and chronic ischemic stroke (see **Table** 1). While a number of these trials employ allogeneic cells isolated from bone marrow or adipose tissue, four of them, including the previously cited CoBIS Phase 1 stroke trial, use UCB cells. Three other trials are based on proprietary modified cell lines (SB623 cells [SanBio, Inc]; CTX cells, [ReNeuron Ltd.]) or a proprietary stem cell product (MultiStem; NCT01436487) derived from mononuclear bone marrow cells.

Table 1: Clinical Studies of Allogeneic Stem Cells in Treatment of Acute and Chronic Ischemic Stroke – ClinicalTrials.gov, accessed June 2016.

Phase	Cell Type	Delivery Route	Intervention	Inclusion Criteria	Number of Patients Planned	Country/ PI	NCT identifier	Status
II	MSC's from adipose tissue	IV	1 x 10^6 cells/kg, within first 2 wk after onset	Acute cerebral infarction (<12h): 60-80 yr old; NIHSS 8-20	20	Spain/ Diez Tejedor	NCT01678534	Recruiting
I/II	Adult BMSC's	IV	0.5-1.5 x 10^6 cells/kg	Chronic ischemic stroke (>6m); >18yr old; NIHSS 6-20	38	US/ Stemedica Cell Tech., Lev Verkh	NCT01297413	Active, Not Recruiting
I/IIA	Modified stromal cells (SB623)	IC	2.5, 5.0, or 10 x 10^6 cells	Ischemic stroke in subcortical region of MCA: > 18 – 75 yr old; (6-60m) or Lenticulostriate artery w/wo cortical involvement	Phase 1 = 18; Phase 2= 156	USA/San Bio Inc., Gary Steinberg, [14]	ACTIsSIMA Phase 1 = NCT01287936 Phase 2 = NCT02448641	Active, Not Recruiting
I/II	Immortalized fetal Neural stem cells, CTX cells	IC	2 x 10^6 cells	Ischemic stroke (2-3m); 40-89 yr old; NIHSS >2 upper extremity	Phase1 = 12 Phase 2 = 41	UK/ ReNeuron, Kevin Muir	PISCES Phase 1 = NCT01151124 Phase 2 = NCT03117635	Phase 1 = Active, Not Recruiting Phase 2 = Recruiting
I/II	BM derived MSC's	IV	MTD max. total dose	Acute ischemic stroke (3-10 days); 18-83 yr old; NIHSS 7-25;	Planned, 48; only got 10	Houston Sean Savitz	SAMCIS NCT01922908	Not Yet Recruiting
II	Adult Stem Cells Product 'Multi Stem'	IV	Low 4-12 x 10^8 MAPCs high dose	Moderate to Moderate severe cortical cerebral ischemic stroke (1-2d); 18-83 yr old	n = 140	USA/Athersys, Inc; David Hess, Robert Mays	NCT01436487	Completed
I/II	Cord Stem Cells; Cord	IV	2 x 10^8 cells; 0-7 days after stroke	19-80 yr old; 0-7 days after stroke; NIHSS 5-20 involving anterior circulatory region	18	Korea/ CHA BioTech Co	NCT02378974	Recruiting
I	Allo UCBs; Cord	IV	0.5-5 x 10^7 cells; 3-10 days	Ischemic Stroke; 18-80 yr old; 3-10 days after stroke; NIHSS 8-18	n =10	US/ Duke University Joanne Kurtzberg	CoBIS NCT02397018	Active, Not Recruiting

Phase	Cell Type	Delivery Route	Intervention	Inclusion Criteria	Number of Patients Planned	Country/ PI	NCT identifier	Status
IIa	Allo UCMSC	IV	2 x 10^7 UCMSCs weekly for 4 weeks	Stroke within 3 mo; 70 yr old	2	China/Sheng et al; PI: Duan Lian	NCT02580019	Not Yet Recruiting
I	Allo UCB mononuclear cells	IC	10-40 x 10^6 cells	35-65 yr old; Ischemic Stroke(6-60 mo) MCA; NIHSS 5-15	12	China/China Spinal Cord Injury Network	NCT01673932	Recruiting
I	Allo UCMSC	IA	2 x 10^7 cells	Stroke within 3 mo; MCA; Patients < 60 years old; included 3 ischemic and 1 hemorrhagic	4	Jinling Hospital[27] Nanjing, China	N/A/China	Completed

PI, Principal Investigator; NCT, National Clinical Trial [identifier number]; MSC, mesenchymal stem cells; IV, intravenous; IC, intracranial; IA intra-arterial; NIHSS, National Institutes of Health Stroke Scale; BMSC, bone marrow stromal cell; US, United States; Allo, allogeneic; SB623, modified allogeneic mesenchymal stem cells derived from bone marrow of healthy human adult donors (SanBio, Inc; Mountain View, CA); NM, neuromuscular; MCA, middle cerebral artery; CTX, CTX0E03 human neural stem cell product (ReNeuron; Surrey, England); UK, United Kingdom; BM, bone marrow; MTD, maximum total dose; MAPC, multipotent adult progenitor cells; UCB, umbilical cord blood; UCMSC, umbilical cord mesenchymal stem cell.

Trial acronyms: ACTIsSIMA – Study of Modified Stem Cells (SB623) in Patients with Chronic Motor Deficit From Ischemic Stroke;
PISCES – Pilot Investigation of Stem Cells in Stroke;
SAMCIS — Mesenchymal Stromal Cells for Ischemic Stroke; MAPC;
CoBIS –Study of Allogeneic Umbilical Cord Blood Infusion for Adults with Ischemic Stroke.

One major advantage of UCB cells in allogeneic hematopoietic stem cell transplantation is that they are immunologically tolerant, rendering them less reactive to human leukocyte antigen (HLA)-mismatch than bone marrow or mobilized peripheral blood grafts.[15] Consequently, candidates for allogeneic cell transplant who do not have a fully HLA-matched adult donor can receive partially HLA-mismatched cord blood donor units without risking unmanageable graft versus host disease (GVHD). This has increased access to transplantation therapy for patients with hematological malignancies, congenital immunodeficiency syndromes, bone marrow failure syndromes, hemoglobinopathies, and certain inherited metabolic diseases affecting the brain, where improvements in neurologic function have been seen.[16,17] Similarly, UCB may represent a more tolerable option for medically fragile stroke patients, who might otherwise need to undergo additional procedures, e.g., bone marrow harvest or peripheral stem cell collection; for collection of autologous cells. Unlike patients with malignant and genetic diseases, UCB infusions in patients with stroke are not intended to durably engraft the hematopoietic or immune systems, but rather to circulate and temporarily survive in vivo for sufficient time to alter behavior of endogenous host cells through paracrine signaling. Permanent engraftment is not a goal and, as such, myelo- and/or immunoablation will not be used in the present study.

1.3 Cord Blood Cells in Animal Models of Stroke

Data from animal models of stroke support the safety of UCB cells, as well as their association with improvements in functional recovery. The most extensively studied models are of rodents with ischemic brain damage resulting from permanent middle cerebral artery occlusion (MCAO) in adult rats or transient MCAO accompanied with hypoxia in neonatal rats or mice. Occlusion in the animal models is followed by sequential pathogenic processes starting with massive release of excitogenic amino acids, microglial and astrocytic activation, immune cell infiltration, a protracted inflammatory cytokine storm, and ultimately neuronal death and tissue infarction.[19-22] Given the xenogenic introduction of human cells into a rodent model, rejection by circulating antibodies and complement would be expected; however, there have been no observations of animal weight loss due to cellular therapy or any indication of GVHD symptoms. In most animal studies of induced brain ischemia and stroke, human UCB (HUCB) treatment has instead been associated with indicators of anti-inflammatory activity and altered balance of splenic lymphocyte populations favoring anti-inflammatory outcomes, suggesting that host response to HUCB therapy may be sustained after the cells have been eliminated.[20] This is consistent with animal studies showing that UCB cells can repair hypoxic/ischemic damage even though the cells do not persist for long periods of time in the brain.[23]

1.3.1 Possible Mechanisms of UCB Mediated Neuroprotection and Repair

The ability of UCB to ameliorate various types of central nervous system (CNS) damage has been shown in tissue culture and animal studies. While the exact pathways by which UCB cells lead to neural sparing and recovery have yet to be completely elucidated, several mechanisms have been hypothesized on the basis of animal studies.[24] Transplanted cells may migrate to the

affected ischemic area and deliver trophic factors that provide anti-inflammatory and neuroprotective effects and enhance the potential for survival of host cells.[25,26]

- UCB cells may result in increased plasticity of the injured brain by enhancing synaptogenesis, neovascularization, and endogenous repair mechanisms and by inducing migration and proliferation of endogenous neural stem cells.[27] Evidence of the angiogenic potential of human bone marrow cells is supported by mouse studies demonstrating revascularization of surgically-induced ischemic limbs after IV transplantation.[28] Stem cells may also infiltrate, proliferate, and differentiate into "replacement" neuronal and glial cells and play a role in remyelination.

- Human hematopoietic progenitor cells derived from multiple sources and administered via the IV, intraperitoneal, and intracerebral routes have demonstrated the ability to enhance functional recovery in multiple animal models of stroke.[29]

Significantly, in certain contexts, UCB cells dampen, rather than augment, inflammatory responses through an increase of regulatory T cell populations and the generation of monocyte/dendritic cell anti-inflammatory cytokines. In this regard, it is plausible to consider a population of UCB cells as an anti-inflammatory agent. In animal models of stroke, pro-inflammatory cytokines increase after ischemic brain damage in the affected areas and can be readily measured. Conversely, at both the protein and ribonucleic acid (RNA) level, IL-2, TNFα and IL-1-ß were shown to be decreased after HUCB treatment in a rat model of stroke.[23]

There is evidence that the effects of cord blood cells in the regeneration and repair processes following ischemic stroke are through signaling that increases neurogenesis and angiogenesis in areas proximal to the infarct.[30]

Additional preclinical work has shown that within the HUCB milieu, monocytes (CD14+) are the critical cellular component responsible for neuroprotective and anti-inflammatory effects following ischemic brain injury. In a model of stroke induced by MCAO, rats post occlusion were injected with HUCB preparations depleted either of monocytes (CD14+), stem cells (CD133+), T cells (CD2+) or B cells (CD19+). The group receiving the CD14+ depleted cells had the largest and most consistent decrements in behavioral recovery and increased infarct size, with activity levels and infarct size similar to untreated MCAO rats.[18]

1.3.2 Optimization of Doses, Route and Timing of UCB

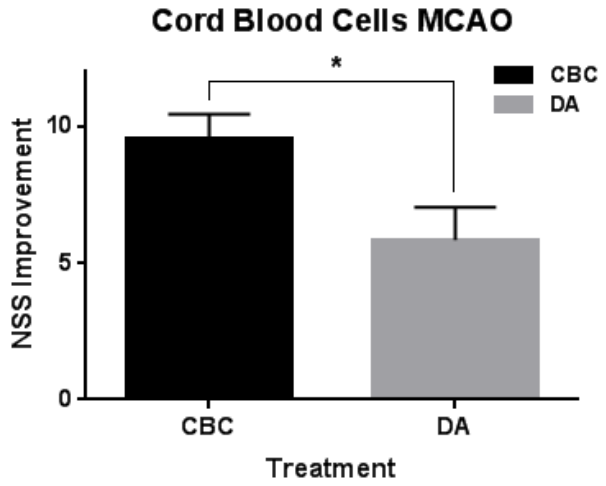
The dose dependence of HUCB effect on neurologic recovery has been demonstrated by Vendrame et al.[31,32] Rats were treated 24 hours after MCAO with doses of HUCB ranging from 1×10^5 to 5×10^7 cells. Behavioral and histological evaluations of the infarct volume clearly showed a dose-dependent effect: only the groups dosed with $\geq 1 \times 10^6$ cells were associated with positive outcomes.

