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ORIGINAL ARTICLE

Motor function and safety after allogeneic cord blood and cord tissue-derived mesenchymal stromal cells in cerebral palsy: An open-label, randomized trial

Jessica M. Sun[1] | **Laura E. Case**[2] | **Colleen McLaughlin**[1] | **Alicia Burgess**[1] | **Natalie Skergan**[1] | **Sydney Crane**[1] | **Joan M. Jasien**[3] | **Mohamad A. Mikati**[3] | **Jesse Troy**[1,4] | **Joanne Kurtzberg**[1]

[1]Marcus Center for Cellular Cures, Duke University School of Medicine, Durham, NC, USA

[2]Department of Physical and Occupational Therapy, Duke University School of Medicine, Durham, NC, USA

[3]Division of Pediatric Neurology, Duke University School of Medicine, Durham, NC, USA

[4]Department of Biostatistics and Bioinformatics, Duke University School of Medicine, Durham, NC, USA

Correspondence
Jessica M. Sun, Marcus Center for Cellular Cures, Duke University School of Medicine, DUMC 3850, 2400 Pratt Street, Durham, NC 27705, USA.
Email: jessica.sun@duke.edu

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Abstract

Aim: To evaluate safety and motor function after treatment with allogeneic umbilical cord blood (AlloCB) or umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC) in children with cerebral palsy (CP).

Method: Ninety-one children (52 males, 39 females; median age 3 years 7 months [range 2–5 years]) with CP due to hypoxic–ischemic encephalopathy, stroke, or periventricular leukomalacia were randomized to three arms: (1) the AlloCB group received 10×10^7 AlloCB total nucleated cells (TNC) per kilogram at baseline ($n = 31$); (2) the hCT-MSC group received 2×10^6 hCT-MSC at baseline, 3 months, and 6 months ($n = 28$); (3) the natural history control group received 10×10^7 AlloCB TNC per kilogram at 12 months ($n = 31$). Motor function was assessed with the Gross Motor Function Measure-66 (GMFM-66) and Peabody Developmental Motor Scale, Second Edition.

Results: Infusions ($n = 143$) were well tolerated, with eight infusion reactions (three in the AlloCB group, five in hCT-MSC) and no other safety concerns. At 12 months, the mean differences (95% confidence intervals [CI]) between actual and expected changes in GMFM-66 score were AlloCB 5.8 points (3.4–8.2), hCT-MSC 4.3 (2.2–6.4), and natural history 3.1 (1.4–5.0). In exploratory, post hoc analysis, the mean GMFM-66 score (95% CI) of the hCT-MSC group was 1.4 points higher than natural history (–1.1 to 4.0; $p = 0.27$), and the AlloCB group was 3.3 points higher than natural history (0.59–5.93; $p = 0.02$) after adjustment for baseline Gross Motor Function Classification System level, GMFM-66 score, and etiology.

Interpretation: High-dose AlloCB is a potential cell therapy for CP and should be further tested in a randomized, blinded, placebo-controlled trial.

This original article is commented on by Clowry on pages 1434–1435 of this issue.

Abbreviations: AlloCB, allogeneic umbilical cord blood; GMQ, Gross Motor Quotient; hCT-MSC, human umbilical cord tissue-derived mesenchymal stromal cells; HLA, human leukocyte antigen; PDMS-2, Peabody Developmental Motor Scales, Second Edition; TNC, total nucleated cells.

Cerebral palsy (CP) is the most common and costly chronic motor disorder in childhood, affecting 1 to 3 of every 1000 live births.[1–4] It is often caused by a static injury to the developing brain sustained in utero, at the time of birth, or in the first 2 years of life. Treatment focuses on early and consistent physical, occupational, and other therapies. Certain surgical procedures and medications may be used to address contractures and hypertonicity, but there is an unmet need for therapies that can help the brain repair and/or compensate for the sustained injury.

A previous randomized, placebo-controlled trial of autologous umbilical cord blood dosed at 1×10^7 to 5×10^7 total nucleated cells (TNC)/kg in children with spastic CP did not show evidence of an effect on motor function 1 year after randomization. However, a post hoc analysis demonstrated that children infused with a dose of at least 2×10^7 TNC/kg had greater improvement in gross motor function 1 year later than children who received a lower cell dose or placebo.[5] This led to the hypothesis that umbilical cord blood may be effective, but at higher doses than administered in that trial. Subsequently, we demonstrated safety of sibling umbilical cord blood infusion in a small, open-label, phase 1 study of 15 children with CP who received a median dose of 3.3×10^7 TNC/kg (range 1.8×10^7 to 5.2×10^7).[6] As many children with CP do not have autologous or sibling umbilical cord blood units available that could provide a pre-cryopreservation dose of at least 3×10^7 TNC/kg, use of an unrelated donor product would expand access for affected children should such therapies prove effective.

Preclinical models of brain injuries suggest that cell therapies may exert paracrine effects in the brain that can enhance neuroprotection, angiogenesis, synaptogenesis, endogenous repair mechanisms, and/or migration and proliferation of existing neural stem cells as well as potentially decrease inflammation.[7] The present trial evaluated two candidate unrelated donor cell-therapy products: allogeneic umbilical cord blood (AlloCB) and unrelated donor umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC).

