

A Randomized, Placebo-Controlled, Phase II Trial of Intravenous Allogeneic Non-HLA Matched, Unrelated Donor, Cord Blood Infusion for Ischemic Stroke

Daniel T. Laskowitz^{*.1}, Jesse Troy², Emily Poehlein², Ellen R. Bennett¹, Elizabeth J. Shpall³, John R. Wingard⁴, Brian Freed⁵, Samir R. Belagaje⁶, Anna Khanna⁷, William Jones⁸, John J. Volpi⁹, Eric Marrotte¹⁰, Joanne Kurtzberg¹¹ 

¹Department of Neurology, Duke University School of Medicine, Durham, NC, USA

²Department of Biostatistics and Bioinformatics, Duke University School of Medicine, Durham, NC, USA

³The MD Anderson Cord Blood Bank, Houston, TX, USA

⁴LifeSouth Cord Blood Bank, University of Florida, Gainesville, FL, USA

⁵ClinImmune Labs, University of Colorado Cord Blood Bank, Aurora, CO, USA

⁶Departments of Neurology and Rehabilitation Medicine, Emory University School of Medicine, Atlanta, GA, USA

⁷Department of Neurology, University of Florida, Gainesville, FL, USA

⁸Department of Neurology, University of Colorado, Aurora, CO, USA

⁹Department of Neurology, Houston Methodist, Houston, TX, USA

¹⁰Department of Neurology, Wake Forest University Baptist Medical Center, Winston-Salem, NC, USA

¹¹Marcus Center for Cellular Cures, Duke University School of Medicine, Durham, NC, USA

*Corresponding author: Daniel T. Laskowitz, MD, Department of Neurology, Duke University School of Medicine, 227B Bryan Research Building, Durham, NC 27710, USA. Tel: +1 919 684 6514. Email: daniel.laskowitz@duke.edu

Abstract

Stroke remains a leading cause of death and disability in the US, and time-limited reperfusion strategies remain the only approved treatment options. To address this unmet clinical need, we conducted a phase II randomized clinical trial to determine whether intravenous infusion of banked, non-HLA matched unrelated donor umbilical cord blood (UCB) improved functional outcome after stroke. Participants were randomized 2:1 to UCB or placebo within strata of National Institutes of Health Stroke Scale Score (NIHSS) and study center. Study product was infused 3–10 days following index stroke. The primary endpoint was change in modified Rankin Scale (mRS) from baseline to day 90. Key secondary outcomes included functional independence, NIHSS, the Barthel Index, and assessment of adverse events. The trial was terminated early due to slow accrual and logistical concerns associated with the COVID-19 pandemic, and a total of 73 of a planned 100 participants were included in primary analyses. The median (range) of the change in mRS was 1 point (–2, 3) in UCB and 1 point (–1, 4) in Placebo ($P = 0.72$). A shift analysis comparing the mRS at day 90 utilizing proportional odds modeling showed a common odds ratio of 0.9 (95% CI: 0.4, 2.3) after adjustment for baseline NIHSS and randomization strata. The distribution of adverse events was similar between arms. Although this study did not suggest any safety concerns related to UCB in ischemic stroke, we did not show a clinical benefit in the reduced sample size evaluated.

Key words: stroke; cellular therapy; umbilical cord blood; stem cells; clinical trials.

Graphical Abstract

Title: A Randomized, Placebo-Controlled, Phase 2 Trial of Intravenous Allogeneic Non-HLA matched, unrelated donor, Umbilical Cord Blood Infusion for Adults with Ischemic Stroke

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Primary Objective:

To determine, in a multicenter, randomized, placebo-controlled trial, the feasibility, safety, and efficacy of a single intravenous (IV) infusion of banked, non-HLA matched, unrelated donor umbilical cord blood (UCB) in patients with ischemic stroke. This clinical trial represented the collaboration of 4 cord blood banks and 6 stroke treatment centers to execute the study.

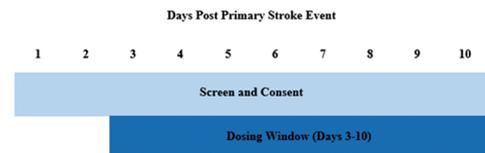
Treatment:

Intravenous infusion of a single non-HLA matched, banked umbilical cord blood unit or placebo to a participant with acute ischemic stroke within 3-10 days of the stroke event.



Duke CoBIS Study

Study Flow Chart

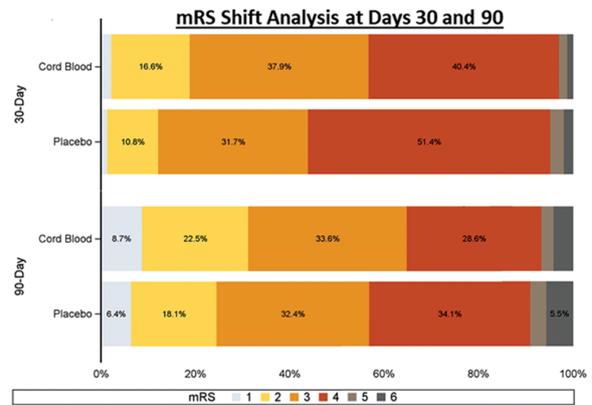
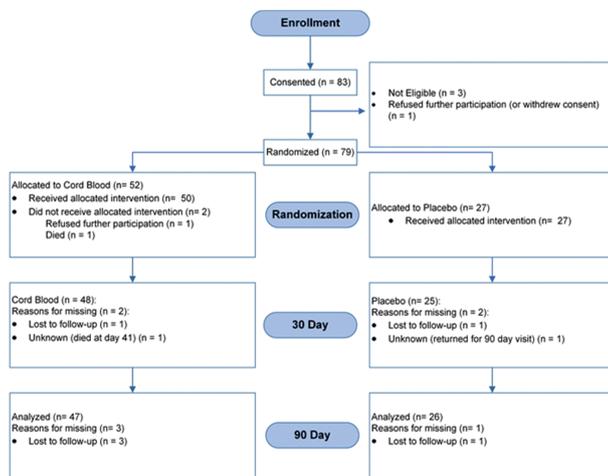


Potential subjects can be screened on the day of the primary stroke event, after confirmation of ischemic stroke, but neurological status must be confirmed within 24 hours prior to infusion. Consent may be obtained ≥ 24 hours following stroke onset (Day 1), but no later than 10 days after the index stroke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

Primary Endpoint:

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

Enrollment Schema



Significance Statement

Stroke remains a leading cause of death and disability in the US with limited treatment options. In this phase II study, we demonstrate the safety and feasibility of infusing banked, non-human leukocyte antigen matched, unrelated allogeneic umbilical cord blood into adults during the 3-10 days window following acute ischemic stroke. These observations are consistent with results of a recent phase I study. This study was not adequately powered for efficacy and further reduced secondary to the COVID-19 pandemic and no clinical benefit was observed in the patients enrolled.

Introduction

Every year, ~800 000 people in the US suffer a stroke, of which ~90% are ischemic.¹ Although there has been an emphasis on evaluating pharmacological and mechanical interventions to promote reperfusion and reduce secondary tissue injury after acute stroke, these strategies are time limited, and stroke remains a leading cause of long-term disability, affecting one in six people worldwide. To date, there are no Food and Drug Administration (FDA)-approved pharmacological interventions targeting neuroprotection in acute ischemic stroke, and the only approved therapy to promote early reperfusion is intravenous administration of tissue plasminogen activator (tPA).² Unlike traditional neuroprotective and reperfusion therapies, cell-based interventions may modulate inflammation and improve long-term plasticity and functional recovery.³ Preclinical data suggest that the delivery of human stem cells into animal models of stroke may reduce infarct volume and improve functional outcomes.^{4,5} Although

the exact mechanisms of these beneficial effects remain poorly defined, it has been demonstrated that in a number of animal models of brain injury, stem cells are capable of migration and immunomodulation, secrete neurotrophic factors, and modulate neuroinflammatory responses after acute ischemic injury.⁶ If successful, such therapies would allow for interventions in the subacute setting and offer the potential for reducing the burden of disability in a much larger population than is eligible for reperfusion.

Although cell-based interventions offer the potential to improve outcomes after subacute brain injury, there remain a number of unique challenges associated with this strategy, including defining the optimal cell-based intervention, mode of administration, and timing of intervention relative to the index stroke. For example, several recent early phase trials have been performed evaluating the use of bone marrow-derived mononuclear cells, mesenchymal stem cells, and modified cell lines in the setting of subacute stroke.⁷⁻¹²

The studies, which were associated with intravenous, intra-arterial, intrathecal, and intraparenchymal modes of delivery have been associated with favorable safety profiles and challenging logistics. To date none have demonstrated definitive improvements in functional outcomes.