In a rat model of collagenase-induced intracerebral hemorrhage (ICH), neurologic deficits were ameliorated by a single IV injection of cryopreserved human UCB cells (HUCB): animals receiving 2.4–3.2 x 10^6 mononucleated HUCB cells IV demonstrated significant improvements in the neurologic severity test, the stepping test and elevated body-swing test (EBST) compared with saline-injected controls.[34]

In a murine stroke model, male mice injected at 5 days after transient (25 min) MCAO with 1 x 10^6 HUCB cells IV had significant reduction in neurological deficit (improved NeuroSeverity Score [NSS]) (p=0.0193), improved 10-day survival (83% vs. 50%, respectively), and a reduction in infarct volume, relative to vehicle control (**Figure 1**). No difference in Rotarod (RR) performance was observed between the two cohorts (preliminary results from the authors of this protocol (unpublished)). The same authors conducted a second study to determine if the time of HUCB treatment after ischemic brain injury affected outcomes: mice subjected to transient MCAO (15 min) were randomized to injection of HUCB at either 2 or 5 days post MCAO, or injection of vehicle at 2 days post MCAO. At 10 days post MCAO-induced stroke, animals infused with HUCB on either Day 2 or Day 5 exhibited a significant reduction (~2-fold) in neurological deficit (improved NSS) (p=0.0329 and 0.0352, respectively) as well as functional improvement by RR performance (p=0.0361 and 0.0006, respectively), relative to controls (**Figure 2**). However, no significant difference in infarct size was observed among the three cohorts.

Figure 1: IV administration of Human UCB in an MCAO Mouse Model of Ischemic Stroke

A.



B.

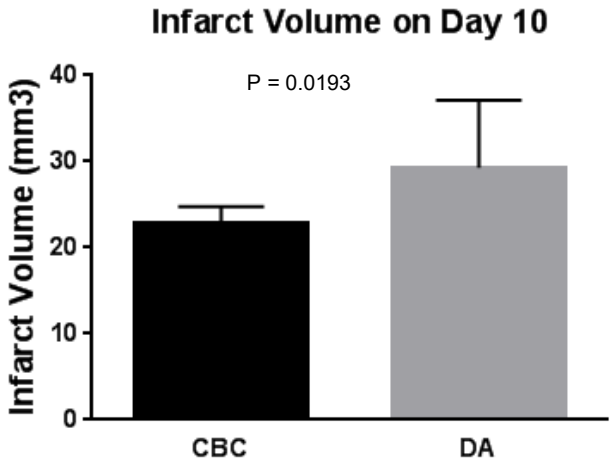
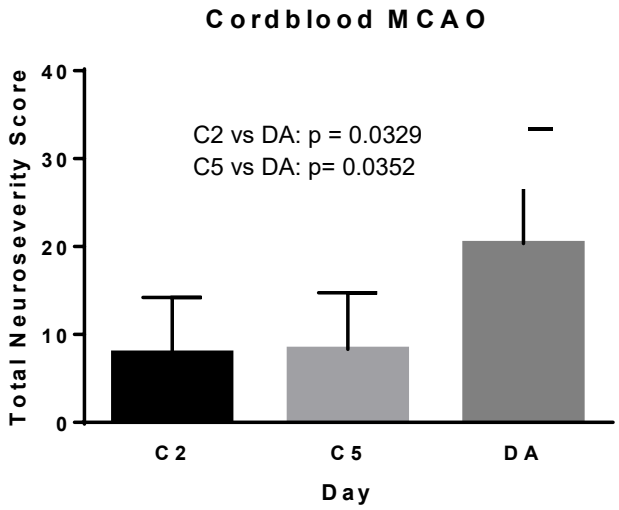


Figure 1. Thirty 10-12 week old male C57BL6 mice were subjected to MCAO for 25 min. and randomized into two groups: an experimental group (UCB) received 1 x 10^6 HUCB cells (CBC) and vehicle group (DA) received dextran/5% albumin 5 days after MCAO. Outcome was assessed 10 days post MCAO**. A.** Animals receiving HUCB showed a reduction in neurological deficit (improved NSS) relative to vehicle controls (P=0.0193). **B.** Animals treated with HUCB (CBC) had a reduction in infarct size relative to controls (DA).

Figure 2: Mouse Model of Stroke infused with HUCB on either Day 2 or Day 5 Post MCAO

A.



B .

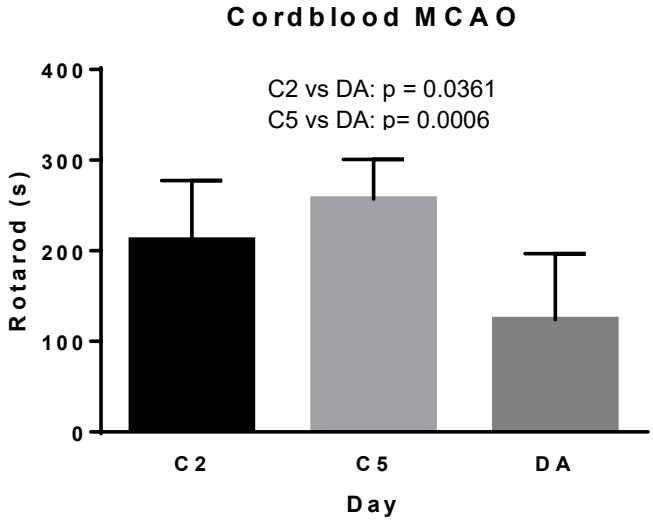


Figure 2. Thirty 10-12 week old male C57BL6 mice were subjected to MCAO for 15 min. and randomized into 3 groups:: cohorts 1 and 2 were administered 1 x 10^6 HUCB at Day 2 (C2) or Day 5 (C5) post MCAO; control group received vehicle alone (DA). **A.** Animals infused with HUCB on days 2 and 5 exhibited a significant reduction in neurological deficit (improved NSS; p=0.0329 and 0.0352) 10 days post MCAO. **B.** Both groups exhibited functional improvement on RR performance relative to vehicle controls (p=0.0361 and 0.0006) 10 days post MCAO.

Among rats injected with 3 x 10^6 HUCB IV at either 1 or 7 days after MCAO-induced ischemic brain injury, HUCBs were found to have survived and migrated to the damaged brain areas, primarily to the ischemic boundary zone. Significant improvements were observed in the group administered HUCB 1 day after MCAO but not at 7 days post MCAO, suggesting that early administration of HUCB was more efficacious than delayed treatment.[23]

Various delivery routes for stem cells have been used in the treatment of neurological disorders in both preclinical and clinical studies, including stereotactic transplantation into the brain, intra-arterial, intrathecal, IV and intranasal delivery, and evidence has been mixed as to whether route of cell transplantation impacts the extent of behavioral recovery.[35-39] In a rat MCAO model, Willing et al compared the effect of HUCBs injected intravenously into the femoral vein or directly into the striatum. Although both groups demonstrated improvements in several measures of hyperactivity and passive avoidance tests, only the group treated by femoral vein injection had significant improvement in the stepping test, an indicator of recovery from asymmetric motor deficits.[25] Similar conclusions have been made regarding stem cell delivery to treat stroke in humans: among the current stem cell stroke trials listed on ClinicalTrials.gov, the favored method of stem cell delivery is intravenous (**Table 1**).

In this regard, recent studies suggest that intravenously injected UCB cells interact with the immune system in the spleen of experimental animals and elicit the formation of cells or cytokines that subsequently have therapeutic effects in the brain.[40] Intravenously injected mesenchymal cells have similar therapeutic allocrine effects on the brains of animals with stroke or traumatic brain injury. Acosta et al recently showed in a rat model of chronic stroke that human bone marrow stromal cells (hBMSC) delivered intravenously preferentially migrated to the spleen over the brain.[41] Furthermore, cells survived better in the spleen relative to the brain, which in turn correlated with improvement of neurostructural deficits and inflammation. Coupled with down-regulation of TNFα density in the spleen, cell transplantation was associated with reduced infarct size and attenuated stroke-induced inflammation in the brain, supporting the hypothesis that stem cells provide a therapeutic benefit by acting in the spleen to dampen inflammation and subsequent cell death associated with chronic stroke. A (optional) spleen sub-study will be conducted to verify the natural history of splenic response in stroke patients, and investigate the effect of UCB cells in altering the splenic response and correlating this with peripheral cytokine and WBC counts.

In this Phase 2 trial, UCB will be administered intravenously to patients within 3–10 days following ischemic stroke. Following IV injection, UCB may elicit local and/or allocrine responses to ongoing inflammatory or tissue damage signals that can down regulate brain pathology. The activities of UCB that have been documented in laboratory studies are consistent with data demonstrating their ability to dampen an ongoing autoimmune and/or inflammatory response in the brain and promoting CNS repair.[42] Furthermore, our Phase 1 study indicated that delivery of UCB intravenously was safe and well tolerated in 10 adults with acute ischemic stroke.

In summary, literature suggests xenogenic infusion of human UCB cells in animals following ischemic injury offers changes in animal behavior representative of recovery. The proportional doses of cells used in animal studies have exceeded the proposed human dose in this current study with no documentation of animal safety issues. Optimal timing of dose and route of administration remain poorly defined.

1.4 Clinical Trial Experience with Cord Blood Cells in Treatment of Stroke and Other Ischemic Brain Injuries – Duke University

The rationale for cord blood therapy is based on the hypothesis that UCB cells act through paracrine and allocrine mechanisms to modulate and down regulate inflammation and/or immune pathology, possibly protecting neurons from further damage and promoting neurorepair. In ongoing clinical trials at Duke University, autologous UCB cells have been administered to patients with cerebral palsy (CP) and patients with hypoxic ischemic encephalopathy (HIE), both diseases thought to arise from an acute hypoxic/ischemic event leading to a pathological cascade that closely parallels events in the animal models that have been successfully treated with UCB.[39,43] In addition, 15 children have been infused with haploidentical or matched sibling cord blood on a Phase 1 study over the past year and no safety concerns were identified.

