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CORD BLOOD

Effect of Autologous Cord Blood Infusion on Motor Function and Brain Connectivity in Young Children with Cerebral Palsy: A Randomized, Placebo-Controlled Trial

JESSICA M. SUN,[a] ALLEN W. SONG,[b] LAURA E. CASE,[c] MOHAMAD A. MIKATI,[d] KATHRYN E. GUSTAFSON,[e] RYAN SIMMONS,[a] RICKI GOLDSTEIN,[f] JODI PETRY,[c] COLLEEN MCLAUGHLIN,[a] BARBARA WATERS-PICK,[g] LYON W. CHEN,[b] STEPHEN WEASE,[h] BETH BLACKWELL,[h] GORDON WORLEY,[d] JESSE TROY,[a] JOANNE KURTZBERG[a]

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ABSTRACT

Cerebral palsy (CP) is a condition affecting young children that causes lifelong disabilities. Umbilical cord blood cells improve motor function in experimental systems via paracrine signaling. After demonstrating safety, we conducted a Phase II trial of autologous cord blood (ACB) infusion in children with CP to test whether ACB could improve function (ClinicalTrials.gov, NCT01147653; IND 14360). In this double-blind, placebo-controlled, crossover study of a single intravenous infusion of 1–5 × 10^7 total nucleated cells per kilogram of ACB, children ages 1 to 6 years with CP were randomly assigned to receive ACB or placebo at baseline, followed by the alternate infusion 1 year later. Motor function and magnetic resonance imaging brain connectivity studies were performed at baseline, 1, and 2 years post-treatment. The primary endpoint was change in motor function 1 year after baseline infusion. Additional analyses were performed at 2 years. Sixty-three children (median age 2.1 years) were randomized to treatment ($n = 32$) or placebo ($n = 31$) at baseline. Although there was no difference in mean change in Gross Motor Function Measure-66 (GMFM-66) scores at 1 year between placebo and treated groups, a dosing effect was identified. In an analysis 1 year post-ACB treatment, those who received doses ≥2 × 10^7/kg demonstrated significantly greater increases in GMFM-66 scores above those predicted by age and severity, as well as in Peabody Developmental Motor Scales-2 Gross Motor Quotient scores and normalized brain connectivity. Results of this study suggest that appropriately dosed ACB infusion improves brain connectivity and gross motor function in young children with CP. STEM CELLS TRANSLATIONAL MEDICINE *2017;00:000–000*

SIGNIFICANCE STATEMENT

Results of this trial suggest that when adequately dosed, an intravenous infusion of autologous umbilical cord blood improves whole brain connectivity and motor function in young children with cerebral palsy.

INTRODUCTION

Cerebral palsy (CP), the most prevalent motor disorder of childhood, affects two to three per 1,000 live births [1]. CP typically results from in utero or perinatal brain injury such as hypoxic insult, hemorrhage, or stroke. Affected children have varying degrees of functional impairments from mild limitations in advanced motor skills to severely limited self-mobility despite use of assistive technology resulting in a lifelong inability to function independently. Currently, the cornerstone of treatment is various therapies to optimize function and quality of life. However, no curative therapies are available.

Improved motor function has been demonstrated in animal models of ischemic brain injury and CP after administration of human umbilical cord blood cells [2, 3]. Evidence suggests that cord blood cells act via paracrine signaling endogenous cells to facilitate repair. This led us to hypothesize that intravenous (IV) infusion of autologous cord blood (ACB) would improve motor function in young children with CP. After demonstration of safety in 184 children [4], we conducted a randomized,

Authored by a member of CORD BLOOD ASSOCIATION

[a]The Robertson Clinical and Translational Cell Therapy Program, [b]The Brain Imaging and Analysis Center, [c]Department of Physical and Occupational Therapy, [d]Division of Pediatric Neurology, [e]Department of Psychiatry, [f]Division of Neonatology, [g]Stem Cell Transplant Laboratory, Duke University, Durham, North California, USA; [h]The Emmes Corporation, Rockville, Maryland, USA

Correspondence: Jessica Sun, M.D., Robertson Clinical and Translational Cell Therapy Program, Duke Translational Research Institute, Duke University Medical Center, Box 3850 Durham, North California 27710, USA. Telephone: 919-668-1119; e-mail: jessica.sun@duke.edu