The objectives of this open-label, randomized study were to evaluate the change in motor function 12 months after treatment and to assess the safety of these therapies in children with CP. A concurrently randomized natural history control group was included who did not receive an infusion until 12 months. The intent was to use this trial as a basis for deciding whether either of the donor-derived products has potential as cellular therapy for CP.

What this paper adds

- Unrelated donor allogeneic umbilical cord blood (AlloCB) and human umbilical cord tissue-derived mesenchymal stromal cell infusion is safe in young children with cerebral palsy.
- Significant changes in motor function were not observed 6 months after treatment.
- One year later, treatment with AlloCB was associated with greater increases in Gross Motor Function Measure-66 scores.

METHOD

This single site, prospective, randomized, open-label, phase 2 trial was performed at Duke University, North Carolina, USA. Written informed consent was obtained for screening and treatment phases of the trial (ClinicalTrials.gov NCT03473301), which was approved by the Duke Hospital Institutional Review Board and conducted under Investigational New Drug Application #17921 from the United States Federal Drug Administration. The study began in April 2018. All participants had initial evaluations, all but one had 6-month follow-up visits, and 12-month visits were underway when in-person evaluations were halted in March 2020 because of the COVID-19 pandemic. The trial protocol is available from the authors on request.

Participants

Children aged 2 to 5 years with hypertonic CP due to hypoxic–ischemic encephalopathy, periventricular leukomalacia, or in utero stroke or bleed were eligible to participate if they were classified in Gross Motor Function Classification System (GMFCS) levels I to IV and their history and routine brain imaging did not suggest a genetic condition or brain malformation as the cause of their CP. Exclusion criteria included autism spectrum disorder, legal blindness, hypsarrhythmia, intractable seizures, uncontrolled infections, progressive neurological or genetic diseases, impaired organ function, immunosuppression, classification in GMFCS level V, or hypotonic or ataxic CP without hypertonicity. Children with an available autologous umbilical cord blood unit or who had previously received any form of cellular therapy were also ineligible.

Randomization and blinding

A stratified, blocked randomization table was generated before conducting the trial, and treatment assignments were allocated from this table using an electronic system. Participants were randomized within strata of CP etiology (stroke/bleed vs other) and severity (GMFCS levels I/II vs III/IV) in an even allocation to one of three arms: (1) single dose of 10×10^7 TNC/kg of AlloCB at baseline; (2) three doses of 2×10^6 cells/kg of hCT-MSC given at baseline, 3 months, and 6 months; or (3) natural history in which 10×10^7 TNC/kg of AlloCB were administered at 1 year. Participating families were not blinded to treatment assignment, but the physical therapist performing the motor assessments was.

Interventions

AlloCB units and tissue to manufacture hCT-MSC used for this trial were obtained from donations to the Carolinas Cord Blood Bank, a US Food and Drug Administration-licensed (DUCORD) Public Cord Blood Bank at Duke University Medical Center, which is also a member of the National Cord Blood Inventory of the CW Bill Young Cell Transplantation Program administered by the US Health Resources and Services Administration. Donor screening and testing was performed according to Carolinas Cord Blood Bank standard operating procedures to meet all requirements in 21 CFR Part 1271. Maternal donors were screened and/or tested for human immunodeficiency virus (HIV)-1, HIV-2, HIV-O, hepatitis B virus (surface antigen and core antibody), hepatitis C virus antibody, Treponema pallidum (syphilis), Creutzfeldt–Jakob disease (screening only), Chagas disease, human T-lymphotropic virus types 1 and 2, and total antibodies against cytomegalovirus. Nucleic acid testing for HIV-1/2/O, hepatitis B, West Nile virus, and hepatitis C was also performed on maternal blood. Screening and testing for Zika virus may have been performed, depending on the year of donation.

AlloCB units

The best available (at least 4 out of 6) human leukocyte antigen (HLA)-matched AlloCB unit, using intermediate-level matching at HLA class I, A and B, and high-resolution allele-level matching at HLA class II, DRB1, with a pre-cryopreservation TNC count of at least 10×10^7 TNC/kg, was selected. Units and recipients were not ABO matched, but Rh-negative units were selected for Rh-negative females to avoid Rh sensitization. HLA confirmatory typing was performed on a segment of the AlloCB unit, as was potency testing.[8] Units were acceptable for the trial if potency testing on an attached segment met specifications (CD45 viability ≥ 40%, CD34 viability ≥ 70%). On the day of infusion, AlloCB was thawed and washed in dextran/albumin in the standard fashion.[9] A targeted dose of 10×10^7 TNC/kg was prepared for infusion in a total volume of 1.25 ml/kg of recipient body weight.