1.5 Human Clinical Data Using Autologous Stem Cells in the Treatment of Stroke

There are no Food and Drug Administration (FDA)-approved biomedical treatments using stem cells for the core symptoms of stroke. Numerous groups have conducted Phase 1 and 2 clinical trials and reported safety of infusion of various autologous cell populations delivered to patients with acute (1 day – 9 days), subacute (~10 – 30 days), and chronic (6 – 60 months) stroke. To date, approximately 8 such studies have been reported on ClinicalTrials.gov as ‘completed’ (**Table 2**). The majority of these included stem cells isolated from bone marrow, although one completed study in chronic stroke patients conducted in China used stem cells isolated from peripheral blood (NCT00950521). The results from these clinical trials reported safety of infusion of autologous stem cells for the treatment of stroke. Banerjee et al reported significant improvement in clinical functional scores and reductions in lesion volume with intra-arterial immunoselected CD34+ cells from autologous bone marrow in 5 patients ages 30–80 with acute ischemic stroke, with no significant treatment- related adverse effects.[44] In a separate study, Savitz et al found IV delivery of autologous BMSCs to be safe in the treatment of 10 patients ages 18–80 with acute ischemic stroke.[45]

Table 2: Completed Stroke Studies Using Autologous Stem Cells

Clinical Trials Identifier	Cell	Delivery	Number of subjects	Acute	Subacute	Chronic	Reference
NCT00761982 Phase 1/2a	BMSC	IA	20	X			China Medical University Hospital Shinn-Zong Lin Moniche et al. Stroke, 2012 [46]
NCT00535197 Phase 1	BMSC	IA	5	X			Banerjee et al. Stem Cells Transl Med 2014 [44]
NCT00859014 Phase 1	BMSC	IV	25	X			Vahidy et al. Stem Cells Dev. 2013 ; Savitz et al. Ann Neurol 2011 [47,48]
NCT02425670 Phase 2	BMSC	IV	120		X		Prasad et al. Stroke 2014 [49]
NCT01501773 Phase 1	BMSC	IV	9		X		Prasad et al. Indian J. Med Res 2012 [50]
NCT00950521 Phase 2	Peripheral blood CD34+ cells	IC	30			X	Mackie & Losordo Tex Heart Institution J. 2011 [51]
NCT02065778 Phase 1	BMSC	IT	30			X	Borlongan, Leukemia 2011 [52]
NCT01028794 Phase 1/2a	BMSC	IV	6	X			Taguchi et al. Stem Cells and Dev 2015 [53]

BMSC, bone marrow stromal cells, IA, intra-arterial injection; IV, intravenous injection. IC, intracranial

In a Phase 1 study conducted in India, favorable outcomes were described for 9 patients ages 30–70 with subacute stroke after treatment with IV injection of autologous BMSCs.[54] The study was expanded to a Phase 2, multicenter, parallel group, randomized trial (n=120), with 58 patients receiving BMSCs at a median of 18.5 days after stroke.[49] In the larger Phase 2 study, although the cells were shown to be safe, response based on the mRS and Barthel Index (BI) at 180 days showed no beneficial effect of treatment on stroke outcome.

1.5.1 Clinical Trials of Allogeneic Stem Cells in Patients with Stroke

Treatment with allogeneic HUCB cells without prior immunosuppression has been reported by Yang et al in a variety of degenerative conditions including paraplegia, ataxia, multiple sclerosis, ALS, brain disease and brain injury.[55] Cells from digested cord were evaluated for safety in 114 humans with no SAEs reported for hematological, immunological and biochemical endpoints. By contrast, more approved therapies have been reported for the use of allogeneic UCB in patients with inherited metabolic diseases and oncology using prior immunosuppression.[39]

There are several clinical trials in various stages that are investigating the use of allogeneic cell therapy using selected populations of cells for the treatment of ischemic stroke (**Table 1**). Cell products used in these trials are derived from adipose tissue, BMSC's, umbilical cord, UCB and fetal neural stem cells.

- One completed pilot study in 4 patients with chronic stroke (3 ischemic, 1 hemorrhagic) showed treatment with allogeneic mesenchymal stem/stromal cells (MSC's) isolated from umbilical cord was safe and associated with no adverse events (AEs).[56]

- Furthermore, in patients with ischemic stroke, but not intracranial hemorrhage, MSCs improved the neurological function as assessed by the mRS,[57] although the small sample size is a major limitation of this study.

- In contrast, a second completed Phase 2 randomized study using MultiStem, a proprietary stem cell product derived from mononuclear bone marrow cells in the setting of acute stroke (n=140), found no differences in 90 day outcome between therapy and placebo group, although treatment was associated with lower rates of mortality and life threatening AEs.[58,59]

- An additional group using modified stromal cells (SB623 cells) transplanted into the brains of patients suffering from chronic stroke recently reported improvement in clinical outcome endpoints at 12 months in a Phase 1 study.[14]

- The PISCES stroke trial uses immortalized fetal neural stem cells surgically implanted into the brains of patients with chronic stroke and is currently recruiting for a Phase 2 trial.[60]

- Five studies use stem cells derived from UCB to treat chronic (3) or acute (2) stroke, including Duke University's Phase 1 CoBIS study (**Table 1**). Compared with BMSCs, stem cells from UCB offer improved plasticity, faster growth time, lower immunological response against host, and ready availability without the need for invasive procedures in compromised patients. Furthermore, umbilical cord stem cells are biologically closer to embryonic stem cells than adult BMSCs.

1.5.2 Phase 1 Clinical Trial Data in Ischemic Stroke using Allogeneic Cord Blood Cells

Ten adult male patients were treated in a Phase 1 clinical study (CoBIS) at Duke University and Houston Methodist Hospital. **Table 3** is a summary of patient demographics, dose of UCB cells, and window of treatment post the primary stroke event. The participants had a mean age of 61.5 years (range 45–79); the range of cell doses infused was 0.83–3.34 x 10^7 TNCC/kg, and infusions were administered from 3 to 9 days post stroke. The mRS was used to characterize outcome associated with stroke recovery at baseline, 1 month, and 3 months following stroke. The NIHSS was used to measure impairments due to stroke at baseline and 3 months after stroke; the BI was used to measure basic activities of daily living. Other measures, such as the EQ-5D-3L and PHQ-8 were used to measure health status. **Table 4** shows change in scores from baseline to 3 months. All 10 patients were independent prior to the stroke; 9 patients had an historic mRS of 0 (no symptoms at all) and one patient (0101) had an historic mRS of 1 (no significant disability despite symptoms; able to carry out all usual activities). Safety was assessed at 24 hours, days 3–10, and at 3, 6, and 12 months post infusion. As of June, 2016, a total of 115 AEs were reported in 10 of 10 patients (**Table 4**); 8 of these were serious (6 occurring in one study patient and 2 in a second patient), were unrelated to study therapy and not reportable. The SAE incidence rate, defined as the number of patients experiencing an event divided by the number receiving treatment, was 20% (2/10 patients experienced an SAE). All 10 patients shifted at least one grade in mRS from baseline (time of infusion) to 3 months after stroke (range 1-3 points), indicating improved outcome.

Most stroke patients will show the fastest improvement following a stroke within the first month,[61] and in one of the few studies examining change in mRS over time, 58% of patients disabled by stroke (mRS 3–5) improved at least one grade in their mRS after 3 months.[62] The CoBIS Phase 1 study results are in agreement with this and show that 50% of the patients exhibited a 1 grade increase (improvement) in mRS at 3 months relative to baseline (infusion). However, 4 out of 10 patients improved by 2 grades and one patient by 3 grades, suggesting that the UCB therapy may further benefit ischemic stroke patients. Similarly, all patients demonstrated improved NIHSS from time of infusion (mean 11.2 ± 1.6; range 9-14) to 3 month assessment (mean 5.3±2.2; range 3-9) with a shift down (improvement) in at least 4 points (mean 6.1±1.7; range 4-9 points), and showed improvement in activities of daily life as measured by an increase in points on the BI (mean 52.0±24.7; range 10-80).

The results from this Phase 1 study satisfy our primary endpoint by demonstrating a favorable safety profile for IV infusion of UCB in adult patients with ischemic stroke. Furthermore, they suggest that outcome measures including the mRS, NIHSS, and BI are appropriate assessment tools for patients with ischemic stroke. These results support a controlled and randomized Phase 2 Study using human UCB and placebo in patients with ischemic stroke.

Table 3: CoBIS Phase 1 – Patient Demographic and Cell Infusion Characteristics

Number	Subject	Site	Age	Gender	Ethnicity	Race	Infusion Date	Infused Cell Dose $x10^7$/kg	Number of days post stroke for infusion
1	0 101	Duke University	62	Male	Non Hispanic or Latino	White (Non Hispanic)	20-Jul-15	1.52	8
2	0 102	Duke University	69	Male	Non Hispanic or Latino	White (Non Hispanic)	28-Jul-15	2	9
3	0 103	Duke University	79	Male	Non Hispanic or Latino	White (Non Hispanic)	5-Oct-15	1.09	6
4	0 104	Duke University	47	Male	Non Hispanic or Latino	Black/ African American	3-Dec-15	0.83	7
5	0 105	Duke University	47	Male	Non Hispanic or Latino	White (Non Hispanic)	3-Feb-16	0.84	3
6	0 1016	Duke University	51	Male	Non Hispanic or Latino	White (Non Hispanic)	3-Mar-16	0.9	9
7	0 301	Houston Methodist	70	Male	Non Hispanic or Latino	American Indian/ American Native, White (Non Hispanic)	9-Nov-15	3.34	7
8	0 302	Houston Methodist	70	Male	Non Hispanic or Latino	Black/ African American	21-Jan-16	2.78	9

Number	Subject	Site	Age	Gender	Ethnicity	Race	Infusion Date	Infused Cell Dose $x10^7$/kg	Number of days post stroke for infusion
9	0 303	Houston Methodist	75	Male	Non Hispanic or Latino	White (Non Hispanic)	23-Jan-16	3.16	7
10	0 304	Houston Methodist	45	Male	Non Hispanic or Latino	Black/ African American	25-Jan-16	1.56	5

Table 4: CoBIS Phase 1 – Outcomes and Adverse Events

		Baseline versus 3 Month Outcomes				All AEs	SAE's				
Number	Subject	mRS (baseline/ infusion)	mRS (3 months)	NIHSS (baseline/ infusion)	NIHSS (3 months)		BI (baseline/ infusion)	BI (3 months)	Number	Therapy related (Y/N)	Reportable (Y/N)
1	0 101	5	4	11	7	34*	5	15	6	N	N
2	0 102	4	2	11	6	10*	10	90	0	N/A	N/A
3	0 103	4	2	11	5	4*	30	85	0	N/A	N/A
4	0 104	4	2	10	3	3*	20	95	0	N/A	N/A
5	0 105	4	2	11	3	11*	30	95	0	N/A	N/A
6	0 106	4	3	10	5	7*	15	90	2	N/A	N/A
7	0 301	5	4	14	9	11*	10	25	0	N/A	N/A
8	0 302	5	4	14	8	15*	0	35	0	N/A	N/A
9	0 303	5	2	11	3	4*	30	80	0	N/A	N/A
10	0 304	4	3	9	4	16**	35	95	0	N/A	N/A

mRS, modified Rankin Scale; NIHSS, NIH Stroke Scale; BI, Barthel Index.

* None of the adverse events related to study drug.