One promising intervention that mitigates the risk inherent in invasive intraparenchymal and intra-arterial modes of delivery or bone marrow harvest in critically ill patients, is the intravenous administration of banked unrelated allogeneic umbilical cord blood (UCB).^{13,14} Preclinical studies have demonstrated both neurotrophic¹⁵ and immunomodulatory¹⁶ properties of UCB, which are associated with functional improvements in experimental stroke models.¹⁷ Moreover, there are a number of clinical advantages to the use of UCB which facilitate translation to the clinical setting. For example, UCB cells are immunologically tolerant, and associated with a long history of safety in the setting of partially human leukocyte antigens (HLA)-mismatched hematopoietic stem cell transplantation in unrelated donors.^{14,16} UCB is a readily available, cryopreserved, banked blood product which does not require collection of autologous cells via bone marrow harvest or peripheral stem cell collection; this is a particular advantage in the setting of medically vulnerable patients following stroke. The safety and feasibility of UCB in subacute stroke was recently demonstrated in a pilot trial in which banked, nonhuman leukocyte antigen matched UCB were administered intravenously into adults 3-10 days following a middle cerebral artery stroke.¹⁸ The current randomized, placebo-controlled trial was designed to evaluate the feasibility and efficacy of a single intravenous (IV) infusion of non-HLA matched, unrelated donor UCB for improving functional outcomes in patients with ischemic stroke.

Methods

Study design

COBIS 2 was designed as a multicenter, placebo controlled, randomized, double-blinded phase II study in 100 subjects 18-90 years of age who had sustained a recent ischemic stroke in the middle cerebral artery (MCA) distribution without a clinically significant midline shift prior to infusion. Volunteers could be screened on the day of the primary stroke event after confirmation of ischemic stroke, but neurological status was confirmed within 24 hours prior to infusion. After obtaining informed consent, patients were randomized 2:1 to receive treatment with umbilical cord blood (UCB) cells or placebo, which was administered intravenously as a single infusion 3-10 days after the patient's index stroke.

Participants

Eligible patients were male and female adults 18-90 years of age who experienced an acute cortical ischemic stroke in the middle cerebral artery (MCA) distribution that was verified by diffusion-weighted imaging (DWI) abnormality on magnetic resonance imaging (MRI). Eligible patients were required to 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) were not eligible for infusion. Patients were required to have a platelet count >100 000/ μ L, hemoglobin > 8 gm/dL, white blood cell count (WBC) > 2500/ μ L, and an absolute lymphocyte count (ALC) \geq 1200 for African-American patients and \geq 1500 for all other racial-ethnic groups, and WBCs > 2500/ μ L. If ALC

upon presentation was disqualifying, patients were eligible for enrollment if they had a historical pre-stroke of ALC \geq 1200 for African American and \geq 1500 for all other racial-ethnic groups within 6 months of stroke and a post stroke ALC value of \geq 1000. Patients with pre-morbid mRS > 1, hemorrhagic conversion or midline shift, isolated brainstem or lacunar stroke, prolonged need for mechanical ventilation, coagulopathy, systemic infection, pregnancy active malignancy within 3 years, autoimmune disease or any disease or therapy that would compromise immune function were excluded. Patients were also excluded from the study if they had an active malignancy or autoimmune disease requiring immunosuppressive therapy within 3 years prior to the start of screening (excluding skin cancers other than melanoma). Patients who received tissue plasminogen activator (tPA) or endovascular reperfusion were eligible for inclusion included in the study if they met all other inclusion and exclusion criteria. A detailed list of inclusion/exclusion criteria is provided in [Supplementary Table S1](#).

Intervention and Allogeneic UCB Infusion

Subjects were not HLA typed and did not receive immunosuppressive or myeloablative medications between the time of consent and the infusion. UCB units were selected from an accredited U.S. public cord bank (The Carolinas Cord Blood Bank, Durham, NC; The MD Anderson Cord Blood Bank, Houston, TX; LifeSouth Cord Blood Bank, Gainesville, FL; The University of Colorado Cord Blood Bank, Aurora, CO) based on blood type and a targeted cell dose ranging between 0.5 and 5 \times 10⁷ total nucleated cell count (TNCC)/kg. Emory, Wake Forest, and Duke participants received cord blood units (CBU) from the Carolinas Cord Blood Bank, MD Anderson participants received CBU from MD Anderson Cord Blood Bank, University of Florida participants received CBU from LifeSouth Cord Blood Bank, and University of Colorado participants received CBU from the University of Colorado Cord Blood Bank. On the day of infusion, UCB was thawed in a 37 degree C waterbath, and washed in an automated device (Sepax 2 RM) (Cytiva Life Sciences, Marlborough, MA) with dextran 40 with 5% human serum albumin. The washed product was deposited into a transfer bag on the washing kit (Cytiva, Marlborough, MA) in 50 mL Dextran 40 and 5% human serum albumin. Thawed UCB units were tested for enumeration of total nucleated cell count (TNCC), viable CD34+ cells, colony forming units, cell viability via trypan blue, confirmatory HLA typing, and sterility cultures. A final 50 mL volume of the cellular product was transported to the care site of the participant in the transfer bag using a container validated to maintain 20.8-24.8 °C for administration.

Stroke participants were premedicated with diphenhydramine 0.5 mg/kg/dose IV (maximum 50 mg), hydrocortisone 1 mg/kg/dose i.v. (maximum 100 mg), and acetaminophen 10-15 mg/kg (maximum 650 mg) by mouth (PO) or per rectum (PR) 30-60 minutes prior to the UCB infusion. Antihypertensive medication was available at the patient's bedside because of the potential risk that hydrocortisone and residual dimethyl sulfoxide in the cell product would elevate blood pressure, although the threshold for permissive hypertension was defined by the treating clinician prior to infusion. A peripheral IV was used to administer allogeneic UCB over a period of 5-30 minutes, at a maximum rate of 5 mL/kg per hour; this was performed under direct physician supervision. Participants received IV hydration of normal saline infused at a minimum of 75 mL/hour for 2-4 hours post-infusion. When the UCB

infusion was initiated >24 hours after the baseline brain MRI, an uncontrasted head computerized tomography (CT) was obtained within 24 hours prior to infusion, to evaluate for exclusionary pretreatment hemorrhage, increasing edema, or midline shift. All patients received standard of care therapy while enrolled in this study and all subjects were encouraged to participate in rehabilitative therapy.

Randomization and Masking

This study randomized participants 2:1 to cord blood or placebo and utilized a randomly varying block size of 3 or 6 with stratification by study center and NIHSS score (6-11 or 12-18). The 2:1 allocation was selected to facilitate recruitment and expand the safety database for UCB in ischemic stroke. The randomization table was prepared by an independent statistical coordinating center. During the pre-planned interim analysis (see below), an irregularity in the ratio of allocated treatments was observed that led to a discovery of an error in the randomization table. Specifically, when the separate strata of the randomization schedule were merged into a single table there was a mistaken overlap in which the assignments from 10 blocks in the table were split across 2 strata. At the time of discovery, 6 treatment assignments from split blocks had been allocated to patients. Although this caused a deviation from the intended 2:1 allocation ratio at the time of the interim analysis, when the study ended the allocation ratio was correct at 2:1.

All subjects, families and medical staff were blinded. Following randomization, the selected CBU was shipped frozen overnight to the site. Once selected and available on site, each CBU was thawed, washed, tested, released and infused intravenously using common standard operating procedures (SOPs) at all sites. The placebo product was acellular and consisted of tissue culture medium 199 (TC-199 [pink] Gibco, Waltham, MA) with 1% dimethyl sulfoxide (DMSO, Protide, Crystal Lake, IL), which are standard components in cellular products. This product is similar in both appearance and odor as CBU. The volume of placebo was 50 mL \pm 5mL, the same range for UCB infusion. The placebo was delivered to the clinic in the same final container as used for the CBU infusion with the same masking cover.

Primary and Secondary Outcomes

The primary outcome measure was the Modified Rankin Scale (mRS), a validated 7-level ordinal scale ranging from 0 (no symptoms) through 6 (dead) that is commonly used to assess global functional outcome after stroke.¹⁹ The mRS is highly correlated with health-related quality of life and a score of ≤ 2 is considered compatible with functional independence. The mRS is scored for each patient as: 0 = no symptoms; 1 = no significant disability despite symptoms; able to carry out all usual duties and activities; 2 = slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance; 3 = moderate disability; requiring some help, but able to walk without assistance; 4 = moderately severe disability; unable to walk and attend to bodily needs without assistance; 5 = severe disability; bedridden, incontinent and requiring constant nursing care and attention; or 6 = dead.