hCT-MSC

hCT-MSC is a product of allogeneic cells manufactured from digested umbilical cord tissue that is expanded in culture, cryopreserved, and banked in the Marcus Center for Cellular Cures Good Manufacturing Practice cell manufacturing laboratory (Investigational New Drug Application #17313) at Duke University.[10] Briefly, cord tissue was harvested from male infants delivered by elective Cesarean section after a normal, full-term pregnancy. The cord tissue was transported to the Good Manufacturing Practice laboratory in plasmalyte A and cut and digested on the Miltenyi Biotec GentleMacs Octo Dissociator (Bergisch Gladbach, Germany) in four Good Manufacturing Practice grade enzymes (proprietary). The resulting cellular preparation was further manufactured in a series of two passages to generate a master cell bank, a working cell bank, and the study product. The product for each step was cryopreserved in a controlled rate freezer and stored in the vapor phase of liquid nitrogen. The final product was thawed and assessed for identity, purity, sterility, potency, and quality. A representative sample of each lot was thawed and tested for viability, cell count, sterility, endotoxin, mycoplasma, and in vitro adventitial viruses. On the day of treatment, cells were thawed and diluted in 40 ml of plasmalyte A plus 5% human serum albumin. Release specifications after thaw and dilution included total nucleated cell count and viability of at least 70% via Cellometer (Nexcelom; Lawrence, MA, USA). Participants were dosed with 2×10^6 hCT-MSCs/kg on the basis of the post-thaw count, and three infusions were performed at 3-month intervals (baseline, 3 months, and 6 months).

Cellular infusions

All infusions were given through a peripheral intravenous catheter after premedication with diphenhydramine (0.5 mg/kg intravenously) and methylprednisolone (0.5 mg/kg intravenously). AlloCB was infused over 10 to 25 minutes, and hCT-MSC over 30 to 60 minutes under direct supervision and continuous pulse oximetry. Participants were hydrated with standard intravenous fluids as tolerated, observed for at least 1 hour post-infusion, and reassessed in-person the following day. In the event of a minor infusion reaction, the infusion was temporarily halted, the child was treated with additional diphenhydramine and/or steroids and other medical interventions as indicated and, if the reaction abated, the infusion was restarted and completed within the expiry of the product.

Outcomes

Gross motor function was assessed with the Gross Motor Function Measure-66 (GMFM-66) at baseline, 6 months, and 12 months. The GMFM-66 is a standardized criterion-referenced instrument with validity, reliability, and responsivity to change in children with CP. Developmental curves of expected progression have been published for children aged 2 to 12 years,[11,12] allowing the calculation of future expected scores according to baseline age, GMFCS level, and GMFM-66 score. The primary endpoint was defined as the difference between a child's actual and expected changes in GMFM-66 score 12 months after the initial study infusion and was prespecified to be estimated within each study arm using 95% confidence intervals (CIs).

Exploratory endpoints included change in GMFM-66, Peabody Developmental Motor Scales, Second Edition (PDMS-2), and Pediatric Evaluation of Disability Inventory-Computer Adaptive Test scores at 6 and 12 months. Safety

assessments were performed at 6, 12, and 24 months in-person or remotely. A subset of participants underwent brain magnetic resonance imaging with diffusion tensor imaging to assess brain connectivity, which will be reported separately.

Statistical methods

A sample size of 30 participants per arm (n = 90 total) was selected for 95% CI estimation of the mean 12-month observed-minus-expected GMFM-66 change score in each of the study arms (SD 5.16) with a margin of error of no more than 2 points, assuming CIs based on the t-distribution. The study was not powered for between-arm comparisons of the primary or exploratory outcomes. On the basis of binomial probabilities, 30 participants provided a 95.8% probability of identifying one or more product-related adverse events occurring in 10% of infusions. All data analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC, USA).

Safety analyses

Descriptive methods were used to analyze adverse events. Serious adverse events were summarized separately from non-serious adverse events. All events were summarized according to MedDRA system organ class, preferred term, relationship to study product, and severity. Severity was assigned on the basis of the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Analysis of the primary outcome

The primary outcome was summarized within each study arm using 95% CIs based on the t-distribution. The prespecified statistical analysis plan was amended during the conduct of the trial to include an analysis of the primary outcome with and without multiple imputation when it was realized that the COVID-19 pandemic would affect the number of participants completing the study. Multiple imputation (30 data sets) was based on the fully conditional specification method.[13]

Analysis of the exploratory outcomes

While this trial was ongoing, there were multiple communications with subject matter experts and regulatory authorities about the design of a future phase 3 trial of a cell therapy for treatment of motor function in CP. An important outcome of those discussions was that an exploratory endpoint of this trial, the GMFM-66, would be the most appropriate endpoint for a future, placebo-controlled trial (see Discussion). Therefore, additional non-prespecified analyses of this endpoint and another exploratory motor function outcome, the PDMS-2, were designed. Most importantly, analyses comparing the treatment arms, for which the study was not powered a priori and that are not specified in the protocol, were performed. These analyses are delineated in the Results.

Post hoc analyses of the exploratory motor function outcomes, GMFM-66, and PDMS-2 were accomplished using analysis of covariance (ANCOVA; separate models for outcomes at months 6 and 12). The ANCOVA models estimated the mean (GMFM-66 or PDMS-2) at month 12 conditional on the baseline value, assigned treatment, GMFCS level, and etiology of CP. For the GMFM-66, ANCOVA models were also fitted with and without inverse probability weighting as a sensitivity analysis to assess the impact of missing GMFM-66 at month 12 caused by the COVID-19 pandemic.[14] The propensity model for the inverse probability weighting included the study design variables (treatment arm and the randomization strata) as well as baseline variables (Table 1) related to missingness as assessed by a standardized difference greater than 0.2 comparing participants with and without missing outcomes at month 12 (GMFCS level, etiology of CP, and the baseline and 6-month GMFM-66 scores).