** One (1) adverse event related to study drug, but it was expected and not reportable; the event resolved on the same day as onset.

1.6 Study Rationale and Hypotheses

The major goal of this Phase 2 study is to study the effects of allogeneic, unrelated donor, UCB IV infusion in patients following ischemic stroke. Magnetic resonance imaging (MRI) will be used to confirm ischemic stroke in the middle cerebral artery (MCA) distribution. Various clinical assessments and outcome measures will be used to evaluate neurological and physical function and functional outcomes. The rationale for this study proposes:

- The brain exhibits plasticity with the ability to recover from injuries due to stroke.
- Cellular therapy using UCB may facilitate repair of damaged areas of the brain through paracrine signaling. UCB cells may recruit endogenous host cells to aid in enhancing neural pathway connectivity restoring functional pathways damaged by the stroke.
- Cellular therapy may decrease inflammation which may, in turn, limit local tissue damage and subsequently increase functional recovery from injury.

To have a lasting effect on stroke symptoms, treatment with UCB cells must stimulate endogenous mechanisms to repair damaged neuronal pathways and ultimately permit formation of normal patterns leading to changes in the cognitive symptomology of stroke. It is important to stress that in this view, for which there is strong preclinical and clinical evidence, UCB cells may not be required to proliferate or persist in the recipient. Given that the recipients in this study with ischemic stroke will not be pre-conditioned before cord blood infusion and because these patients will have normal immune function prior to treatment, we feel it is highly unlikely that durable engraftment of stem and progenitor cells from the allogeneic UCB treatment will be required to contribute to any expected therapeutic effect.

1.7 Potential Risks and Benefits

1.7.1 Known Potential Risks

UCB cells or placebo will be prepared and infused using SOPs that have been used in over 30,000 individuals worldwide. The potential risks associated with infusion of allogeneic UCB include infusion related reactions, transmission of infection, Graft vs. Host Disease (GVHD), and secondary malignancy. Infusion related reactions include anaphylaxis, urticaria, dyspnea, hypoxia, cough, wheezing, bronchospasm, nausea, vomiting, hives, fever, hypertension, hypotension, bradycardia, tachycardia, rigors, chills, infection, and hemoglobinuria. Symptoms of GVHD, e.g. rash or diarrhea, are unlikely and would not be evident until 14 to 100 days post infusion. It is expected that the cells will be immunologically rejected within days to weeks of administration, eliminating a risk of GVHD or aberrant cell proliferation post-infusion. All UCB units are screened for

sterility and risk of infection or genetic disease transmission and must meet donor eligibility screening and potency release criteria prior to banking to minimize any risks.

1.7.2 Known Potential Benefits

Potential benefits of cord blood cell infusions include the possibility that the UCB cells may, via direct or indirect mechanisms, reduce inflammation and also induce neurogenesis and other processes in the brain that decrease the impact of tissue damage resulting in improved mental and physical function thereby improving the quality of life for the participant and their families following stroke.

2 OBJECTIVES AND PURPOSE

The primary purpose of this Phase 2 study is to determine whether IV infusion of banked allogeneic umbilical cord blood (UCB) improves functional outcome in patients with acute ischemic stroke in addition to best medical therapy. The study is designed to determine whether a single dose of UCB administered to patients within 3 to 10 days following acute ischemic stroke increases the probability of improved functional outcomes as compared to placebo therapy. Improved functional outcome will be measured by the shift in mRS performed at 90 days following stroke. Patients will not be pre-treated with immunosuppressive drugs, and each cord blood unit (CBU) will be obtained from an accredited U.S. public cord bank and selected based on blood type and cell dose, targeting a dose range of 0.5–5 x 10^7 total nucleated cell count (TNCC)/kg.

2.1 Primary Objective

The primary objective is to determine, in a randomized, placebo controlled trial, the efficacy of a single IV infusion of unrelated donor UCB for improving functional outcomes in patients with ischemic stroke.

2.2 Secondary Objectives

The secondary objectives are as follows:

1. To describe the safety and tolerability of a single IV infusion of unrelated donor UCB in patients with ischemic stroke
2. To evaluate the efficacy of a single IV infusion of unrelated donor UCB for improvement of neurological symptoms following ischemic stroke
3. To evaluate the efficacy of a single IV infusion of unrelated donor UCB for improvement in quality of life and emotional and cognitive status in patients with ischemic stroke

2.3 Exploratory Objectives

To determine whether biomarkers of brain inflammation, neuronal injury, and repair, are differentially expressed as a function of UCB administration in addition to apolipoprotein E (APOE) genotype.

2.4 Study Endpoints

2.4.1 Primary Endpoint

The primary endpoint is the shift in modified Rankin Scale (mRS) from baseline to 3 months post infusion.

2.4.2 Secondary Endpoints

1. Safety and tolerability of donor UCB infusion in adults with ischemic stroke will be assessed by the following:
    a. Incidence and severity of infusion reactions
    b. Incidence and severity of product-related infections
    c. Evidence of alloimmunization via anti-HLA and anti-RBC antibodies and nonspecific markers of systemic inflammation (ESR, CRP)
    d. Incidence and severity of graft vs. host disease
    e. Incidence and severity of unexpected AEs, by relation to study product
    f. Mortality
2. Functional independence at 90 days defined as a 90-day mRS score of 0, 1, or 2
3. mRS shift score at 30 and 180 days post infusion
4. The National Institutes of Health Stroke Scale (NIHSS) at 90 days
5. The Barthel Index (BI) at 90 days
6. Stroke Impact Scale-16 (SIS-16) at 90 days
7. The European Quality of Life (EQ-5D-3L) survey at 90 days
8. Patient Health Questionnaire Scale (PHQ-8) at 90 days
9. Telephone Interview for Cognitive Status (TICS) total score at 30 and 365 days

10. Stroke Inventory Test Battery scores at 90 days

2.4.3 Exploratory Endpoints

1. Inflammatory cytokines and other biomarkers of brain injury and repair (e.g., S100B, TNFα, IL 1β, IL-4 and IL-10, VEGF, BDNF, MMP9) in peripheral blood at baseline, within 6-24 hours after UCB infusion, and 90 days

2.5 Description of Outcome Measures

2.5.1 Primary Outcome Measure

Mental and physical function will be assessed by the following:

2.5.1.1 Modified Rankin Scale (mRS)

The mRS is a commonly used scale for measuring the degree of disability or dependence in the daily activities of people who have suffered a stroke, and it has become the most widely used clinical outcome measure for stroke clinical trials. The mRS is scored from 0 to 6, and describes a clinically relevant continuum from perfect health without symptoms (0) to death (6).[57,63] Administration of the mRS takes approximately 5 minutes.

All study personnel performing the mRS must have current certification.

2.5.2 Secondary Outcome Measures

2.5.2.1 Safety Assessments

Safety will be assessed by documenting the frequency and severity of AEs occurring within 24 hours of cord blood infusion and within the 12-month period post infusion.

2.5.2.2 National Institutes of Health Stroke Scale (NIHSS)

The NIHSS is a common method used by neurologists, nurses, and other medical professionals to determine the level of neurological impairment created by a stroke. The NIHSS is a 15-item neurologic examination stroke scale used to evaluate the effect of acute cerebral infarction on the levels of consciousness, language, neglect, visual-field loss, extraocular movement, motor strength, ataxia, dysarthria, and sensory loss.[64] A trained observer rates the patient's ability to answer questions and perform activities. Ratings for each item are scored with 3 to 5 grades with 0 as normal, and there is an allowance for untestable items. A patient with a completely normal neurological exam and normal mental status will have an NIHSS of 0. The maximum recordable NIHSS score is 42. The NIHSS can be administered in less than 10 minutes and should be administered by the same currently trained and experienced personnel throughout the study.

2.5.2.3 The Barthel Index (BI)

The BI is a standard measure of disability, widely used in the assessment of patients with stroke. The 10-item scale covers activities involving self-care (feeding, grooming, dressing), ability to use the bathroom, and acts of mobility. The maximum score is 100 with a higher score reflecting greater independence in the patient. Administration of the BI takes 5 minutes.[65]

2.5.2.4 European Quality of Life (EQ-5D-3L)

The European Quality of Life (EQ-5D-3L) is a standardized measure of health status developed by the EuroQoL Group in order to provide a simple, generic measure of health for clinical and economic appraisal.[66] The assessment comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 3 levels: no problems, some problems, severe problems. The EuroQoL visual analogue scale (EQ VAS) records the patient's self-rated health on a visual analogue scale where the endpoints are labeled 'Best imaginable health state' and 'Worst imaginable health state'. The EQ-5D-3L takes less than 5 minutes to complete. A proxy (spouse, family member, or caregiver) is allowed to assist the subject if they are unable to complete the survey alone.

2.5.2.5 Patient Health Questionnaire Scale (PHQ-8)

The PHQ-8 is an eight-item questionnaire depression scale established as a valid diagnostic and severity measure for depression disorders including stroke. The questionnaire takes approximately 2 min.[67]

2.5.2.6 Stroke Impact Scale–16 (SIS-16)

The Stroke Impact Scale–16 (SIS-16) is a composite measure of physical function developed by Rasch analysis from the 4 physical domains of the SIS: strength, activities of daily living/instrumental activities of daily living (ADL/ IADL), mobility, and hand function.[68] It is designed to measure the impact of physical function on disability after stroke. The SIS-16 is a valid and reliable measure tool and because of a less pronounced ceiling effect, it is more sensitive to change than the BI and the Short Form-36. The SIS-16 takes less than 10 minutes to complete.

2.5.2.7 Telephone Interview for Cognitive Status (TICS)

The Telephone Interview for Cognitive Status™ (TICS™) is a brief, standardized test of cognitive functioning that was developed for use in situations where in person cognitive screening is impractical or inefficient (e.g., large-scale population screening, epidemiological surveys, with patients who are unable to appear in person for clinical follow-up).[69] Although the TICS is designed to be administered using the telephone, it also may be administered face-to-face. Because it does not require vision, the TICS is

particularly useful for examining visually impaired individuals and individuals who are unable to read or write. Research has demonstrated that psychological data obtained over the telephone are as reliable and valid as those obtained through face-to-face interaction. The TICS correlates highly with the Mini-Mental® State Examination (MMSE®). It has high test-retest reliability and excellent sensitivity and specificity for the detection of cognitive impairment. The 11 test items usually take less than 10 minutes to administer and score.

All examinee responses are recorded verbatim. If a patient is aphasic BUT capable of writing answers which in turn can be communicated over the phone to the CRC by test proctor then the TICS may be administered and responses accepted. For TICS questions that require speech (e.g., Repeat after me 'no ifs, ands or buts' and 'Methodist Episcopal') patient will score 0 points. If a patient is aphasic and unable to communicate via voice OR by writing answers then the TICS should be left blank. CRC's should include an explanation such as "patient unable to participate due to aphasia and/or inability to write responses".

The individual item scores are summed to obtain the TICS Total score. The TICS Total score provides a measure of global cognitive functioning and can be used to monitor changes in cognitive functioning over time. The impairment ranges have been shown to adequately distinguish between normal participants and patients with cognitive impairment. The appropriate normative reference group for interpretation will depend on the reason for the evaluation and on the examinee's age and level of education.