The mRS was assessed at baseline, day 30, and day 90. The primary outcome of the study was the difference in mRS between baseline and day 90, which yields a 13-level ordinal scale where positive numbers indicate improved functional outcome at day 90 as compared to the time of the stroke, 0 indicates no change, and negative values indicate worse

functional outcome at day 90 than at the time of the stroke. Key secondary outcomes were the mRS at days 30 and 90, functional independence at day 90 (defined as mRS 0, 1, or 2), the NIHSS,²⁰ activities of daily living measured using the Barthel index,²¹ and a series of patient reported outcome measures at days 30, 90, and 1 year: The Stroke Impact Scale,²² European Quality of Life Health Questionnaire,²³ the Patient Health Questionnaire Scale,²⁴ and the Telephone Interview for Cognitive Status (TICS).²⁵

Secondary safety and tolerability endpoints included incidence of infusion reaction, infection, alloimmunization, and graft vs host disease. A cognitive battery, comprised primarily of Stroke Common data elements,²⁶ including Trail Making Task,^{27,28} Montreal Cognitive Assessment,²⁹ Controlled Oral Word Association Task,³⁰ and the Hopkins Verbal Learning Test-Revised³¹ were performed at day 30.

Safety Monitoring

Safety was evaluated during the infusion, the first 24 hours post-infusion, and at scheduled visits or telephone calls performed 30 days, and at 3, 6, and 12 months after treatment. During the day of treatment urine output was monitored and vital signs were reviewed pre-infusion, every 5 minutes during the infusion, every 15 minutes for 1 hour post-infusion, every 30 minutes for 2 hours post-infusion, and then hourly until 6 hours post-infusion. Patients were observed during the infusion, and 6- and 24-hours post-infusion to document adverse events (AE) and any change to their functional statuses. Additional safety monitoring included assessing alloimmunization by direct and indirect Coombs and HLA reactive antibody testing, prior to and 3 months post-infusion. Clinical symptoms for graft-versus-host disease (GVHD), infection, and hypersensitivity were monitored at the 3-month visit, and at 6 and 12 months by remote follow-up. Additional AEs were identified in person at the 3-month clinic visit, and through phone interviews with participants at 1, 6, and 12 months post-treatment. For analysis, verbatim AE terms were mapped onto standard terminology defined by the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and summarized according to severity and relationship to the intervention, as judged by the investigator. An independent Data and Safety Monitoring Board (DSMB) was chartered to assess participant safety during the trial according to a charter established prior to the start of the study.

Sample Size

This study was designed to test the null hypothesis that there is an equal probability of improved functional outcome at day 90 in patients treated with UCB and placebo. Under the alternative hypothesis, there is a greater than 0.5 probability that a randomly selected participant treated with UCB will have a more favorable change in mRS than a randomly selected patient treated with placebo. An alternative hypothesis probability of 0.67 was selected based on clinical judgement as the minimal clinically important difference (MCID) that the study should be powered for. This effect size is equivalent to an odds ratio (OR) of 2 comparing UCB to placebo. We used Noether's formula³² to estimate that $N = 100$ participants would be required for 80% power to detect the MCID assuming a 2:1 allocation (67 on UCB and 33 on placebo) and 2-sided 5% type I error rate. This approach to powering the trial was chosen because it does not rely on assumptions about the control group distribution as required by methods

based on the proportional odds model³³ although it underestimates the required sample size if ties are present.³⁴

Statistical Analysis

The Wilcoxon rank sum test was used to compare the distribution of change in mRS from baseline to day 90 between UCB and placebo. We also compared the distribution of mRS at days 30 and 90 between UCB and placebo using the proportional odds (cumulative logit) model, which is commonly referred to as a “shift analysis” in stroke trials. The common odds ratio reported from this model is interpreted as the odds of a better outcome in UCB as compared to placebo, where better outcomes are lower values on the mRS. These models were adjusted for the randomization strata (NIHSS and study center). Analysis of other secondary outcomes used common statistical tests for binary, ordinal, or continuous data as appropriate. All analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC). The procedure defined by Benjamini and Hochberg was prespecified to control the false discovery rate at 5%. However, we ultimately decided to present the results without correction because none of the analyses were statistically significant at the pre-specified 5% alpha level.

Interim Analysis

A preplanned interim futility analysis was conducted with 60% of the target accrual (24 on Placebo and 36 on UCB; see Randomization and Masking) using the conditional power method.³⁵ Conditional power was 6.6% for the planned

treatment effect, indicating a low probability of detecting clinical benefit, if it were present, given the data accumulated to that point in time. However, the study did not have a binding futility rule. The DSMB recommended the study continue for evaluation of secondary safety and efficacy endpoints.

Results

The study was initially powered to accrue 100 patients, but due to COVID and other logistical challenges, recruitment was halted after 79 patients (Fig. 1). Of the 83 participants who consented, 3 were ineligible and one withdrew consent prior to randomization. Therefore, 79 participants were randomized, 52 to Cord Blood and 27 to Placebo. All 27 participants randomized to placebo received their allocated intervention; however, 2 participants randomized to Cord Blood did not, both of which were withdrawn for clinical reasons. At the 30-day follow-up visit, 2 participants in each treatment group were missing mRS evaluations. In the Cord Blood treatment group, one participant was lost to follow up and one participant had an unknown reason for missing the visit. In the Placebo group, one participant was lost to follow up and the other had an unknown reason for missing the visit; however, this participant returned for a 90-day visit. Ultimately, 47/52 participants in the Cord Blood group and 26/27 participants in the Placebo group with an mRS evaluation at 90 days were included in the efficacy analysis (Fig. 1). Characteristics of participants excluded from the primary analysis of mRS at

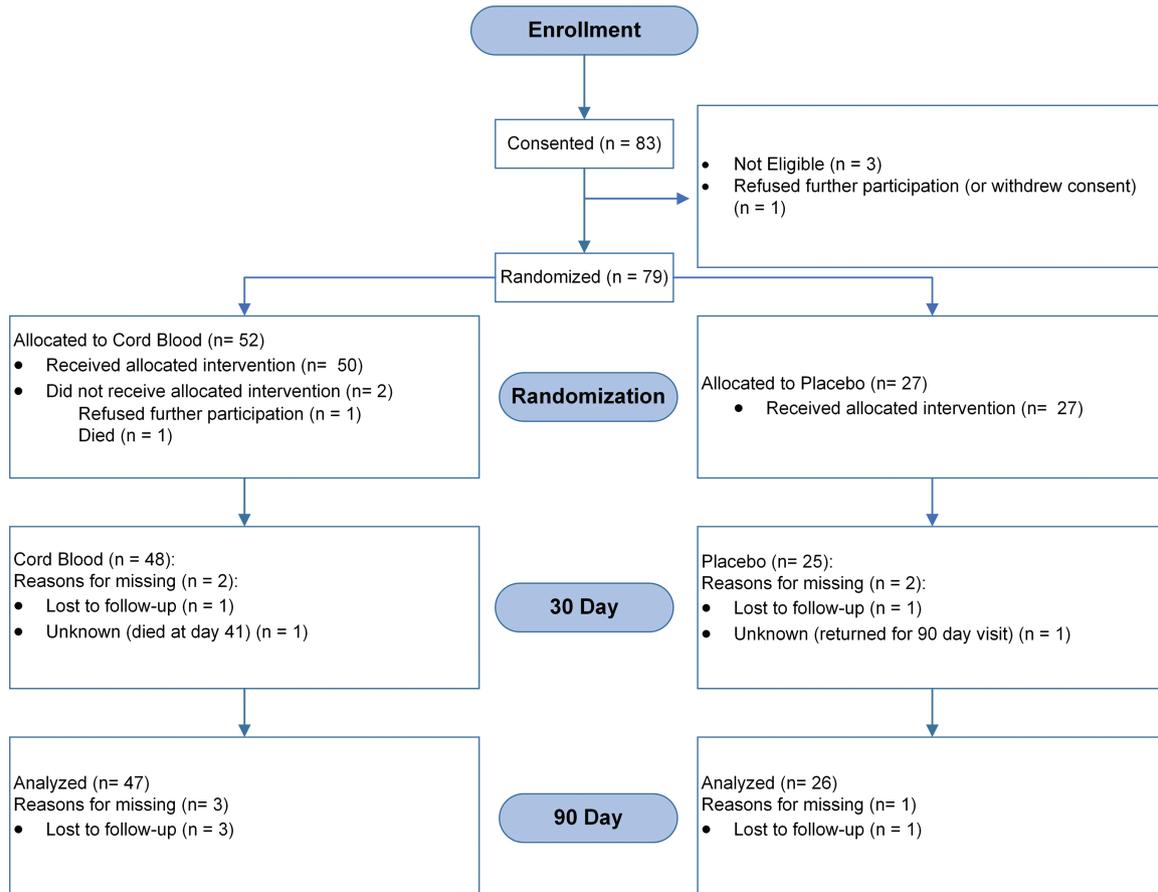


Figure 1. Consort diagram.