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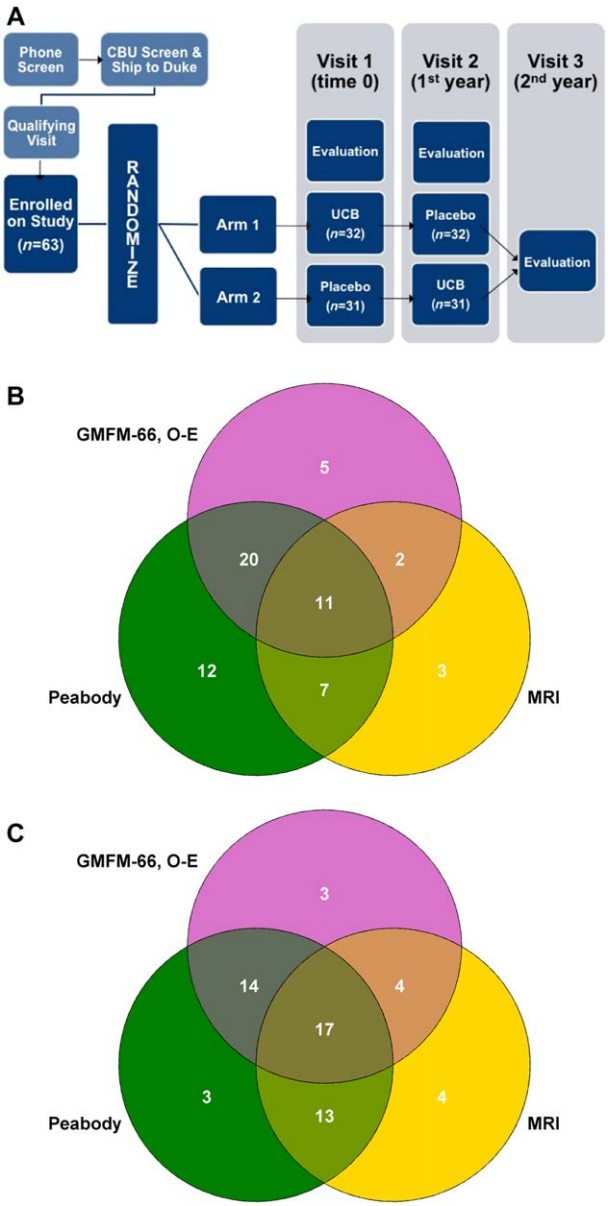

Figure 1. Study schema and distribution of subjects in analysis cohorts. **(A):** Gross motor evaluations (GMFM-66, Peabody Developmental Motor Scales-2 [PDMS-2]) and brain MRI were performed at each visit. **(B):** Diagram of subjects evaluable via change in observed-expected GMFM-66 score (pink), PDMS-2 score (green), and MRI (yellow) from baseline to 1 year. **(C):** Diagram of subjects evaluable via change in observed-expected GMFM-66 score, PDMS-2 gross motor quotient score, and MRI 1 year after cord blood treatment (given at either baseline or 1 year). Abbreviations: GMFM-66, Gross Motor Function Measure-66; MRI, magnetic resonance imaging.

placebo-controlled trial testing the efficacy of a single IV ACB infusion in young children with CP.

Materials and Methods

Study Design

We conducted a single-center, Phase II, prospective, randomized, double-blind, placebo-controlled, crossover study of a single IV ACB infusion in children ages 1 to 6 years with CP (Fig. 1) at Duke University. The study was approved by the Duke Institutional Review Board and conducted under FDA IND14360.

Table 1. Qualifying characteristics of autologous umbilical cord blood units

Characteristic	Specification
Precryopreservation characteristics	
Total nucleated cell count (TNCC)	$\geq 1 \times 10^7$/kg
Viability (total or CD34)	≥80%
Sterility culture	Negative
Maternal infectious disease screening[a]	Negative
Test sample available for confirmatory HLA typing	Yes
Cord blood test sample characteristics	
Identity confirmation via HLA testing of subject and cord blood sample	Confirmed
CD34 viability	≥60%
Colony forming units	Growth

[a]All mothers/units were tested for Hepatitis B, Hepatitis C, HIV, and syphilis. Most were also tested for HTLV I/II.
Abbreviation: HLA, Human Leukocyte Antigen.