For the GMFM-66 analysis, model coefficients (and 95% CI) for the two cell-therapy arms (i.e. the mean difference between each active therapy and the common control) are reported. For the GMFM-66 and PDMS-2, the predicted mean outcomes from the ANCOVA models at the mean of the baseline values are reported. The p-values and Bonferroni-corrected p-values for the between-arm comparisons of each active therapy to the common control are reported in all post hoc analyses (i.e. p-values are multiplied by 2). This represents strong type I error control (α = 0.025 for comparison of each treatment arm with natural history) for analysing individual outcomes but weak trial-wide type I error control across the exploratory outcomes.

RESULTS

Participants' characteristics

Between April 2018 and May 2019, 91 children (52 males, 39 females) with hypertonic CP were enrolled and randomized (Figure S1). The median age at study entry was 3 years 7 months (interquartile range [IQR] 2 years 8 months–4 years 3 months; range 2–5 years) and median baseline GMFM-66 score was 47 (IQR 36–56; range 24–84). One participant who randomized to the hCT-MSC arm withdrew before treatment. Owing to the COVID pandemic, in-person evaluations were suspended according to institutional guidelines in March 2020. At that time, all infusions in the AlloCB and hCT-MSC arms and all but one 6-month evaluation had been completed. A total of 68 participants were able to complete in-person 12-month evaluations as planned and contributed to the analysis of the primary outcome. Baseline characteristics, including GMFCS level and etiology of CP, were not statistically different between treatment arms among the 68 participants who completed the 12-month assessments (Tables 1, S1, and S2).

TABLE 1 Baseline characteristics

	AlloCB ($n = 20$)	hCT-MSC ($n = 23$)	Natural history ($n = 25$)	All ($n = 68$)[a]
Age, years:months				
Mean (SD)	3:6 (1:0)	3:8 (0:10)	3:6 (0:9)	3:7 (0:10)
Median (minimum, maximum)	3:10 (2:2, 5:0)	3:9 (2:1, 5:0)	3:8 (2:1, 4:10)	3:9 (2:1, 5:0)
Sex, *n* (%)				
Female	10 (50.0)	6 (26.1)	12 (48.0)	28 (41.2)
Male	10 (50.0)	17 (73.9)	13 (52.0)	40 (58.8)
Race,[b] *n* (%)				
Asian	0	2 (8.7)	3 (12.0)	5 (7.4)
White	20 (100.0)	21 (91.3)	20 (80.0)	61 (89.7)
Other[c]	0	0	2 (8.0)	2 (2.9)
Ethnicity, *n* (%)				
Hispanic	2 (10.0)	2 (8.7)	5 (20.0)	9 (13.2)
Non-Hispanic	18 (90.0)	21 (91.3)	20 (80.0)	59 (86.8)
Baseline GMFM-66 score				
Mean (SD)	48.95 (11.7)	50.26 (12.8)	48.12 (13.7)	49.09 (12.7)
Median (minimum, maximum)	47.50 (29.0, 72.0)	45.00 (30.0, 73.0)	48.00 (24.0, 70.0)	47.50 (24.0, 73.0)
Randomization strata, *n* (%)				
GMFCS level I/II, other	3 (15.0)	5 (21.7)	6 (24.0)	14 (20.6)
GMFCS level I/II, stroke	8 (40.0)	8 (34.8)	8 (32.0)	24 (35.3)
GMFCS level III/IV, other	7 (35.0)	9 (39.1)	8 (32.0)	24 (35.3)
GMFCS level III/IV, stroke	2 (10.0)	1 (4.3)	3 (12.0)	6 (8.8)
GMFCS level at randomization, *n* (%)				
I	7 (35.0)	7 (30.4)	9 (36.0)	23 (33.8)
II	4 (20.0)	6 (26.1)	5 (20.0)	15 (22.1)
III	1 (5.0)	5 (21.7)	3 (12.0)	9 (13.2)
IV	8 (40.0)	5 (21.7)	8 (32.0)	21 (30.9)
Etiology of CP, *n* (%)				
Hypoxic–ischemic encephalopathy	6 (30.0)	5 (21.7)	3 (12.0)	14 (20.6)
In utero or perinatal stroke/hemorrhage	10 (50.0)	9 (39.1)	12 (48.0)	31 (45.6)
Periventricular leukomalacia	4 (20.0)	9 (39.1)	10 (40.0)	23 (33.8)

Abbreviations: AlloCB, allogeneic cord blood; CP, cerebral palsy; GMFCS, Gross Motor Function Classification System; GMFM-66, Gross Motor Function Measure-66; hCT-MSC, human umbilical cord tissue-derived mesenchymal stromal cells.

[a]This table shows only the participants who contributed the primary outcome at month 12 (see Figure S1 for details). Refer to Tables S1 and S2 for baseline characteristics of all randomized participants. There are no statistically significant differences between the treatment arms ($p > 0.20$ for all baseline factors, Fisher's exact test).

[b]For both transplant and cord blood banking purposes, we are required to report both race and ethnicity (as two separate categories) for federal reporting purposes.