2.5.2.8 Stroke Inventory Test Battery (SITB)

This abbreviated neuropsychological test battery utilizes objective measures that are sensitive to cognitive dysfunction, with an emphasis on tests sensitive to deficits in learning and recall and of executive control. The test battery utilizes most measures from the Stroke Common Data Elements (CDEs).[70] Stroke cognitive CDEs were specifically selected to be sensitive indicators of cognition in stroke. Individuals administering these tests will first be trained by a board certified clinical neuropsychologist.

Trail Making Test[71,72] The first part of this test, Trails A, requires the patient to rapidly sequence a straightforward series. The second part, Trails B, is a more difficult cognitive flexibility task requiring the patient to follow a sequential pattern while shifting cognitive sets. Trails A and B together take approximately 15 min to perform.

Montreal Cognitive Assessment (MoCA)[73] The Montreal Cognitive Assessment (MoCA) was designed as a rapid screening instrument for mild cognitive dysfunction. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. Time to administer the MoCA is approximately 10 minutes. The total possible score is 30 points; a score of 26 or above is considered normal.

Hopkins Verbal Learning Test-Revised (HVLT-R)[74] The HVLT-R is a brief assessment of verbal learning and memory (recognition and recall) for individuals 16 years and older. It is easy to administer and score and is well tolerated even by significantly impaired individuals. The HVLT-R requires recall of a series of 12 words over three learning trials, free recall after a delay, and a recognition trial. The assessment takes approximately 5-10 minutes with a 25-minute delay to complete.

Patient Health Questionnaire (PHQ-8) (see above)

Short Form 36 Health Survey (SF-36)[75] The Short Form-36 is one of the most widely used generic measures of health-related quality of life and has been shown to discriminate between subjects with different chronic conditions and between subjects with different severity levels of the same disease. The survey is a structured, self-report questionnaire that the patient can generally complete with little or no intervention from an interviewer. However, patients with visual or upper extremity impairments may need to have the SF-36 administered by a trained interviewer. Administration time is approximately 10 minutes.

Controlled Oral Word Association Test (COWAT)[76] The COWAT is a verbal fluency test that measures spontaneous production of words belonging to the same category or beginning with a designated letter. The examination takes approximately 5 minutes.

Oral Symbol Digit Modalities Test (SDMT)[77] The oral SDMT is a neuropsychological test used to assess information processing speed and is a sensitive measure for identifying deficits due to the presence of cerebral dysfunction.

2.5.2.9 Rehabilitation Survey – see Appendix 1

This is a self-reporting survey for patients to document type and frequency of rehabilitation services they received while on study.

2.5.2.10 Neuroimaging

As part of normal standard of care, MRI is typically obtained in patients with acute stroke within several days of presentation. The initial brain MRI imaging performed on patients enrolled in this study will use the routine stroke imaging protocol and imaging equipment particular to each institution.

In the event that cell infusion is scheduled to be initiated >36 hours after the baseline brain MRI or other neuroimaging study as performed as standard of care, non-contrast computed tomography (CT) of the head will also be obtained within 24 hours prior to infusion using routine institutional scan protocol, in order to evaluate for changes in the volume of infarcted brain parenchyma and for complications of potential clinical significance such as hemorrhage or increasing mass effect.

3 STUDY DESIGN

3.1 Study Design Overview and Duration

This is a multicenter, placebo controlled, randomized, double-blinded Phase 2 study in 100 patients 18-90 years of age who have sustained a recent ischemic stroke. Potential patients can be consented ≥ 24 hours following stroke onset (Day 1), but no later than 10 days after the index stroke event. Treatment with UCB cells or placebo will be administered intravenously as a single infusion as early as 3 days after the patient's stroke, but no later than 10 days after the stroke. Administration of cord blood or placebo will only be done after confirmation of eligibility criteria. See **Figure 3** for the study flow chart.

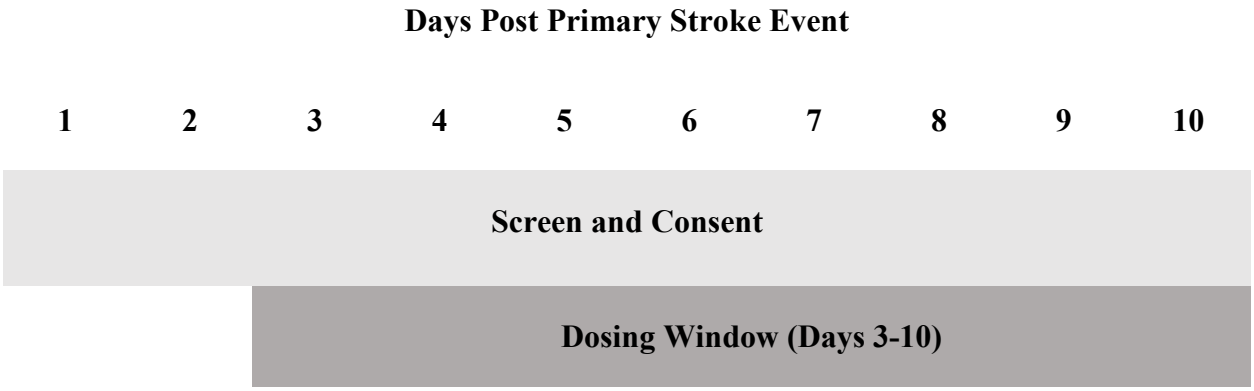
Patients will not receive immunosuppressive or myeloablative medications prior to infusion of the UCB cells or placebo.

For patients randomized to active treatment, the CBUs will be selected from an accredited U.S. public cord bank based on blood type and a targeted cell dose ranging between 0.5 to 5 x 10^7 TNCC/kg. For patients randomized to UCB cells at a clinical site that has no cord blood bank, the CBUs will be shipped frozen overnight to the site. Once a unit is selected and available on site, the cells will be thawed, washed, tested, released and infused intravenously using common SOPs at all sites.

For patients randomized to placebo, a container of diluent with the same appearance and odor as the CBU will be prepared so all patients, families and medical staff are blinded to treatment arm.

Patients will have a baseline brain MRI and will be assessed at 1, 3, 6, and 12 months for functional outcomes. All patients will receive standard of care therapy at the discretion of the Investigator and their other providers while enrolled in this study and all patients will be strongly encouraged to participate in rehabilitative therapy.

Figure 3: Study Flow Chart



Potential subjects can be screened on the day of the primary stroke event, after confirmation of ischemic stroke, but neurological status must be confirmed within 24 hours prior to infusion. Consent may be obtained ≥ 24 hours following stroke onset (Day 1), but no later than 10 days after the index stroke event.

3.2 Research Participant Selection and Withdrawal

3.2.1 Study Population

100 patients ages 18-90 years old with ischemic stroke documented by MRI

3.2.2 Inclusion Criteria

An individual is eligible for inclusion if **all** of the following apply:

1. 18-90 years old
2. Recent, acute, cortical, hemispheric, ischemic stroke in the MCA distribution without a clinically significant midline shift as detected by MRI as a DWI abnormality
3. NIHSS 6-15(R) and 6-18 (L) at the time of informed consent. Subjects with a >4-point increase of NIHSS from time of consent (worsening of score) will not be eligible for infusion.
4. Patients must have a platelet count >100,000/uL, hemoglobin >8gm/dL, absolute lymphocyte count (ALC) ≥ 1200 for African American patients and ≥1500 for all other racial-ethnic groups, and WBC >2,500/uL

   OR

   Historical pre-stroke value of ALC ≥ 1200 for African American and ≥1500 for all other racial-ethnic groups within 6 months of stroke

And a post stroke ALC value of ≥ 1000, platelet count >100,000/uL, hemoglobin >8gm/dL and WBC >2,500/uL.

5. Patients who received tPA or underwent endovascular reperfusion may be included in the study

6. Able to provide consent to study or consent is obtained from the patient's legal representative

7. Patients of childbearing potential must practice effective contraception during the study, and be willing to continue contraception for at least 6 months after intervention so that, in the opinion of the Investigator, they will not become pregnant during the course of the study

8. Is a good candidate for the trial, in the opinion of the Investigator

9. Agrees to participate in follow-up visits

10. ABO/Rh matched CBU(s) with a minimum of 0.5 x 10^7 TNCC/kg based on the pre-cryopreservation TNCC is available for infusion

11. Has not had a disease or therapy that would compromise current immune function.

3.2.3 Exclusion Criteria

An individual is ineligible to participate if **any** of the following apply:

3.2.3.1 Exclusionary Medical Conditions:

1. Medical history of neurological or orthopedic pathology with a deficit as a consequence that results in a modified Rankin Scale >1 before stroke or has a pre-existing cognitive deficit

2. Clinically significant and/or symptomatic hemorrhage associated with stroke

3. Evidence of significant midline shift as assessed by CT or MRI who are felt to be at high risk for neurological decompensation or need for decompressive hemicraniectomy due to hemispheric edema

4. New intracranial hemorrhage, edema, or mass effect that may place patient at increased risk for secondary deterioration when assessed prior to infusion

5. Hypotension as defined as the need for IV pressor support of SBP <90

6. Isolated brain stem stroke

7. Pure lacunar stroke

8. Requires mechanical ventilation. An exception may be patients who were electively intubated for endovascular reperfusion and then extubated immediately following the procedure.
9. Requires a craniotomy
10. Serious psychiatric or neurological disease which could alter evaluation on functional or cognitive scales
11. Active systemic infection that is felt, at the discretion of the Investigator, to place the patient at increased risk for participation in this study
12. Documentation of human immunodeficiency virus positive (HIV+) status in the medical record
13. Active malignancy within 3 years prior to the start of screening excluding skin cancers other than melanoma
14. Known hypercoagulable state or coagulopathy deficiencies such as Factor V Leiden, Antiphospholipid Syndrome (APC), Protein C, Protein S, anticardiolipin antibody, phospholipid syndrome or Sickle Cell Disease
15. History of or currently active autoimmune disease, or current immunomodulatory therapy or a recipient of immunomodulatory therapy in the past year.
16. Concurrent illness or condition that in the opinion of the Investigator might interfere with treatment or evaluation of safety
17. Current or recent history of alcohol or drug abuse, or stroke associated with drug abuse that Investigator feels may impair therapy or assessments
18. Pregnant as documented by blood test or lactating

3.2.3.2 Prohibited Concomitant or Prior Therapies

1. Patients currently receiving immunosuppressant drugs, not including glucocorticoid taper, topical/inhaled glucocorticoids
2. History of prior transfusion reaction
3. Currently on dialysis
4. Recipient of bone marrow or organ transplant
5. Renal insufficiency with serum creatinine > 2.0 mg/dL

6. Hepatic insufficiency (bilirubin >2.5mg/dL or transaminases >5x the ULN) Patients with Gilberts syndrome are eligible for the study if other liver function tests are normal, regardless of bilirubin level

7. Previous or current treatment with angiogenic growth factors, cytokines, gene or stem cell therapy

8. Participating in another interventional clinical trial of an investigational therapy within 30 days of consent

3.3 Early Withdrawal of Research Participants

The following criteria will be used for removal from protocol therapy:

- Diagnosis of a genetic disease while under evaluation or on study
- Significant change in medication and therapy as determined by the PI
- Change in medical condition that precludes study participation

3.3.1 Off-study Criteria

Patients who are off protocol therapy are to be followed for the duration of the study or until they meet off-study criteria as below:

- Death
- Lost to follow-up
- Withdrawal of consent for any further data collection

4 STUDY PRODUCT

4.1 Allogeneic Umbilical Cord Blood

Patient enrollment will be dependent on the availability of adequately dosed ABO/Rh matched banked CBUs that have been stored at an accredited U.S. public cord bank. The unit must meet donor screening and product characterization as detailed below.