day 90 ($N = 6$) and those who were included ($N = 73$) were similar (not shown). Based on this observation, and the small number of participants with missing data, we determined the impact on the final analysis was likely minimal and no imputation or other missing data analyses were done. In the group of analyzed participants, the initial severity of stroke, patient demographics and comorbidities were balanced between groups (Table 1). Seventy seven of the 79 enrolled subjects received their baseline infusion. Both participants who did not receive infusions were withdrawn from the study prior to infusion. All infused subjects received pre-infusion medication per-protocol.

Primary and Secondary Neurological Endpoints

The median [range] of the change in mRS was 1 point (-2, 3) in UCB and 1 point (-1,4) in Placebo ($P = .72$), indicating no difference between the groups in global functional outcome. The shift analysis based on the proportional odds model showed similar results, with an odds ratio of 0.9 (95% CI: 0.4, 2.3) after adjustment for baseline stroke severity and randomization strata (Fig. 2). A post hoc descriptive analysis suggested increasing latency to treatment after index stroke might be associated with the impact of treatment, although this was not statistically significant (interaction P -value = .19) (Fig. 3). However, the study was not designed to test for this effect so the results of this analysis should be interpreted as hypothesis generating.

In addition to this primary analysis, a series of secondary analyses were performed. When 90-day mRS was treated as a dichotomized variable, 32% of UCB treated patients had a favorable mRS associated with functional independence (mRS 0-2), as compared to 23% of placebo treated patients ($P = .85$). Ninety-day NIHSS was unchanged between groups (median NIHSS of 6 at 90 days in both groups). There were also no significant differences in the functional activities of daily living as assessed by the Barthel Index (BI of 80 in treatment group vs. 85 in placebo treated group (Table 2). There was no correlation between Infused TNCC/kg and CD34/kg with improvement on the mRS at day 30 or 90 ($P > .5$ for all analyses; details not shown).

Safety

There were 55 serious adverse events (SAE), including 5 deaths, reported in 26 participants. The majority of serious adverse events were severe ($n = 33$, 60%). All but one serious adverse event was unrelated to the investigational product, which was possibly related (thromboembolic event). There were no instances of graft versus host disease or product-related infections (Fig. 4).

There were 292 non-serious AEs in 53 participants. All but 4 events were either unrelated or unlikely to be related. The 4 events hypertension ($n = 3$) and seizure ($n = 1$) and all were assessed as possibly related to the study product (Fig. 4). The distribution of SAEs and non-serious AEs was similar between arms. There were 17 mild infusion reactions (13 in the UCB arm). Additional detail on SAE and AE is available in the [Supplementary Material](#).

Discussion

Although traditionally used for pediatric hematological malignancies and metabolic disease, the ready availability, low immunogenicity, and safety profile of umbilical cord blood has

found a growing application in regenerative medicine, with clinical studies performed in patients with hepatic,³⁶ cardiac,³⁷ inflammatory bowel,³⁸ and renal disease.³⁹ In particular, UCB may hold promise in neurological disorders,¹³ and preliminary clinical trials have recently been performed in stroke,¹⁸ cerebral palsy,^{40,41} neonatal hypoxic ischemic encephalopathy,^{42,43} and spinal cord injury.⁴⁴ The current trial demonstrates that infusion of banked, non-HLA matched, unrelated, human, allogeneic UCB could be safely administered to patients within 3-10 days after index stroke. The availability of a therapeutic intervention that can be administered in the subacute setting would represent a significant advance, as currently available reperfusion therapies are time limited. Unfortunately, the planned enrollment of COBIS2 was truncated due to COVID and other logistical challenges. However, with approximately 70% of the planned enrollment, we were not able to demonstrate that administration of UCB was associated with improvement in global functional outcome as assessed by shift in 90-day mRS.

There remain a number of unique challenges associated with the design of studies designed to test the potential of cell-based interventions to improve recovery in subacute stroke. These include optimization of cell type, dosing, route of administration, timing relative to stroke, and functional endpoints sensitive to the therapeutic intervention. Although a number of clinical trials have used different modes of delivery to evaluate the safety and efficacy of administering mesenchymal and neural stem cell following stroke, the use of intravenous UCB has several distinct advantages. UCB cells have low immunogenicity, and thus do not require complete HLA matching. Importantly, UCB cells do not require ex vivo clonal expansion or bone marrow aspiration, which may be suboptimal in medically fragile patients following acute stroke. Moreover, there is long clinical track record of safety with UCB infusion, and the intravenous route avoids the invasiveness inherent with intraparenchymal,^{8,45} intrathecal,^{46,47} or intra-arterial^{9,48-50} routes of administration.

Unlike traditional neuroprotective and reperfusion therapies which must be used within hours of ischemia, cell-based interventions have the potential to improve long term plasticity and recovery. This allows administration in the subacute setting and the opportunity of reducing the burden of disability for a much larger population. Thus, an important variable that would help to inform the clinical design of a cell-based intervention is the optimal timing of the cell-based intervention relative to index stroke. Traditionally, intervention within the first week of stroke is considered the acute phase, whereas treatment within the first 6 months may be considered subacute, and treatment beyond 6 months chronic.⁵¹ However, as a practical matter, the use of intravenous tPA is limited to the first 4.5 hours after stroke, and mechanical thrombolysis is traditionally only performed within the first 6 hours, although this time window may be extended to 24 hours in select situations. Thus, treatment within a 3-10 day time period represents a unique time window in which patients may still be in-hospital, but not eligible for other disease modifying interventions.

Unfortunately, preclinical studies have not been definitive in defining the optimal timing of UCB administration. Although several studies have demonstrated functional improvements following UCB and other cell-based interventions, the mechanism(s) and timing by which this occur remain poorly defined.^{52,53} For example, although UCB has been

Table 1. Baseline Characteristics

	Cord Blood (N = 47)	Placebo (N = 26)	Total (N = 73)
Age (years)			
N	47	26	73
Mean (SD)	62.6 (12.1)	64.4 (11.2)	63.2 (11.7)
Median	64.0	64.0	64.0
Q1, Q3	55.0, 70.0	57.0, 73.0	56.0, 71.0
Range	(22.0-85.0)	(42.0-83.0)	(22.0-85.0)
Sex			
Male	29 (61.7%)	16 (61.5%)	45 (61.6%)
Female	18 (38.3%)	10 (38.5%)	28 (38.4%)
Race			
American Indian/American Native	1 (2.1%)	1 (3.8%)	2 (2.7%)
Black/African American	10 (21.3%)	5 (19.2%)	15 (20.5%)
Asian	3 (6.4%)	1 (3.8%)	4 (5.5%)
White (Non-Hispanic)	26 (55.3%)	17 (65.4%)	43 (58.9%)
White (Hispanic)	7 (14.9%)	2 (7.7%)	9 (12.3%)
Ethnicity			
Hispanic or Latino	6 (12.8%)	2 (7.7%)	8 (11.0%)
Not Hispanic or Latino	41 (87.2%)	24 (92.3%)	65 (89.0%)
tPA Administered			
Missing	2 (4.3%)	2 (7.7%)	4 (5.5%)
Yes	16 (34.0%)	11 (42.3%)	27 (37.0%)
No	29 (61.7%)	13 (50.0%)	42 (57.5%)
Institution			
Duke	8 (17.0%)	6 (23.1%)	14 (19.2%)
Grady/Emory	5 (10.6%)	4 (15.4%)	9 (12.3%)
Houston Methodist	20 (42.6%)	11 (42.3%)	31 (42.5%)
University of Colorado	7 (14.9%)	2 (7.7%)	9 (12.3%)
University of Florida	5 (10.6%)	2 (7.7%)	7 (9.6%)
WFU	2 (4.3%)	1 (3.8%)	3 (4.1%)
Infused dose (TNCC/kg)			
N	47	0	47
Mean (SD)	13.2 (6.0)		13.2 (6.0)
Median	11.9		11.9
Q1, Q3	10.1, 15.1		10.1, 15.1
Range	4.1, 43.5		4.1, 43.5
NIHSS Stroke Score			
Low (6-11)	18 (38.3%)	11 (42.3%)	29 (39.7%)
High (12-20)	29 (61.7%)	15 (57.7%)	44 (60.3%)
Raw NIHSS Score			
N	47	26	73
Mean (SD)	12.3 (3.6)	12.2 (3.4)	12.3 (3.5)
Median	13.0	13.0	13.0
Q1, Q3	9.0, 15.0	9.0, 15.0	9.0, 15.0
Range	(6.0-19.0)	(6.0-18.0)	(6.0-19.0)
MRS at randomization			
1 = No Significant Disability	0 (0.0%)	0 (0.0%)	0 (0.0%)
2 = Slight Disability	2 (4.3%)	0 (0.0%)	2 (2.7%)
3 = Moderate Disability	4 (8.5%)	3 (11.5%)	7 (9.6%)
4 = Moderately Severe Disability	27 (57.4%)	8 (30.8%)	35 (47.9%)
5 = Severe Disability	14 (29.8%)	15 (57.7%)	29 (39.7%)