[c]The other reported races are 'Asian/White' and 'Mediterranean/Asian Mix'.

Product characteristics

Unrelated donor AlloCB units were 4 out of 6 ($n = 44$), 5 out of 6 ($n = 14$), or 6 out of 6 ($n = 1$) HLA-matched with the recipient. The median infused TNC dose was 9.3×10^7 TNC/kg (IQR 7.7–10.4; range 7.1×10^7 to 15.3×10^7 TNC/kg), with a median viability of 97% (IQR 95–98; range 82–99). A total of 81 hCT-MSC infusions across 27 participants had a median infused dose of 2×10^6 TNC/kg (range 1.9×10^6 to 2.2×10^6 TNC/kg) based on the post-thaw count, with a median viability of 76.5% (range 62.8–90.6). All products had negative post-thaw sterility cultures.

Safety of cellular infusions

There were eight acute infusion reactions in total related to the study products in eight unique participants across all treatment groups (three AlloCB, five hCT-MSC). All infusion reactions responded promptly and completely to medical intervention, which included additional diphenhydramine ($n = 5$), methylprednisolone ($n = 2$), acetaminophen ($n = 1$), prophylactic ceftriaxone ($n = 1$), and transient supplemental oxygen ($n = 3$). There were no long-term sequelae. The three AlloCB-associated infusion reactions (one in the AlloCB arm, two in the natural history arm when crossed

over to AlloCB) were all grade 2, consisting of fever (n = 1), tachycardia (n = 1), cough (n = 2), dyspnea (n = 1), and hypoxia (n = 1). Of the five hCT-MSC-associated infusion reactions (one grade 3, four grade 2), two occurred with the first infusion and three occurred with the second infusion. These were characterized by fever (n = 1), vomiting (n = 2), dyspnea (n = 1), and hypoxia (n = 2). Despite these reactions, all cellular infusions were able to be completed, and all participants tolerated subsequent infusions after increasing the premedication regimen to 1 mg/kg each of diphenhydramine and methylprednisolone and adding ondansetron for one participant and hydroxyzine for another. The only other related adverse event was the development of an asymptomatic donor-specific anti-HLA class I antibody in two participants in the hCT-MSC group.

In the 24-month study period, there were a total of 143 unrelated adverse events in 66 participants (Table S3). Forty-one unrelated serious adverse events occurred across treatment groups, most commonly hospitalizations due to planned surgery, seizures, or infections. One hundred and two non-serious, unrelated adverse events occurred over the 2-year period, also most commonly due to surgery, seizures, and infections. Three participants (one each in the AlloCB, hCT-MSC, and natural history groups) developed a new, asymptomatic anti-M antibody at the 12-month time point.

Motor evaluations

Primary outcome: actual-minus-expected change in GMFM-66

One year after baseline, the primary endpoint of the mean differences between a child's actual and expected changes in GMFM-66 score (95% CI) were 5.8 (3.4–8.2) in the AlloCB group, 4.3 (2.2–6.4) in the hCT-MSC group, and 3.1 (1.4–5.0) in the natural history group (Figure 1a). These results indicate that each group, even the natural history group, had significant improvement in motor function over what is expected for the general population of children with CP. The pattern of means suggests the greatest difference from expectation was in the AlloCB group followed by hCT-MSC and natural history. However, this study was not powered to detect differences in the group means, as indicated by the substantial overlap of the CIs for each group. Results were similar when all participants were analyzed using multiple imputation (Table S4).

Post hoc analysis of exploratory outcomes

The observed mean changes in GMFM-66 score from baseline to 12 months (95% CI) were 9.5 (6.6–12.3) in the AlloCB