4.2 Selection of Allogeneic Cord Blood Units

CBUs will be selected based on blood type and the number of cells in the pre-cryopreservation product, targeting a dose range of 0.5 to 5 x 10^7 TNCC/kg. Screening of maternal blood collected within 7 days before or after delivery is used for cord blood donor infectious disease screening. Maternal blood testing must have been

performed in a Clinical Laboratory Improvement Amendments (CLIA) certified donor-testing laboratory and must include the following:

- Hepatitis B surface antigen
- Hepatitis C antibody
- HIV-1 and HIV-2 antibodies
- Human T cell lymphotropic virus (HTLV)-I and HTLV-II antibodies
- Cytomegalovirus (CMV)
- Syphilis

Additional screening, which is dependent on the timing of the cord blood collection, may include nucleic acid testing (NAT) for HIV, hepatitis C virus (HCV), hepatitis B virus (HBV), West Nile Virus, and serological testing for Chagas Disease. The donor mother must screen negative for all tests, except for CMV or hepatitis B core antibody (anti-HBc). Units from mothers who test positive for anti-HBc but who test negative for HBsAg and HBV NAT may be used. Records for maternal testing are reviewed at the cord blood bank prior to release of the unit for treatment. In addition, for recently banked units, donor screening, through the medical history, for Zika virus is performed.

4.3 Cord Blood Unit Characterization Pre-cryopreservation and after Thawing

Results of initial testing at the cord blood bank must meet the following criteria to be acceptable for administration to study patients:

- Pre-cryopreservation TNCC $\geq 0.5x10^7$/kg patient weight
- Viability of CD34+ cells $\geq$ 70% after thawing
- Pre-cryopreservation sterility culture with no growth

All cells recovered post-thaw will be infused.

On the day of infusion, the cord blood will be thawed and washed in an automated fashion using the Sepax device. If able, ABO will be measured after thawing to cross-check pre- cryopreservation data prior to infusion. The cells will be resuspended into dextran 40 + 5% human serum albumin. The final volume of the cellular product to be infused will be 50 +/- 5 mL delivered to the clinic in a Sepax or transfer bag.

Post processing of the CBU, a sample will be removed for standard studies including TNCC, viable CD34+ cell count, confirmatory HLA type, cell viability, Colony Forming

Units (CFU), and sterility cultures to be performed in the Stem Cell Laboratory or by a selected CLIA certified laboratory. This data will not be available prior to infusion. A residual sample of the UCB will be retained for potential future testing.

4.4 Preparation of Placebo

In order for the patients, family, and medical staff to be truly blinded to the type of infusion they are receiving, the placebo product must be similar in both appearance and odor. Therefore, the placebo product will be acellular and will consist of tissue culture medium 199 (TC-199 [pink]) with 1% dimethyl sulfoxide (DMSO), which are standard components in cellular products. The volume of placebo product will be 50 +/- 5 mL, which is in the range of a typical UCB unit that has been washed and thawed after cryopreservation. The 50 +/- 5 mL of placebo solution to be infused will be delivered to the clinic in a Sepax or transfer bag matching the final container used for the suspended UCB cells, so that the clinical staff remains blinded to study product or placebo.

4.5 Transport of Study Product

All study products will be transported from the laboratory to the bedside for infusion in a validated container to maintain room temperature. Once thawed and washed, the cells or placebo have an expiry of 6 hours at room temperature.

4.6 Administration of Allogeneic Umbilical Cord Blood or Placebo

On the day of infusion, a peripheral IV will be placed by the patient's care team if venous access has not been previously established.

Premedication for the cord blood or placebo infusion will be administered 30-60 minutes prior to the start of the infusion. Premedication with diphenhydramine 0.5mg/kg/dose IV (maximum 50 mg), hydrocortisone 1mg/kg /dose IV (maximum 100 mg), and acetaminophen 10-15mg/kg (maximum 650 mg orally [PO] or per rectum [PR]) will be administered. Antihypertensive medication may be advised for hypertensive patients or made available if needed in a timely manner, in light of the risk that premedications, specifically hydrocortisone, as well as residual DMSO in the cell product may contribute to elevation in blood pressure. Staff caring for the patient and the patient's family should be aware that residual DMSO in the UCB product often causes a temporary notable odor in patients' breath and from their skin for up to 24 hours post infusion.

The allogeneic cord blood will be administered intravenously over 5 to 30 minutes not exceeding a maximum of 5 mL/kg/hr; administration will be under direct physician supervision for the duration of infusion. The infusion rate will be reduced if the fluid load is not tolerated. Vital signs (heart rate, blood pressure, temperature, respiratory rate, pulse oximetry) will be checked before the infusion, every 5 minutes during the infusion, at the

end of the infusion, then as scheduled in MOP-01 until 4 hours post infusion. Patients will be observed for at least 4 hours post infusion and receive IV hydration for a minimum of 2 hours post infusion. Hydration will consist of normal saline (0.9% NS) infused at a minimum of 75 ml/hr. If the patient is receiving at least this rate of hydration (NS or other fluid) as part of their clinical care, no additional fluids will be given.

If the patient is discharged before 24 hours have passed since the start of infusion, there will be a phone call or observation by the study staff within 24 hours after discharge to document AEs or any change in status.

4.7 Care During Unexpected Events

In the event that a patient develops signs or symptoms of allergic reaction or anaphylaxis including urticaria, difficulty breathing, cough, wheezing, or vomiting during their infusion, the infusion will be paused, slowed, or terminated. Mild reactions may be treated with additional diphenhydramine, solumedrol, and/or inhaled albuterol. Other medications may be given as clinically indicated by the patient's physician.

If a patient has evidence of illness on the day of planned infusion, including but not limited to fever >38.1° C (100.5°F), active infection, progressive rash, vomiting, diarrhea, or respiratory distress, the infusion will be postponed. If the temperature drops and patient stabilizes to 38.1° C (100.5°F) and remains no higher than this for 24 hours, then the infusion may proceed as planned.

5 STUDY PLAN

5.1 Schedule of Procedures and Assessments

The schedule of study procedures and assessments is summarized in **Table 5**:

Table 5: Schedule of Study Procedures and Assessments

	Between Day 1 and Day 10	Between Day 3 and Day 10	Post Infusion				
	Screening	Day of Infusion	24 hours Phone or Visit	30 days ±7 days Phone or Visit	90 days ±14 days Visit at Clinic/Site	6 month ±14 days Phone call	12 month ±14 days Phone call
Consent	X[a]						
Eligibility	X						
Medical History/ Stroke History	X	X					
Physical/ Neurological exam	X[b]	X[b] (before premeds)	X[b]		X		
Vital Signs		X					
Brain MRI or CT	X[c]	X[c]					
Laboratory Testing	X[d]				X[e]		
Buccal swab	X[f]						
Research Blood Samples		X[g,h,i]	X[g,j]		X[g]		
Serum pregnancy test		X[k]					
Pre-medications		X (30-60 minutes prior to infusion)					
Infusion of UCB or placebo		X					
IV hydration		X					
NIHSS	X (daily until infusion)	X (before premeds and post infusion)	X[l]		X		
mRS	X (historical)	X (prior to infusion)		X	X	X	X
BI				X	X	X	X
EQ-5D-3L					X	X	X
SIS-16				X	X	X	X
TICS				X		X	X

	Between Day 1 and Day 10	**Between Day 3 and Day 10**	**Post Infusion**				
	Screening	**Day of Infusion**	**24 hours** Phone or Visit	**30 days ±7 days** Phone or Visit	**90 days ±14 days** Visit at Clinic/Site	**6 month ±14 days** Phone call	**12 month ±14 days** Phone call
PHQ-8					X	X	X
Survey on post-stroke rehab				X	X	X	X
Stroke Inventory Test Battery							
Trail Making Test					X		
MoCA					X		
COWAT					X		
HVLT-R					X		
Oral SDMT					X		
SF-36					X		
PHQ-8					X		
Safety questionnaire			X	X	X	X	X
Adverse events	X	X[h]	X	X	X	X	X
Concomitant medications	X	X[h]	X	X	X	X	X

[a] Potential subjects can be screened on the day of the primary stroke event, after confirmation of ischemic stroke, but neurological status must be confirmed within 24 hours prior to infusion. Consent may be obtained ≥ 24 hours following stroke onset (Day 1), but no later than 10 days after the index stroke event."

[b] Neurological exam is conducted prior to administration of premeds and pre- and post-infusion on the day of infusion. If subject is discharged less than 24 hours post infusion, the physical and neurological exam should be performed after administration of post-infusion fluids but prior to discharge.

[c] Clinical or diagnostic MRI results can be used. CT scan of the brain must be performed within 24h before infusion if brain MRI was not completed within 36h prior to infusion.

[d] Infectious disease panel, CBC and differential, lipid profile, coag panel, HLA typing, serum chemistries, troponin, liver function tests, ABO/Rh typing, direct and indirect Coombs, ESR, CRP and HLA antibody screen (PRA). For HLA typing being sent to MD Anderson, use 2 x 10 mL EDTA (lavender) tube and 1 x 10 ml ACD (yellow) tube.

[e] HLA antibody screen (PRA), ESR, CRP, direct and indirect Coombs. If any of these tests have been done within 2 days, those results can be used for the purpose of this study.

[f] Apolipoprotein E (APOE) genotyping (voluntary) will be performed on a buccal swab sample collected during screening or at any time prior to hospital discharge or at 90 day visit

[g] Collection of blood for research.

[h] Before administration of premeds.

[i] Within 24 h prior to start of infusion.
[j] Within 6-24 h of completing infusion.
[k] Within 48 hours prior to infusion for women of child bearing potential.
[l] NIHSS should be done 24 hours post infusion and daily post infusion for 7 days or until discharge.

5.2 Patient Interview

Patients who are potentially eligible to participate in the study or their legally authorized representatives (LARs) will be interviewed by study personnel who are trained to describe the study, schedule of visits, verify basic eligibility criteria, answer questions, and confirm interest in participation. If the patient and/or the patient's LAR are interested and the patient appears to be eligible after initial discussions, written informed consent will be obtained via the informed consent process.