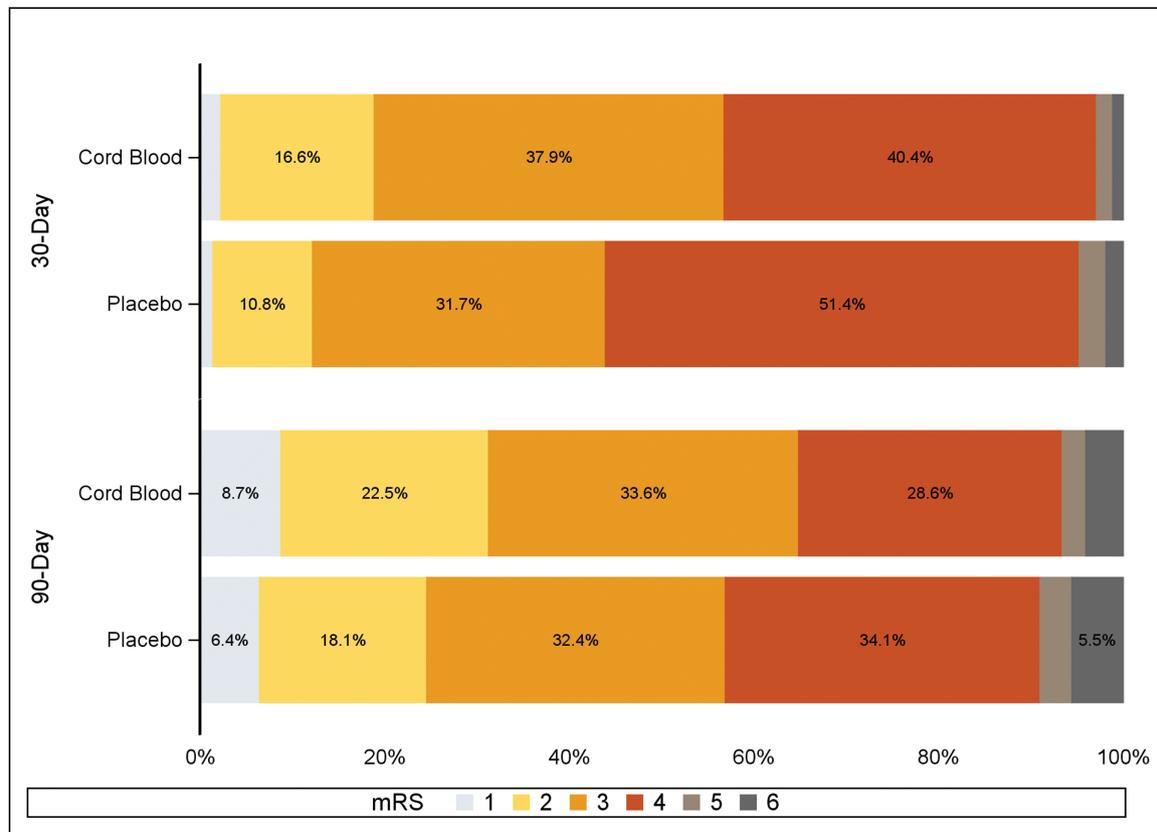


Figure 2. mRS shift analysis at days 30 and 90. Analyses are based on the proportional odds (cumulative logit) model adjusted for NIH Stroke Scale Score and study center.

demonstrated to migrate to the injured central nervous system after ischemia,⁵³ stem cell engraftment does not appear to be necessary for it to exert its beneficial effects. Rather, the primary mechanisms by which UCB are believed to improve outcome after stroke is likely mediated by subpopulations of CD34+ cells, modulating neuroinflammatory response, which is likely most relevant acutely after stroke, and by paracrine effects via release of neurotrophic factors that promote remyelination of damaged axonal tracts.⁵⁴ These latter properties might be expected to be most relevant in improving plasticity and angiogenesis in the subacute phases of stroke recovery. In the current study, UCB cells were administered in a time window from 2 to 11 days following the index stroke. Interestingly, we found a trend toward increasing efficacy with longer latency to intervention (Fig. 3). This might be explained by the fact that the non-HLA matched lymphocytes in the cord blood unit added to the acute inflammatory response to the hypoxic injury a few days post stroke. Over time, as that inflammation subsided, cord blood monocytes stimulated repair of neural circuits as previously reported in children with cerebral palsy.⁴¹

This trial represents the largest randomized controlled trial UCB in the setting of subacute stroke. Consistent with earlier studies, we found that UCB administration was safe and feasible. However, there were a number of limitations to the current trial that should be addressed. Importantly, this trial did not reach full recruitment due to logistic and funding issues associated with COVID; thus, conclusions should be considered preliminary. Traditionally, clinical trials evaluating the efficacy of acute neuroprotectant and reperfusion studies have focused on functional outcome measures such as 90-day

assessment of the modified Rankin score.¹⁹ Although there are a number of advantages to the mRS, including simplicity of use and clinical relevance, potential limitations include the fact that the mRS is largely weighted to ambulation, and may not capture other domain specific endpoints such as upper extremity function that may be sensitive to cell-based interventions. Another potential limitation of the current study is that there was no control for post-stroke rehabilitation. Future studies should determine feasibility of integrating standardizing physical therapy and rehabilitative programs into future protocols.

It is also possible that other cellular products derived from cord blood, cord tissue, or placenta, could improve functional outcomes after stroke. These include ex vivo expanded cord blood monocytes, DUOC—an expanded cord blood macrophage capable of inducing remyelination in early phase clinical trials, and cord tissue derived mesenchymal stromal cells (MSC) given intrathecally or intravenously.⁵⁵⁻⁵⁷ While these products are in development, none have been systematically studied in ischemic stroke. Banked cord blood, although, has the advantage of rapid availability in the acute stroke setting. Perhaps combination cellular therapy will ultimately be the best approach.

Conclusion

In summary, COBIS 2 demonstrated the safety of infusing non-HLA matched UCB to adults with acute ischemic stroke. The primary efficacy endpoint did not demonstrate benefit in this underpowered sample size. An observed trend of improved functional outcomes in recipients of UCB after 5 days post stroke could be explored in future clinical trials.