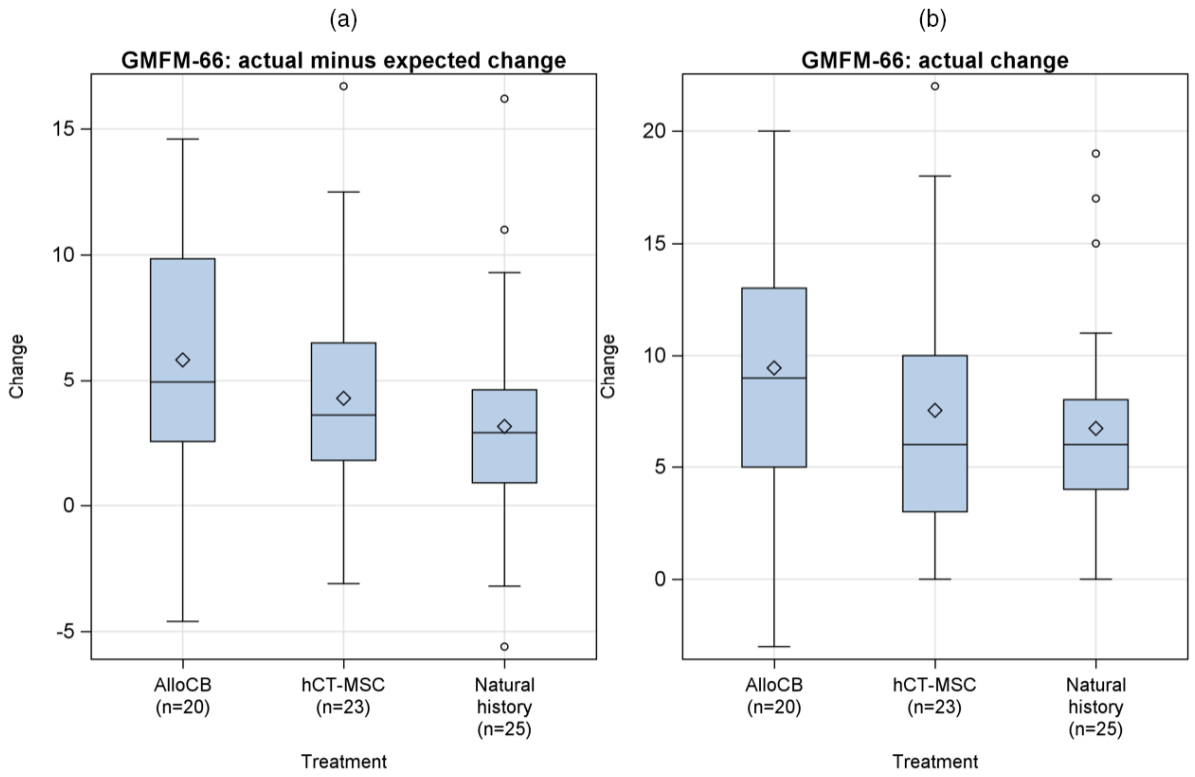

FIGURE 1 Gross Motor Function Measure-66 (GMFM-66) change from baseline to month 12. Diamonds indicate the mean whereas the line in the center of the box indicates the median. (a) The primary outcome of the study is the actual-minus-expected change in GMFM-66 from baseline to month 12. The mean changes (and 95% confidence intervals) are 5.8 (3.4–8.2), 4.3 (2.2–6.4), and 3.1 (1.4–5.0) for the allogeneic umbilical cord blood (AlloCB), human umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSC), and natural history arms respectively. (b) Distribution of the change in the actual GMFM-66 score from baseline to month 12.

TABLE 2 Results of post hoc motor function analyses: predicted mean GMFM-66 and PDMS-2 outcomes at 12 months

		Treatment		
		AlloCB	MSC	Natural history
Exploratory outcome				
GMFM-66	*n*	20	23	25
	Mean (95% CI)	57.99 (55.93, 60.05)	56.17 (54.34, 58)	54.72 (52.76, 56.69)
	P-value compared to Natural History	0.017	0.270	
	Bonferroni-corrected P-Value	0.033	0.540	
Peabody Developmental Motor Scales-2 Domains:				
Gross Motor Quotient	*n*	18	21	24
	Mean (95% CI)	59.68 (57.78, 61.58)	58.81 (57.04, 60.57)	55.6 (53.87, 57.33)
	P-value compared to Natural History	0.001	0.006	
	Bonferroni-corrected P-Value	0.002	0.013	
Locomotion	*n*	20	22	25
	Mean (95% CI)	3.07 (2.7, 3.44)	2.67 (2.32, 3.01)	2.38 (2.03, 2.73)
	P-value compared to Natural History	0.004	0.218	
	Bonferroni-corrected P-Value	0.008	0.436	
Object manipulation	*n*	18	21	24
	Mean (95% CI)	4.04 (3.33, 4.75)	3.97 (3.35, 4.59)	3.41 (2.78, 4.05)
	P-value compared to Natural History	0.165	0.191	
	Bonferroni-corrected P-Value	0.330	0.382	
Stationary	*n*	20	22	25
	Mean (95% CI)	3.88 (3.38, 4.38)	4.25 (3.79, 4.7)	3.75 (3.3, 4.21)
	P-value compared to Natural History	0.699	0.124	
	Bonferroni-corrected P-Value	1.000	0.248	

Abbreviations: AlloCB, allogeneic umbilical cord blood; CI, confidence interval; GMFM-66, Gross Motor Function Measure-66; hCT-MSC, human umbilical cord tissue-derived mesenchymal stromal cells; PDMS-2, Peabody Developmental Motor Scales, Second Edition.

group, 7.5 (5.1–9.9) in the hCT-MSC group, and 6.7 (4.8–8.7) in the natural history group (Figure 1b and Table S4). After adjustment for baseline GMFCS level, GMFM-66 score, and etiology of CP by ANCOVA, there was no evidence of a difference in the mean 12-month GMFM-66 score comparing hCT-MSC with natural history (mean difference 1.5 points, 95% CI −1.1 to 4.0; $p = 0.27$; Bonferroni $p = 0.54$). However, the AlloCB group had a mean 12-month GMFM-66 score that was 3.3 points higher than natural history (95% CI 0.59–5.93; $p = 0.02$; Bonferroni $p = 0.033$). Results from the inverse probability weighting ANCOVA model were similar, suggesting losses, which were primarily related to suspension of follow-up due to COVID-19, did not bias the results (Table S5). Finally, no differences in GMFM-66 scores between groups were observed at the 6-month time point. Predicted means from the ANCOVA models for GMFM-66 are shown in Table 2 (month 12) and Table S6 (month 6).