Participants

Eligible children were 1 to 6 years old had CP with (a) Gross Motor Classification System (GMFCS) level 2–4 or (b) GMFCS level 1 with hemiplegia if they used their affected hand as an assist only. Children also had to have an eligible ACB unit banked at a public or private cord blood bank that was sterile, had a precryopreservation total nucleated cell count (TNCC) $\geq 1 \times 10^7$/kg, and met criteria in Table 1. Children with genetic conditions, intractable seizures, hypsarrhythmia, athetoid CP, severe microcephaly, autism without motor disability, evidence of a progressive neurologic disease or a condition that could require a future allogeneic stem cell transplant, active infection(s), impaired renal, liver or respiratory function, or a history of prior cell therapy were ineligible. Written informed consent was obtained from parent(s)/guardian(s) for patient and ACB screening and study participation.

Randomization and Masking

Patients were randomized to the order in which they received ACB and placebo infusions, given 1 year apart. Those on the ACB arm received an infusion of ACB at baseline. Those on the placebo arm received an infusion of a placebo solution constructed to mimic the color and smell of the ACB at baseline. The placebo product consisted of TC-199 + 1% dimethyl sulfoxide (DMSO). Computer-generated randomization was performed by The Emmes Corporation in a 1:1 ratio, stratified by age and CP typography. Only staff preparing the products were aware of the treatment assignment, and these individuals had no contact with the patients, families, providers, and examiners who were masked to the assigned treatment. Masking was achieved by covering all infusion bags with a dark bag in the laboratory and infusing a similar volume as the placebo product. Cell dose, targeted at $1–5 \times 10^7$ cells per kilogram, was not randomly assigned, but was determined by the number of cells available in each ACB unit and the patient's weight.

Procedures

Patients' medical records and ACB reports were reviewed. If likely to be eligible, an ACB sample was shipped to Duke for potency

and viability testing. Unit identity was confirmed by low-resolution Human Leukocyte Antigen (HLA)-testing of patient and ACB samples. If specifications were met, the cryopreserved ACB unit was shipped to Duke and stored under liquid nitrogen until the day of ACB infusion. Prior to enrollment, all patients were assessed by an independent examiner (M.A.M., G.W., R.G.) to confirm eligibility and assign baseline GMFCS level. On the day of ACB infusion, the product was thawed and washed in dextran 40 + 5% albumin (DA) and placed in 1.25 ml/kg DA for administration [5]. Placebo infusions consisted of TC-199 + 1% (DMSO). The cells or placebo were administered at baseline and 1 year later in a masked manner through a peripheral IV catheter over 5–15 minutes in the outpatient setting after premedication with oral acetaminophen (10–15 mg/kg), IV diphenhydramine (0.5 mg/kg), and IV methylprednisolone (0.5 mg/kg). Subjects received maintenance IV fluids and were monitored for 2–4 hours post-infusion. Safety endpoints were incidences of infusion reactions and infections related to the study treatment. Safety assessments were conducted at 24 hours and 7–10 days after each infusion, as well as annually during return visits. Participants received traditional rehabilitation therapies per their local physicians and therapists throughout the duration of the study.

Motor Assessments

Functional assessments were performed by trained physicians and therapists at baseline, 1- year, and 2-years. GMFCS level was assessed and motor evaluations completed, including the Peabody Developmental Motor Scales-2 (PDMS-2) [6] and GMFM-66 [7], a 66-item measure designed to assess gross motor function in children with CP.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) was performed, under moderate sedation for most participants, at baseline, 1-year, and 2-years. Diffusion weighted images were acquired on a 3 Tesla GE MR750 scanner (Waukesha, WI) using a 25-direction gradient diffusion encoding scheme ($b = 1{,}000$ seconds/mm^2, 3 nondiffusion-weighted images), 70.5 ms echo time (TE), and 12,000 milliseconds repetition time (TR). Isotropic resolution of 2 mm^3 was achieved using a 96 $\times$ 96 acquisition matrix in a field of view (FOV) of 192 $\times$ 192 mm^2. *T1*-weighted images were obtained with an inversion-prepared three-dimensional (3D) fast spoiled-gradient-recalled (FSPGR) pulse sequence with a 2.5 ms TE, 450 ms inversion time (TI), 6.5 ms TR, and 12° flip angle, at 1 mm^3 isotropic resolution.