5.3 Assessment of Patient Capacity to Consent:

Informed consent will be obtained by study staff experienced in working with stroke patients. The Investigator will clinically assess whether or not a patient will be able to provide legally effective informed consent at enrollment. A physician will be at every study visit to re-evaluate the patient's capacity to consent. For patients who are not able to give consent, a LAR must sign on the patient's behalf. In such cases, the patient will be informed of his or her enrollment in the study when he or she has become fully alert: the patient will be told who gave surrogate consent and that the patient has the right to withdraw from the study. The patient will be asked to sign the consent form at that time.

5.4 Screening, Consent, Study Visits and Follow-up Phone Calls

5.4.1 Screening and Consent (Day 1-10)

- Potential subjects can be screened on the day of the primary stroke event, after confirmation of ischemic stroke, but neurological status must be confirmed within 24 hours prior to infusion. Consent may be obtained ≥ 24 hours following stroke onset (Day 1), but no later than 10 days after the index stroke event.
- Medical/Stroke History
- Physical / Neurological exam
- MRI: A standard of care MRI may be used if it was performed within 36 hours prior to infusion. If an MRI was not performed within 36 hours prior to infusion, a CT must be done within 24 hours prior to infusion.
- Laboratory Tests: The laboratory tests listed below should be collected within 48 hours of screening. Exceptions include the ABO/Rh typing, lipid profile, coagulation panel, CBC and differential, and troponin; if these were performed as standard of care upon admission, the results can be used for screening, even if they are greater than 48 hours old.

  -CBC and differential
  -ABO/Rh typing

-Lipid Profile (total cholesterol, triglycerides, HDL, LDL)
-Coagulation Panel (PT/INR, PTT)
-HLA typing
-Serum Chemistries (sodium, potassium, chloride, bicarbonate, urea nitrogen (BUN), creatinine, glucose, calcium, troponin)
-Liver Function Tests (AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin)
-Direct and Indirect Coombs
-HLA antibody screen (Panel Reactive Antibody [PRA]), ESR, and CRP

- Infectious disease screening panel performed in an FDA approved, CLIA certified laboratory: Hepatitis B Surface Antigen, Antibody to Hepatitis C Virus, Antibody to Human Immunodeficiency Virus Types 1 and 2, Antibody to Hepatitis B Core Antigen, Antibody to Human T-lymphotropic Virus Types I and II, *Trypanosoma cruzi*, Antibody to *Treponema pallidum* (syphilis), Antibody to Cytomegalovirus, HIV-1/HCV/HBV NAT (nucleic acid test), West Nile Virus NAT
- (Optional) Buccal swab collected for APOE genotyping at screening, prior to hospital discharge or at 90-day visit.
- NIHSS should be performed daily beginning the day of consent through the day of infusion (prior to infusion of the UCB cells or placebo), within 24 hours of administration of post-infusion fluids, and for up to 7 days post-infusion or until discharge.
- mRS (historical)
- AEs
- Concomitant meds

5.4.2 Day of Infusion (Day 3-10)

- Review of labs (pre-infusion).
  Results of labs related to the inclusion/exclusion criteria should be reviewed by the study staff to verify that the subject remains qualified for the study prior to infusion. Results for the HLA typing, infectious disease panel, direct and indirect Coombs, ESR, CRP and HLA antibody screen do not need to be final prior to infusion. In addition, clinical labs should be reviewed by the PI or designee in order to establish that there are no contraindications to infusion of the cord blood.
- Medical / Stroke History and brief physical exam (pre-infusion before pre-meds)
- Neurological/Physical Exams (before pre-meds)
  Neurological/physical exam is conducted prior to administration of premeds and pre- and post-infusion for day of infusion. If subject is to be discharged less than 24

hours post infusion, the physical and neurological exam should be performed after administration of post-infusion fluids but prior to discharge.

- CT scan should be performed within 24 hours pre-infusion if MRI is not performed within 36 hours of infusion
- (Optional) Collection of research blood samples for biomarkers/immuno-panel analysis within 24 hours prior to infusion
- Serum pregnancy test pre-infusion for women of childbearing potential
- Administration of pre-meds 30-60 minutes prior to the start of the infusion. Premedication consists of diphenhydramine 0.5mg/kg/dose IV (maximum 50 mg), hydrocortisone 1mg/kg /dose IV (maximum 100 mg), and acetaminophen 10-15mg/kg (maximum 650 mg orally [PO] or per rectum [PR]). Antihypertensives may be administered if clinically indicated or should be made available if needed in a timely manner.
- Infusion of study product with cardiac-respiratory hemodynamic monitoring and vital signs (temperature, heart rate, respiratory rate, blood pressure, pulse oximetry) pre-infusion, every 5 minutes during the infusion, at the end of the infusion, and as scheduled in MOP-01 until 4 hours post infusion
- Patients will be treated with standard IV hydration for a minimum of 2 hours post infusion.
- Assessments:
  - mRS, NIHSS (prior to start of infusion and administration of pre-meds)
  - If on the day of infusion NIHSS is <6, patient remains eligible for infusion
  - Subjects with >4-point increase of NIHSS from time of consent (worsening of score) will not be eligible for infusion.
  - NIHSS (within 24 hours after administration of post-infusion fluids)
  - Neurological and physical exam (before pre-meds and infusion and post infusion)
  - AEs, Concomitant meds (post infusion), safety questionnaire

5.4.3 Follow-up phone call or visit 24-hour post infusion

- Physical/Neurological exam (if visit)
- (Optional) Collection of research blood samples for biomarkers/immuno-panel analysis within 6-24 hours of completing infusion. If patient is discharged prior to this time no blood will be collected.

- If the subject is discharged on the day of infusion, then a physical exam and neurological exam should be performed after infusion and fluid administration but prior to discharge.
- NIHSS should be done within 24 hours of administration of post-infusion fluids and daily for 7 days post-infusion or until discharge.
- AEs, Concomitant meds

5.4.4 Follow-Up Phone Call or Visit (Day 30 ± 7 days)

- Assessments: mRS, BI, SIS-16, TICS Survey on Post-stroke Rehabilitation
- Safety questionnaire, AEs, Concomitant meds

5.4.5 Visit 1 – 90 Days Post Infusion (± 14 days)

- Physical/ Neurological exam
- Laboratory Tests: HLA antibody screen (PRA), ESR, CRP, direct and indirect Coombs
- Collection of research blood samples for biomarkers/immuno-panel analysis
- Assessments: mRS, NIHSS, BI, EQ-5D-3L, SIS-16, PHQ-8, Survey on Post-Stroke Rehabilitation, Stroke Inventory Test Battery (SITB)
- Survey on Post-stroke Rehabilitation
- Safety questionnaire, AEs, Concomitant meds

5.4.6 Follow-up phone call (6 months ±14 days)

- Assessments: mRS, BI, SIS-16, EQ-5D-3L, PHQ-8, TICS
- Survey on Post-stroke Rehabilitation
- Safety questionnaire, AEs, Concomitant meds

5.4.7 Follow-up phone call (12 months ±14 days)

- Assessments: mRS, BI, EQ-5D-3L, SIS-16, PHQ-8, TICS
- Survey on Post-stroke Rehabilitation
- Safety questionnaire, AEs, Concomitant meds

6 STATISTICAL CONSIDERATIONS

6.1 Accrual

It is estimated that up to 6 research participants will be enrolled each month at each of the 6 centers and that approximately 20-24 months of accrual will be necessary to enroll 100 patients.

6.2 Study Duration

Research participants will be followed for 12 months after the administration of allogeneic cord blood.

6.3 Demographics and Baseline Characteristics

The number of patients completed and discontinued early from study, and the reasons for discontinuation, will be summarized, if applicable. Demographics and baseline characteristics to include age, gender, and race/ethnicity and baseline status as measured by the mental and physical assessments will be summarized for all patients.

6.4 Description of the Primary Efficacy Outcome Measure

The mRS is a commonly used scale for measuring the degree of disability or dependence in the daily activities of people who have suffered a stroke, and it has become the most widely used clinical outcome measure for stroke clinical trials. The mRS is scored from 0 to 6, running from perfect health without symptoms (0) to death (6)[57,63] Administration of the mRS takes 5 minutes. Personnel performing the mRS must have current certification.

The mRS is:

0 - No symptoms.

1 - No significant disability. Able to carry out all usual activities, despite some symptoms.

2 - Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities.

3 - Moderate disability. Requires some help, but able to walk unassisted.

4 - Moderately severe disability. Unable to attend to own bodily needs without assistance, and unable to walk unassisted.

5 - Severe disability. Requires constant nursing care and attention, bedridden, incontinent.

6 - Dead.

6.5 Sample Size and Power Calculations

The primary endpoint for this study is the shift in mRS from baseline to 90 days, defined for the *i*-th patient as:

$$\delta_i = mRS_0 - mRS_{90}, \qquad i = \{1..N\}$$

Where mRS_0 and mRS_{90} are the baseline and 90-day mRS scores, respectively. Since a shift downward in the mRS scale is considered a clinical improvement, the differences in δ_i are calculated from baseline to insure that, for hypothesis testing purposes, larger shift values represent more clinically desirable outcomes.

Given the above definition we can define testable hypotheses for the primary efficacy analysis as follows.

(Null hypothesis) H0: $P(T_i > P_i) = P(P_i > T_i)$

(Alternative hypothesis) HA: $P(T_i > P_i) \neq P(P_i > T_i)$

Where T_i and P_i are randomly selected 90-day mRS shift scores from the population of patients treated with cord blood (T) or placebo (P), with the differences having the form of δ_i shown above.

The test of this hypothesis can be carried out using a 2-sample Wilcoxon rank sum test. We used Noether's formula[78] to approximate sample size required under various alternatives assuming a 2:1 allocation (cord:placebo), a 5% Type I error rate, and power of 80%. The specific alternative hypothesis, or detectable effect size, in this case is the probability that a randomly sampled patient treated with cord blood has a higher (more favorable) mRS shift score (δ_i) compared with a randomly sampled patient treated with placebo. The probability can also be understood as an odds ratio (OR) comparing treatment to placebo. The following table (**Table 6**) illustrates sample sizes required to detect various effect sizes (expressed both as probabilities and ORs) assuming the aforementioned design parameters.

Table 6. Sample Size Parameters

Probability under HA	OR under HA	N_{Total}	$N_{Placebo}$	$N_{Treatment}$
0.55	1.2	1,461	483	978
0.57	1.3	700	231	469
0.58	1.4	429	142	287

Probability under HA	OR under HA	N_{Total}	$N_{Placebo}$	$N_{Treatment}$
0.60	1.5	295	98	197
0.62	1.6	223	74	149
0.63	1.7	175	58	117
0.64	1.8	144	48	96
0.66	1.9	123	41	82
0.67	2.0	102	34	68

An effect size of p=0.67 (OR=2.0) was selected for this study as it is felt to be the minimal clinically relevant effect size and is detectable with a reasonable sample size. The total sample size required is N=102 (68 on cord blood and 34 on placebo). For simplicity we round the number to N=100.