One year after baseline, the mean change in the PDMS Gross Motor Quotient (GMQ) was 3.6 points in the AlloCB group (95% CI 0.3–6.9), 3.2 points in the hCT-MSC group (95% CI 0.9–5.6), and 0.2 points in the natural history group (95% CI −1.5 to 1.8). In an ANCOVA model, participants with GMQs near the cohort mean at baseline had a predicted average GMQ at 12 months of 59.68 (95% CI 57.78–61.58) in the AlloCB group and 58.81 (95% CI 57.04–60.57) in the hCT-MSC group, both of which were higher than that of the natural history group (mean 55.6, 95% CI 53.87–57.33) (Table 2). The AlloCB group also demonstrated improvement in the locomotion domain at 12 months compared with the natural history group (mean 3.07 [95% CI 2.7–3.44] vs 2.38 [95% CI 2.03–2.73]). There was no statistically significant difference in change at 12 months in the other PDMS domains (stationary, object manipulation) between treatment groups. Changes in domains of the Pediatric Evaluation of Disability Inventory-Computer Adaptive Test were also not statistically significantly different between groups (Table S7).

DISCUSSION

This trial evaluated two different sources of donor cells in the treatment of young children with hypertonic CP due to stroke, hypoxic–ischemic encephalopathy, or periventricular leukomalacia. Three important observations emerged in the study results: (1) there was no evidence of safety concerns related to administration of high-dose AlloCB or repeated doses of hCT-MSC in these children; (2) the primary endpoint, the mean difference between a child's actual and expected changes in GMFM-66 score at 12 months, was highest

in the AlloCB (5.8 points) followed by hCT-MSC groups (4.3 points) and lowest in the natural history group (3.1 points) although CIs overlapped substantially as the study was not powered for between-arm differences; and (3) post hoc analyses of the GMFM-66 exploratory outcome suggested that participants treated with AlloCB had greater improvement in motor function than children who did not receive cell therapy. All of these findings have important implications for the design of future cell-therapy trials in CP.

AlloCB and hCT-MSC infusions were generally well tolerated. However, reactions occurred in 3 out of 57 AlloCB infusions (all grade 2) and 5 out of 81 hCT-MSC infusions (one grade 3; four grade 2). While some of these required minimal or no intervention, three required administration of oxygen, three required additional doses of diphenhydramine, and two required additional doses of solumedrol. With early recognition and prompt intervention, all reactions resolved completely, all cellular infusions were able to be completed in their entirety, and participants in the hCT-MSC group were able to successfully tolerate subsequent hCT-MSC infusions without additional reactions. This was possible because the clinical site was appropriately staffed with personnel capable of recognizing and responding to infusion reactions. Although the incidence of infusion reactions is low, it is essential that such infusions are performed in a setting with necessary emergency equipment and the oversight of experienced personnel who can recognize and treat reactions promptly.

Regarding the primary endpoint, the actual-minus-expected change in GMFM-66, improvements over expectation were observed even in the control arm. This is consistent with observations from our previous trial of autologous umbilical cord blood in CP and implies that children with CP who participate in clinical trials have, on average, greater change in motor function than expected according to their age and GMFCS level. This agrees with what is known about clinical trial participants generally. Therefore, benchmarking against the general population is not helpful for interpreting treatment effects in children with CP as has been suggested previously.[15] Furthermore, benchmarking against the general population is not necessary given the establishment of minimal clinically important differences for the GMFM-66.[16] Therefore, we plan to use the GMFM-66 as the primary motor function outcome in future trials, without comparing with expected values.

Our conclusion about choice of motor function endpoint also reflects discussions with experts and regulatory authorities while the trial was ongoing, which led to the post hoc analyses reported here. In fact, one of the most interesting findings from this trial was from a post hoc analysis that suggested patients treated with AlloCB had greater mean GMFM-66 scores at month 12 than participants randomized to the natural history arm. Importantly, the entirety of the 95% CI for the mean difference in 12-month GMFM-66 scores comparing AlloCB with natural history (0.59–5.93) includes established minimal clinically important differences in the GMFM-66 among ambulatory children with CP,[6] indicating that this observation is potentially clinically relevant. On the other hand, in our post hoc comparison of hCT-MSC with natural history, the 95% CI overlapped with no benefit (95% CI −1.1 to 4.0). It should also be noted that much of this interval also overlapped the interval estimate for the AlloCB arm. Therefore, these results should not be considered a definitive comparison of the two potential cellular therapies.

The results of our post hoc analysis of the GMFM-66 are not entirely consistent with the Peabody GMQ. On the Peabody GMQ, the hCT-MSC and AlloCB groups showed a similar amount of improvement relative to natural history. The differences in results may, in part, reflect the fact that the GMFM-66 is designed specifically for children with CP with a focus on quality of movements assessed whereas the Peabody GMQ is normalized to motor function of typically developing children without CP, and we might reasonably expect children with CP to fall further behind typically developing peers over time. Not surprisingly, the mean Peabody scores of participants in this trial fell into the lowest descriptive category, indicating 'very poor' gross motor function. Nonetheless, both treatment groups demonstrated greater gains in the Peabody GMQ at 12 months, raising the possibility that both hCT-MSCs and AlloCB may have potential as cellular therapy for CP. The primary mechanism of action of MSCs is thought to result from immunomodulatory effects on humoral and cell-mediated immune responses including inhibiting B-, T-, NK-, dendritic-cell, and microglial proliferation, decreasing pro-inflammatory cytokine production, and blocking neutrophil recruitment.[17–20] In children with CP, the sustained brain injury is static and, by the age of 2 to 5 years, is in the chronic phase. As such, the degree of ongoing inflammation is probably minimal and may be one explanation for the observation that hCT-MSCs were not associated with significant motor gains on the GMFM-66. Our group and others continue to investigate hCT-MSCs in the acute treatment of hypoxic brain injury.