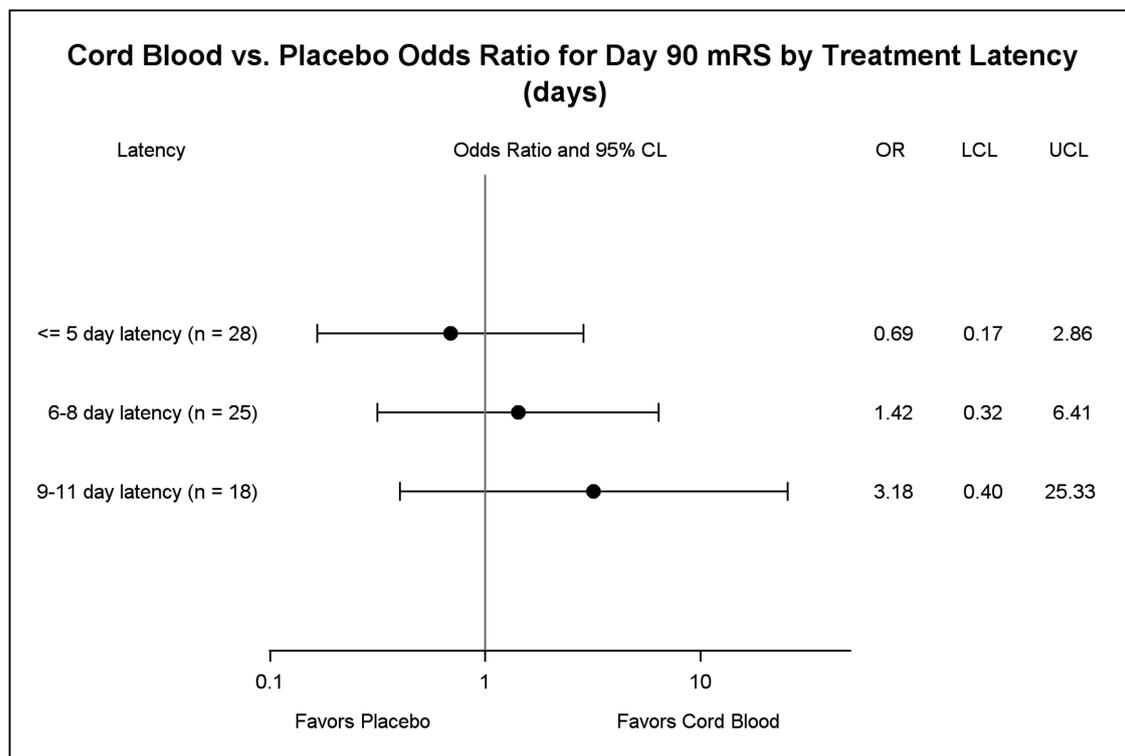


Figure 3. Post hoc analysis of treatment efficacy at day 90 as a function of time from stroke to treatment. Effect estimates are common odds ratios reported from a proportional odd (cumulative logit) model adjusted for NIH Stroke Scale Score, Study Center, and that includes an interaction between treatment assignment and time from stroke to intervention (labeled as “latency” in the figure).

Table 2. Summary of secondary endpoints

Endpoint	Statistical test method	Cord blood	Placebo	P-value
Functional independence at 90 days defined as a 90-day mRS score of 0, 1, or 2	Chi square test (df = 1)	31.9%	23.1%	.42
National Institutes of Health Stroke Scale score at 90 days	Wilcoxon rank sum test	6 (0, 18)	6 (1, 22)	.76
Barthel Index Score at 90 days	Wilcoxon rank sum test	80 (15, 100)	85 (0, 100)	.87
EuroQoL Visual Analogue Scale Score at 90 days	Wilcoxon rank sum test	70 (0, 100)	75 (35, 98)	.7
Patient Health Questionnaire Scale 8 (PHQ-8) Score at 90 days	Wilcoxon rank sum test	4 (0, 22)	5 (0, 18)	.98
Telephone Interview for Cognitive Status (TICS) Total Score at 30 days	Wilcoxon rank sum test	26.5 (2, 41)	20.5 (0, 34)	.11
Telephone Interview for Cognitive Status (TICS) Total Score at 6 months	Wilcoxon rank sum test	28 (2, 40)	29 (0, 40)	.57
Telephone Interview for Cognitive Status (TICS) Total Score at 12 months	Wilcoxon rank sum test	31 (6, 39)	30 (2, 40)	.37
Stroke Impact Scale 16 Score at 90 days	Wilcoxon rank sum test	59 (29, 80)	64 (16, 80)	.94
Trail making test (A) at 90 days	Log-Rank test (df = 1)	78 (20, 181)	62 (26, 181)	.35
Trail making test (B) at 90 days	Log-Rank test (df = 1)	278 (48, 300)	300 (60, 300)	.79
Montreal Cognitive Assessment Total Score at 90 days	Wilcoxon rank sum test	21 (2, 28)	20 (2, 26)	.5
SF-36: Physical Functioning at 90 days	Wilcoxon rank sum test	30 (0, 100)	60 (0, 95)	.19
SF-36: Role Limitations Due to Physical Health at 90 days	Wilcoxon rank sum test	0 (0, 100)	0 (0, 100)	.94
SF-36: Role Limitations Due to Emotional Problems at 90 days	Wilcoxon rank sum test	66.66 667 (0, 100)	100 (0, 100)	.57
SF-36: Energy/Fatigue at 90 days	Wilcoxon rank sum test	55 (10, 90)	65 (10, 100)	.21
SF-36: Emotional Well-being at 90 days	Wilcoxon rank sum test	72 (12, 100)	76 (28, 100)	.43
SF-36: Social Functioning at 90 days	Wilcoxon rank sum test	62.5 (0, 100)	62.5 (0, 100)	.55
SF-36: General Health at 90 days	Wilcoxon rank sum test	60 (20, 90)	70 (30, 95)	.37
Controlled Oral Word Association Test Total Score at 90 days	Wilcoxon rank sum test	14 (0, 43)	9.5 (0, 36)	.16
Oral Symbol Digit Modalities Test Total Score at 90 days	Wilcoxon rank sum test	13 (0, 50)	21 (0, 56)	.2

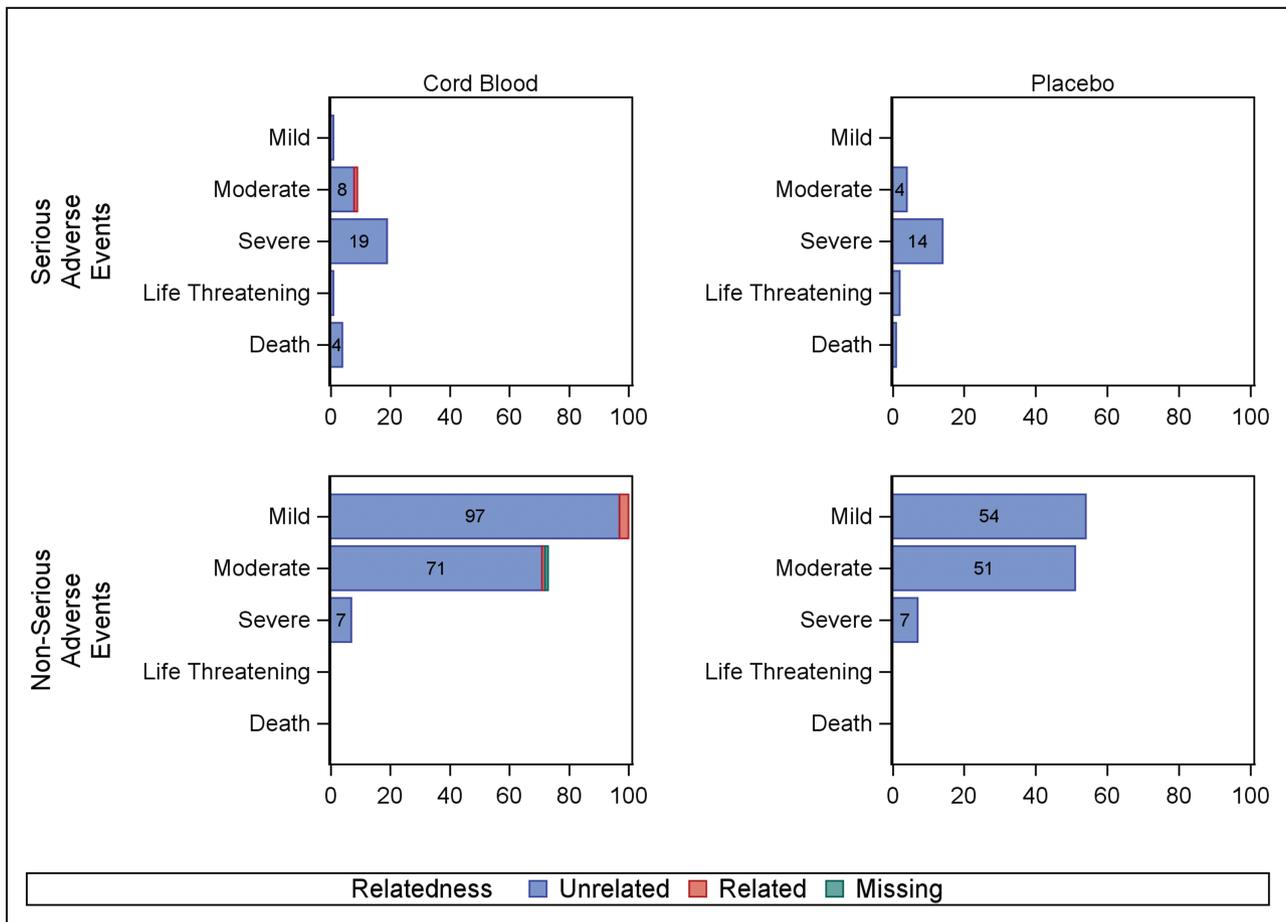


Figure 4. Summary of serious and non-serious adverse events. Numbers on the plot show the number of events in each category. Some participants experienced more than 1 event. There are 47 participants in the cord blood group and 26 participants in the placebo group. The relationship to study product was not determined for 1 moderate non-serious event in the cord blood group.