Therefore, with a total sample size of N=100 (67 on cord blood and 33 on placebo), our study will have approximately 80% power to detect a significant difference between cord blood and placebo when the true probability of a greater mRS shift in cord blood patients (compared to placebo) is 67%. This equates to a doubling of the odds that a randomly selected patient treated with cord blood will have a more favorable mRS shift score compared with a randomly selected patient treated with placebo. These calculations assume a Type I error rate of 5%.

6.6 Correction for Multiple Testing

The primary efficacy analysis will use an alpha of 0.05. Statistically significant results on the secondary efficacy endpoints will be identified using the False Discovery Rate (FDR) procedure. The primary efficacy comparison will be included in the FDR procedure. Analysis of safety outcomes and exploratory endpoints will be primarily descriptive and thus will not include correction for multiple testing. Further details are available in the Statistical Analysis Plan (SAP).

6.7 Randomization Plan

This study will randomize participants 2:1 to cord blood or placebo and will utilize a randomly varying block size with stratification by study center (up to 6 centers) and NIHSS score (6–11 or 12-18). The NIHSS score collected closest to randomization will be used.

6.8 Analysis Plan

Full details of the planned analysis are available in the accompanying SAP. Briefly, safety analyses will be primarily descriptive and will use graphical displays and tabulated descriptive statistics. The efficacy analysis for the primary endpoint is based on the normal approximation to a two-sample Wilcoxon rank sum test. Additional analyses are described in the SAP.

6.8.1 Analysis Populations (see SAP for further details):

Full Analysis Population – This population includes all enrolled participants and is used for descriptive purposes; e.g. to identify patients who do not provide data for the primary efficacy analysis.

Modified Intention to Treat (mITT) Population – This analysis set will include only patients who complete the mRS at both baseline and 90 days. Calculation of the mRS shift score is not possible in patients who are missing either the baseline or 90-day mRS evaluation. Patients will be analyzed according to the treatment they were assigned (regardless of what they actually receive).

Safety Population – The safety population will include all patients who received an infusion. Analyses of the Safety Population will be conducted using an as-treated approach, which considers each patient according to the treatment actually received rather than the treatment they were assigned.

6.9 Interim Analysis

An interim estimation of conditional power[79] will be conducted when the primary endpoint is evaluable on approximately 50% of the participants (17 patients in the placebo group 34 in the cord blood group). Conditional power will be evaluated assuming future data are consistent with the observed trend at the time of the interim analysis as well as the planned alternative. There is no a priori threshold for curtailing the trial for futility. Rather, the interim analysis is intended to provide information to the DSMB and study sponsor to assist with determining whether the trial should proceed as designed, or whether the plans detailed in this protocol are realistic under reasonable alteration to the pre-specified sample size. The interim analysis will be accomplished by an independent, unblinded statistician.

7 SAFETY AND ADVERSE EVENT REPORTING

7.1 Adverse Events

Adverse event means any untoward medical occurrence associated with the use of the investigational product, whether or not considered related to the investigational product.

7.1.1 Serious Adverse Events

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

7.1.2 Grade/Severity

Grade/Severity will be performed per Common Terminology Criteria for Adverse Events (CTCAE) guidelines.

7.1.3 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the investigational product and the adverse event.

7.1.4 Causality

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

7.2 Adverse Event Reporting

All AEs reported or observed during the study beginning at the time of the allogeneic cord blood infusion must be recorded and maintained in the study participant's paper files or electronic case report forms. Information to be reported includes when the site became aware of the event, Investigator-specified assessment of severity and relationship to study therapy, whether there is an alternative etiology, seriousness, as well as any required treatment for evaluations, and outcome. In general, investigators should report AEs as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequelae. Severe adverse reactions (fatal or life threatening) that are unexpected and related will be reported to the FDA by Duke University within seven calendar days of receipt of the information by telephone, mail or fax, following FDA guidelines. All non-life threatening serious, related and unexpected AEs will be reported to the FDA via a written report within 15 days of receipt of the information (21CFR 312.32) by Duke University. If the Principle Investigator assesses the event to be unrelated to the study, then the event will not require expedited reporting but will be included in a summary report.

7.3 Serious Adverse Event Reporting

Any protocol deviation, SAE which is directly related to the study product, or safety event that is reportable for any other reason should be communicated via phone or email to the protocol PIs with Erin Arbuckle (study coordinator) and Jessica Chapman (Duke Regulatory Affairs) also notified.

Contact Information for Safety Reporting:
Joanne Kurtzberg, MD: joanne.kurtzberg@duke.edu, 919-668-1100
Daniel Laskowitz, MD: danl@neuro.duke.edu, 919-684-5650
Erin Arbuckle: erin.arbuckle@dm.duke.edu, 919-684-3293
Jessica Chapman, PhD: jessica.chapman@duke.edu, 919 668-7962

The Principal Investigator or its representative will be responsible for telephone, mail or fax reporting of suspected unexpected serious adverse reactions (SUSARs) to the FDA according to 21 CFR 312.32. The Principal Investigator or its representative will notify the FDA by telephone, mail or fax of any fatal or life threatening experience (expedited report) associated with the use of the study therapy as soon as possible but no later than 7 calendar days after receipt of the information. Initial notification will be followed by a written report within 15 calendar days. For non-life threatening SUSARs, the Principal Investigator will notify the FDA as soon as possible, but no later than 15 days of the initial receipt of the information. All reportable events per Duke HRPP policies, whether at the Duke site or elsewhere, will be reported to the Duke IRB in accordance with HRPP and/or FDA reporting requirements. The Principal Investigator or Sub-Investigator is responsible for informing the Institutional Review Board (IRB) and Data Safety Monitoring Board (DSMB). Copies of SAE correspondence with all Principal Investigators or Sub-Investigators, governing authorities, ethics committees, and the sponsor must be submitted to Duke University for filing.

7.4 Eliciting Adverse Event Information

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

7.5 Stopping Guidelines

Two stopping guidelines based on safety will be monitored during the duration of the study. The stopping guidelines will be monitored by the study personnel, and are to be used to indicate boundaries requiring discussion by the DSMB. Accrual will be temporarily suspended if:

- Any patient experiences a grade 4-5 infusion related reaction within 24 hours of infusion;

OR

- Any patient experiences a blood stream infection deemed related to the cord blood infusion within 6 months of infusion
- Any patient develops grade III-IV GVHD within 6 months of infusion

A consensus decision to stop the study will be made by the Investigators and the DSMB. Such a decision with its supporting documentation and possible future plans for the study will be submitted to, and discussed with, the FDA.

7.6 Patient Replacement

Treatment assignments will not be replaced for patients who are not infused, lost to follow-up, or are discontinued from the study. However, additional patients may be randomized per decision of the study sponsor.

8 DATA SAFETY MONITORING BOARD (DSMB)

A DSMB will be formed and charter established. Faculty of Duke University Medical Center or other participating clinical sites are exempted from participation on this study DSMB. Members of the DSMB will comprise neurologists and a physician with experience in cell therapy. A non-voting statistician will be invited to meetings or calls as needed. The DSMB will be notified immediately for all SAEs directly related to study product throughout the study. A total safety assessment will be prepared on an annual basis and forwarded to the DSMB for review as well. Policies of the DSMB will be described in the DSMB charter, and signed by all members.

9 DATA HANDLING AND QUALITY ASSURANCE

9.1 Case Report Forms

As part of the responsibilities assumed by participating in the study, the Principal Investigator or Sub-Investigators agree to maintain adequate case histories of the research participants treated as part of the research under this protocol. The Principal Investigator and Sub-Investigator agree to maintain accurate case report forms (CRFs) and source documentation as part of the case histories. RTI will supply the CRFs electronically (eCRF) through secured electronic data entry systems.

9.2 Inspection of Records

The Principal Investigator and Sub-Investigators at all institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the Principal Investigator or

Sub-Investigator agrees to allow the sponsor, the FDA, or other regulatory agency access to all study records.

The Principal Investigator or Sub-Investigators should promptly notify the sponsor and monitor of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to both.

9.3 Study Record Retention

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

10 ADMINISTRATIVE ASPECTS

10.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain research participant confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the research participant's LAR except as necessary for monitoring and auditing.

The Principal Investigator or Sub-Investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

10.2 Institutional Review Board Approval

Federal regulations and the International Council on Harmonisation guidelines require that approval be obtained from an IRB prior to participation of human research participants in research studies. Prior to the study onset, the protocol, informed consent, any advertisement used to recruit study patients, and any other written information regarding this study to be provided to the research participant or the research participant's LAR must be approved by the IRB. Documentation of all IRB approvals and of the IRB compliance with International Council on Harmonisation Guideline E6 will be maintained by the site and will be available for review by the sponsor or its designee.

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

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

10.3 Modification of the Protocol

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

10.4 Informed Consent

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

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

The Principal Investigator or Sub-Investigator shall provide a copy of the signed informed consent to the research participant and/or LAR. A second original form shall be maintained in the research participant's medical records at the site.

Informed consent will be obtained by study staff experienced in working with stroke patients. The Investigator will clinically assess whether or not a patient will be able to provide informed consent at enrollment. A physician will be at every study visit to re-evaluate the patient's capacity to consent. For patients who are not able to give consent, a LAR must sign on the patient's behalf. In such cases, the patient will be informed of his or her enrollment in the study when he or she has become fully alert: the patient will be told who gave surrogate consent and that the patient has the right to withdraw from the study. The patient will be asked to sign the consent form at that time.

10.5 Protocol Violations and Deviations

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

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

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the IRB and agreed to by the Principal Investigator or Sub-Investigator. Deviations usually have an impact on individual research participants or a small group of research participants and do not involve inclusion/exclusion or primary endpoint criteria.

A protocol violation occurs when there is nonadherence to the protocol that results in a significant, additional risk to the research participant; when the research participant or Principal Investigator or Sub-Investigator has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the research participant is enrolled without prior sponsor approval; or when there is nonadherence to FDA regulations and/or International Council on Harmonisation GCP guidelines.

Protocol violations and deviations will be documented by the clinical monitor throughout the course of monitoring visits. Principal Investigators or Sub-Investigators will be notified of violations and/or deviations in writing by the monitor. The Principal Investigator should ensure that the IRB is notified of all protocol violations and deviations in a timely manner as required.

10.6 Study Reporting Requirements

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

11 Financial Obligations

Duke University is not financially responsible for further testing/treatment of any medical condition that may be detected during the screening progress. In addition, in the absence of specific arrangements, Duke University is not financially responsible for further treatment of the research participant's disease.

12 Study Conduct

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

13 Publications

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

Data is the property of Duke University but data and publication thereof will not be unduly withheld.

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15 Appendix

Survey on Post-stroke Rehabilitation- Please answer three questions:

1. Did you receive rehabilitative therapy while in the acute-care hospital?

No ☐

Yes ☐

2. Did you receive rehabilitative therapy following discharge form the acute-care hospital?

No ☐

Yes ☐

3. If yes, check the services you received since the last study visit and frequency of treatments:

	FREQUENCY		
	Daily	**≥ 1 X/week**	**<1 X/week**
Rehabilitation Nurses	☐	☐	☐
Physical Therapists	☐	☐	☐
Occupational and recreational Therapists	☐	☐	☐
Speech-Language pathologists	☐	☐	☐