While we plan to use the GMFM-66 as a primary motor function outcome in future trials, heterogeneity in the outcome, probably related to heterogeneity of the study population and/or the intervention, remains a challenge. Our previous randomized controlled trial of autologous umbilical cord blood included children aged 1 to 6 years with spastic CP that was not due to a genetic condition or brain malformation.[5] In that study, heterogeneity in the etiology of CP, age of participants, and cell dose contributed to wide variability in motor outcomes. In the present trial, we limited the etiology of CP to the acquired perinatal brain injuries of stroke, hypoxic–ischemic encephalopathy, and periventricular leukomalacia. We also restricted the age range to 2 to 5 years and attempted to administer a standard, higher dose of AlloCB of 10×10^7 TNC/kg given our previous finding that gross motor improvement was associated with a higher cell dose. This allowed us to glean important information about cell therapies in a relatively small sample. Interestingly, the median change in GMFM-66 score in

the AlloCB group, which received a fivefold higher cell dose than in the autologous trial, was 9 points, consistent with the median change of 8.5 points observed in the high-dose ($>2 \times 10^7$ TNC/kg infused) autologous umbilical cord blood group in our previous study. This suggests a potential dose threshold for clinical efficacy as opposed to a dose–response. However, there have been no randomized studies comparing different doses so the question of whether efficacy is dose-dependent has not been answered definitively.

In this trial, we also chose to examine motor function at two time points, 6 and 12 months. While we observed greater motor improvement at 12 months, there were no significant differences in GMFM-66 scores between any of the groups in any of the measures at the 6-month time point. On the basis of our previous work, one hypothesis about the mechanism of umbilical cord blood cells in the treatment of brain injuries is that they may improve myelination, whole brain connectivity, and connectivity between relevant motor areas of the brain through paracrine effects.[5,21] This process is likely to take time, and results of this trial suggest a period greater than 6 months. Brain diffusion tensor magnetic resonance imaging with tractography was performed in a subset of participants in this trial, and those results will be reported separately.

There are some limitations to this trial, including the fact that the families were not blinded to treatment assignment, the COVID pandemic prevented approximately a quarter of participants from completing their 12-month motor evaluations, multiple etiologies of CP were included, and the sample size was not powered to conduct comparisons between treatment groups. While family members were not blinded because of differences in treatment schedule between the groups, the physical therapists conducting the motor function assessments were blinded to treatment assignment, providing confidence of unbiased evaluation in the GMFM-66 and PDMS-2 measures. Even though the COVID pandemic halted the trial prematurely, multiple imputation statistical techniques indicated that the results were not affected by the missing 12-month data. This is in line with our expectations given that the primary reason for losses was implemented uniformly regardless of assigned treatment. Lastly, although the study population in this trial was more uniform than our previous study, heterogeneity in age and etiology of CP may have affected outcomes. Future studies should continue to stratify participants on the basis of etiology of CP so that any differential effects can be observed.

This study adds to a growing body of evidence from around the world that umbilical cord blood may be beneficial in improving motor function after brain injury.[22–25] As many children in need do not have an adequate autologous or sibling umbilical cord blood unit available, it is clear that a donor product will be necessary to extend treatment to all who might benefit. The main goals of this trial were to evaluate two different sources of donor cells as potential treatment options in children with CP, estimate the magnitude of their improvement in motor function after treatment, and determine the best time interval to assess response. The use of hCT-MSC and unrelated donor AlloCB from a public cord blood bank allowed the provision of consistently manufactured products, a much higher AlloCB cell dose than could be achieved otherwise, and inclusion of all eligible children regardless of whether they had a family umbilical cord blood unit banked. Results of our post hoc analyses suggest that treatment with AlloCB was associated with gross motor gains in young children with CP. This finding should be pursued in a phase 3 randomized, double blind, placebo-controlled trial.

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DATA AVILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

ORCID

Jessica M. Sun https://orcid.org/0000-0001-8085-1013
Joan M. Jasien https://orcid.org/0000-0002-5469-1092
Mohamad A. Mikati https://orcid.org/0000-0003-0363-8715

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SUPPORTING INFORMATION

The following additional material may be found online:

Figure S1: CONSORT diagram.

Table S1: Baseline characteristics (all randomized participants).

Table S2: Baseline characteristics of randomized versus analyzed participants.

Table S3: Adverse events.

Table S4: Summary of GMFM-66 based on multiple imputation.

Table S5: GMFM-66 at month 12: analysis of covariance with and without inverse probability weights for missingness.

Table S6: Results of post hoc motor function analyses: predicted mean GMFM-66 and PDMS-2 outcomes at 6 months.

Table S7: Summary of PEDI-CAT.

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