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Conflict of Interest

J.T. declared U.S. patent holder for 62/470,431 and 16/493,754; Royalties from SinoCell Technologies and Cryo-Cell International; Advisory role with Aegis-CN, LLC; NeurOp, Inc.; Honoraria from Synthetic Biologics, The EMMES Corporation, Navitas Clinical Research; and stock option with NeurOp, Inc. E.J.S. declared License agreements with Takeda, Affimed, Syena; Advisory role with Synthego Corporation, Bayer, ASC Therapeutics, Novartis, Magenta, Cimeio Therapeutics AG, NY Blood Center, Adaptimmune, Navan, Celaid Therapeutics, Zelluna Immunotherapy, FibroBiologics, Axio; Honoraria payments from University of Chicago-Jonas Scientific Advisory Board meeting and Banner MD Anderson Cancer Center-Inaugural Stem Cell Transplant

Cellular Therapy Symposium; Support for attending meetings and/or travel: MD Anderson Cancer Center Office for Training Mentoring of Scientists and the CSO-Inaugural MD Anderson Cancer Center and Weizmann Institute Symposium; NMDP-Committee participation. J.R.W. declared an Advisory role with Celgene, Cidara, Orca, Takeda. W.J.J. declared Research funding in the form of NIH Grant and ACTICOR Grant. J.K. declared leadership position with Duke University School of Medicine; Licensing agreement with Duke and CryoCell; licensing agreement with SinoCell and Duke; Consultant for Neurogene and Mesoblast; Study funded by the Marcus Foundation; Enzyvant—contract manufacturer for thymus—funds to Duke University; Research support from the Marcus Foundation; Celularity stock options. All of the other authors declared no potential conflicts of interest.

Author Contributions

D.T.L.: Conception and design, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. J.T., J.K.: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. E.P.: Collection and/or assembly of data, data analysis and interpretation, manuscript writing. E.R.B.: Provision of study material or patients, collection and/or assembly of data, data analysis

and interpretation, manuscript writing. E.J.S., J.R.W., B.F., S.R.B., A.K., W.J., J.J.V., E.M.: Provision of study material or patients, collection and/or assembly of data, manuscript writing.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supplementary Material

Supplementary material is available at *Stem Cells Translational Medicine* online.

References

- Tsao CW, Aday AW, Almarzooq ZI, et al. Heart disease and stroke statistics-2022 update: a report from the American Heart Association. *Circulation*. 2022;145(8):e153-e639. <https://doi.org/10.1161/CIR.0000000000001052>
- Demaerschalk BM. Alteplase treatment in acute stroke: incorporating food and drug administration prescribing information into existing acute stroke management guide. *Curr Atheroscler Rep*. 2016;18(8):53. <https://doi.org/10.1007/s11883-016-0602-5>
- Hassani Z, O'Reilly J, Pearse Y, et al. Human neural progenitor cell engraftment increases neurogenesis and microglial recruitment in the brain of rats with stroke. *PLoS One*. 2012;7(11):e50444. <https://doi.org/10.1371/journal.pone.0050444>
- Burns TC, Verfaillie CM, Low WC. Stem cells for ischemic brain injury: a critical review. *J Comp Neurol*. 2009;515(1):125-144. <https://doi.org/10.1002/cne.22038>
- Dulamea AO. The potential use of mesenchymal stem cells in stroke therapy - from bench to bedside. *J Neurol Sci*. 2015;352(1-2):1-11. <https://doi.org/10.1016/j.jns.2015.03.014>
- Gervois P, Wolfs E, Ratajczak J, et al. Stem cell-based therapies for ischemic stroke: preclinical results and the potential of imaging-assisted evaluation of donor cell fate and mechanisms of brain regeneration. *Med Res Rev*. 2016;36(6):1080-1126. <https://doi.org/10.1002/med.21400>
- Jeong H, Yim HW, Cho Y, et al. Efficacy and safety of stem cell therapies for patients with stroke: a systematic review and single arm meta-analysis. *Int J Stem Cells*. 2014;7(2):63-69. <https://doi.org/10.15283/ijsc.2014.7.2.63>
- Kalladka D, Sinden J, Pollock K, et al. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. *Lancet*. 2016;388(10046):787-796. [https://doi.org/10.1016/S0140-6736\(16\)30513-X](https://doi.org/10.1016/S0140-6736(16)30513-X)
- P B, S B, M H, et al. Phase I trial of intra-arterial autologous CD341 haematopoietic stem cells in acute ischaemic stroke. *Int J Stroke*. 2011;6(2):24. <https://doi.org/10.1111/j.1747-4949.2011.00684.x>
- Moniche F, Gonzalez A, Gonzalez-Marcos JR, et al. Intra-arterial bone marrow mononuclear cells in ischemic stroke: a pilot clinical trial. *Stroke*. 2012;43(8):2242-2244. <https://doi.org/10.1161/STROKEAHA.112.659409>
- Hess DC, Sila CA, Furlan AJ, et al. A double-blind placebo-controlled clinical evaluation of MultiStem for the treatment of ischemic stroke. *Int J Stroke*. 2014;9(3):381-386. <https://doi.org/10.1111/ijss.12065>
- Taguchi A, Sakai C, Soma T, et al. Intravenous autologous bone marrow mononuclear cell transplantation for stroke: phase I/2a clinical trial in a homogeneous group of stroke patients. *Stem Cells Dev*. 2015;24(19):2207-2218. <https://doi.org/10.1089/scd.2015.0160>
- Sun JM, Kurtzberg J. Cord blood for brain injury. *Cytotherapy*. 2015;17(6):775-785. <https://doi.org/10.1016/j.jcyt.2015.03.004>
- Zhou H, Chang S, Rao M. Human cord blood applications in cell therapy: looking back and look ahead. *Expert Opin Biol Ther*. 2012;12(8):1059-1066. <https://doi.org/10.1517/14712598.2012.691161>
- Chen N, Newcomb J, Garbuzova-Davis S, et al. Human umbilical cord blood cells have trophic effects on young and aging hippocampal neurons in vitro. *Aging Dis*. 2010;1(3):173-190.
- Kim YJ, Broxmeyer HE. Immune regulatory cells in umbilical cord blood and their potential roles in transplantation tolerance. *Crit Rev Oncol Hematol*. 2011;79(2):112-126. <https://doi.org/10.1016/j.critrevonc.2010.07.009>
- Vendrame M, Cassady J, Newcomb J, et al. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke*. 2004;35(10):2390-2395. <https://doi.org/10.1161/01.STR.0000141681.06735.9b>
- Laskowitz DT, Bennett ER, Durham RJ, et al. Allogeneic umbilical cord blood infusion for adults with ischemic stroke: clinical outcomes from a phase 1 safety study. *Stem Cells Transl Med*. Published online 2018;7(7):521-529. <https://doi.org/10.1002/sctm.18-0008>
- Van Swieten JC, Koudstaal PJ, Visser MC, Schouten H, Van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke*. 1988;19(5):604-607. <https://doi.org/10.1161/01.STR.19.5.604>
- Brott T, Adams HP, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. 1989;20(7):864-870. <https://doi.org/10.1161/01.str.20.7.864>
- Mahoney FI, Barthel DW. Functional evaluation: the barthel index. *Md State Med J*. 1965;14:61-65.
- Duncan PW, Lai SM, Bode RK, Perera S, DeRosa J. Stroke impact scale-16: a brief assessment of physical function. *Neurology*. 2003;60(2):291-296. <https://doi.org/10.1212/01.wnl.0000041493.65665.d6>
- Buxton M, Rabin R, Pekurinen M, et al. EuroQol - a new facility for the measurement of health-related quality of life. *Health Policy (New York)*. 1990;16(3):199-208. [https://doi.org/10.1016/0168-8510\(90\)90421-9](https://doi.org/10.1016/0168-8510(90)90421-9)
- Kroenke K, Spitzer TW, Spitzer RL, et al. The PHQ-8 as a measure of current depression in the general population. *J Affect Disord*. 2009;114(1-3):163-173. <https://doi.org/10.1016/j.jad.2008.06.026>
- Brandt J, Spencer M, Folstein M. The telephone interview for cognitive status. *Neuropsychiatry, Neuropsychol Behav Neurol*. 1988;1(2):111-117.
- Saver JL, Warach S, Janis S, et al. Standardizing the structure of stroke clinical and epidemiologic research data: the national institute of neurological disorders and stroke (NINDS) stroke common data element (CDE) project. *Stroke*. 2012;43(4):967-973. <https://doi.org/10.1161/strokeaha.111.634352>
- Tombaugh TN. Trail Making Test A and B: normative data stratified by age and education. *Arch Clin Neuropsychol*. 2004;19(2):203-214. [https://doi.org/10.1016/S0887-6177\(03\)00039-8](https://doi.org/10.1016/S0887-6177(03)00039-8)
- Tamez E, Myerson J, Morris L, et al. Assessing executive abilities following acute stroke with the trail making test and digit span. *Behav Neurol*. 2011;24(3):177-185. <https://doi.org/10.3233/BEN-2011-0328>
- Nasreddine ZS, Phillips NA, Bédirian V, et al. The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53(4):695-699. <https://doi.org/10.1111/j.1532-5415.2005.53221.x>
- Loonstra AS, Tarlow AR, Sellers AH. COWAT metanorms across age, education, and gender. *Appl Neuropsychol*. 2001;8(3):161-166. https://doi.org/10.1207/S15324826AN0803_5

31. Brandt J. The Hopkins Verbal Learning Test: development of a new memory test with six equivalent forms. *Clin Neuropsychol.* 1991;5(2):125-142. <https://doi.org/10.1080/13854049108403297>
32. Noether GE. Sample size determination for some common nonparametric tests. *J Am Stat Assoc.* 1987;82(398):645-647. <https://doi.org/10.1080/01621459.1987.10478478>
33. Whitehead J. Sample size calculations for ordered categorical data. *Stat Med.* 1993;12(24):2257-2271. <https://doi.org/10.1002/sim.4780122404>
34. Divine G, Kapke A, Havstad S, Joseph CLM. Exemplary data set sample size calculation for Wilcoxon-Mann-Whitney tests. *Stat Med.* 2010;29(1):108-115. <https://doi.org/10.1002/sim.3770>
35. Proschan MA, Lan G KK, and Wittes JT. *Statistical Monitoring of Clinical Trials: A Unified Approach.* Springer; 2006.
36. Li YH, Xu Y, Wu HM, et al. Umbilical cord-derived mesenchymal stem cell transplantation in hepatitis B virus related acute-on-chronic liver failure treated with plasma exchange and entecavir: a 24-month prospective study. *Stem Cell Rev Rep.* 2016;12(6):645-653. <https://doi.org/10.1007/s12015-016-9683-3>
37. Medhekar SK, Shende VS, Chincholkar AB. Recent stem cell advances: cord blood and induced pluripotent stem cell for cardiac regeneration- a review. *Int J Stem Cells.* 2016;9(1):21-30. <https://doi.org/10.15283/ijsc.2016.9.1.21>
38. Mao F, Wu Y, Tang X, et al. Human umbilical cord mesenchymal stem cells alleviate inflammatory bowel disease through the regulation of 15-LOX-1 in macrophages. *Biotechnol Lett.* 2017;39(6):929-938. <https://doi.org/10.1007/s10529-017-2315-4>
39. Peng X, Xu H, Zhou Y, et al. Human umbilical cord mesenchymal stem cells attenuate cisplatin-induced acute and chronic renal injury. *Exp Biol Med.* 2013;238(8):960-970. <https://doi.org/10.1177/1535370213497176>
40. Kang M, Min K, Jang J, et al. Involvement of immune responses in the efficacy of cord blood cell therapy for cerebral palsy. *Stem Cells Dev.* 2015;24(19):2259-2268. <https://doi.org/10.1089/scd.2015.0074>
41. Sun JM, Song AW, Case LE, et al. Effect of autologous cord blood infusion on motor function and brain connectivity in young children with cerebral palsy: a randomized, placebo-controlled trial. *Stem Cells Transl Med.* Published online 2017;6(12):2071-2078. <https://doi.org/10.1002/sctm.17-0102>
42. Cotten CM, Murtha AP, Goldberg RN, et al. Feasibility of autologous cord blood cells for infants with hypoxic-ischemic encephalopathy. *J Pediatr.* 2014;164(5):973-979.e1. <https://doi.org/10.1016/j.jpeds.2013.11.036>
43. Penny T, Pham Y, Sutherland A, et al. Multiple doses of umbilical cord blood cells improve long-term perinatal brain injury. *Stem Cells Transl Med.* 2020;9(S1):S3-S3. <https://doi.org/10.1002/sctm.12808>
44. Zhu H, Poon W, Liu Y, et al. Phase I-II clinical trial assessing safety and efficacy of umbilical cord blood mononuclear cell transplant therapy of chronic complete spinal cord injury. *Cell Transplant.* 2016;25(11):1925-1943. <https://doi.org/10.3727/096368916x691411>
45. Muir KW, Bulters D, Willmot M, et al. Intracerebral implantation of human neural stem cells and motor recovery after stroke: multicentre prospective single-arm study (PISCES-2). *J Neurol Neurosurg Psychiatry.* Published online 2020;91(4):396-401. <https://doi.org/10.1136/jnnp-2019-322515>
46. Sharma A, Sane H, Gokulchandran N, et al. Autologous bone marrow mononuclear cells intrathecal transplantation in chronic stroke. *Stroke Res Treat.* 2014;2014:1-9. <https://doi.org/10.1155/2014/234095>
47. Wang L, Ji H, Li M, et al. Intrathecal administration of autologous CD34 positive cells in patients with past cerebral infarction: a safety study. *ISRN Neurol.* 2013;2013:1-6. <https://doi.org/10.1155/2013/128591>
48. Correa PL, Mesquita CT, Felix RM, et al. Assessment of intra-arterial injected autologous bone marrow mononuclear cell distribution by radioactive labeling in acute ischemic stroke. *Clin Nucl Med.* 2007;32(11):839-841. <https://doi.org/10.1097/RLU.0b013e318156b980>
49. Friedrich MAG, Martins MP, Araújo MD, et al. Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke. *Cell Transplant.* 2012;21(Suppl 1):S13-S21. <https://doi.org/10.3727/096368912x612512>
50. Savitz SI, Yavagal D, Rappard G, et al. A phase 2 randomized, sham-controlled trial of internal carotid artery infusion of autologous bone marrow-derived ALD-401 cells in patients with recent stable ischemic stroke (RECOVER-Stroke). *Circulation.* 2019;139(2):192-205. <https://doi.org/10.1161/CIRCULATIONAHA.117.030659>
51. Kawabori M, Shichinohe H, Kuroda S, Houkin K. Clinical trials of stem cell therapy for cerebral ischemic stroke. *Int J Mol Sci.* 2020;21(19):7380. <https://doi.org/10.3390/ijms21197380>
52. Willing AE, Lixian J, Milliken M, et al. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *J Neurosci Res.* 2003;73(3):296-307. <https://doi.org/10.1002/jnr.10659>
53. Chen J, Sanberg PR, Li Y, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke.* 2001;32(11):2682-2688. <https://doi.org/10.1161/hs1101.098367>
54. Taguchi A, Soma T, Tanaka H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest.* 2004;114(3):330-338. <https://doi.org/10.1172/jci200420622>
55. Scotland P, Buntz S, Noeldner P, et al. Gene products promoting remyelination are up-regulated in a cell therapy product manufactured from banked human cord blood. *Cytotherapy.* 2017;19(6):771-782. <https://doi.org/10.1016/j.jcyt.2017.03.004>
56. Sun JM, Dawson G, Franz L, et al. Infusion of human umbilical cord tissue mesenchymal stromal cells in children with autism spectrum disorder. *Stem Cells Transl Med.* 1146;9(10):1137-1146. <https://doi.org/10.1002/sctm.19-0434>
57. Sun JM, Case LE, McLaughlin C, et al. Motor function and safety after allogeneic cord blood and cord tissue-derived mesenchymal stromal cells in cerebral palsy: an open-label, randomized trial. *Dev Med Child Neurol.* 2022;64(12):1477-1486. <https://doi.org/10.1111/dmcn.15325>