Whole brain connnectome analysis was based on MRI diffusion weighted images from all directions. Diffusion tensor in every voxel across the entire brain was derived, and fiber pathways tracked using fiber assignment by continuous tracking streamline tracking algorithm [8, 9] based on a standard fractional anisotropy (FA) threshold (0.2) to limit the pathways within the white matter. A whole-brain connectome analysis, based on all diffusion tensors, was then carried out to investigate brain connectivity and improvement among functional brain regions. These gray matter regions, termed "nodes" in the brain connectome, were defined by the JHU-DTI-MNI "Eve" atlas template [10, 11], and warped into each subject's DTI image space via the Advanced Normalization Tools toolkit [12] for a standardized processing strategy. Connectivity from any given node, or between any pair of nodes, was first measured by determining volumes of the relevant white matter fiber pathways projecting from that node or between a pair of nodes. These volumes were then further normalized by the total white matter volume (derived from a 3D FSPGR T1 weighted MRI) to remove the dependence on brain sizes due to developmental effect.

Statistical Analysis

The primary endpoint was change in motor function from baseline to 1-year assessed by the GMFM-66. A positive change in GMFM-66 score is considered an improvement, and minimal clinically important differences (MCIDs) [13] of medium and large effect sizes have been established. Sample size planning used estimates of this change score in untreated patients derived from a literature review (mean = 6, SD = 3) [14, 15]. The study was originally planned for 60 subjects/group ($n = 120$ total), estimated to provide 78%–97% power to detect a clinically relevant increase of 25%–35% in the mean 1-year GMFM-66 change score comparing ACB to placebo using a two-sided, equal-variance t test and 5% Type I error rate.

Two unplanned interim analyses were conducted for the primary endpoint. Efficacy stopping rules were designed to preserve the overall Type I error rate at 5% using an alpha spending function, $f(t) = \min(\alpha * t^3, \alpha)$. The null hypothesis of no difference between treatment groups was not rejected at either analysis. A simulation study for conditional power conducted after the first interim analysis suggested potential benefit in continuing the trial. However, due to slow accrual, the trial was closed when enrollment reached $n = 63$. When all subjects had completed the 1-year assessment and after verifying assumptions, the test of the primary hypothesis was performed using an equal-variance, two-sample t test with a critical value of 2.00, which maintained an overall two-sided cumulative alpha of 0.05 across interim looks with the final sample size. Results of the interim analyses were reviewed by the primary investigators. Study personnel conducting outcome assessments were not informed of the results.

All analyses (performed by B.B., J.T., R.S. using SAS versions 9.3 and 9.4) followed the intention-to-treat principle. The primary endpoint was compared between ACB and placebo using an equal-variance t test. Additional analyses, involving comparison of outcomes by dose between ACB and placebo 1 year after baseline and among all patients 1 year after treatment with ACB, used the t test, Wilcoxon rank sum test, Fisher's exact test, or Spearman correlation as appropriate. We defined high- and low-dose categories using the cohort median dose. For analyses from baseline to 1 year, the median dose was calculated for the 32 patients randomized to the treatment arm (3.0×10^7/kg precryporeservation, 1.98×10^7/kg infused). For analyses of the composite cohort (all children 1 year post ACB infusion), we used the median infused dose for all 63 patients, 2×10^7/kg. Data for this report were locked on October 25, 2016. This trial is registered with ClinicalTrials.gov, number NCT01147653.

Results

Characteristics of Patients and ACB Units

Between September 27, 2010 and February 14, 2014, 63 patients were enrolled and randomized to receive an initial infusion of ACB ($n = 32$) or placebo ($n = 31$) with a crossover to the alternate infusion 1 year later (Fig. 1). Subjects' etiology of CP was classified as: periventricular leukomalacia ($n = 17$), in utero stroke/bleed ($n = 27$), ischemic injury ($n = 7$), other multifactorial causes

Table 2. Characteristics of patients and autologous cord blood units by randomized treatment assignment and cell dose

	Randomized assignment		Infused cell dose	
	Autologous Cord blood group ($N = 32$)	Placebo group ($N = 31$)	Low ($<2 \times 10^7$/kg) ($N = 31$)	High ($\geq 2 \times 10^7$/kg) ($N = 32$)
Patient characteristics				
Age, years – median (range)	2.1 (1.1–6.2)	2.3 (1.1–7.0)	2.5 (1.1–7.0)	2.1 (1.1–5.3)
Sex – no. (%)				
Male	20 (62.5)	22 (71)	20 (64.5)	22 (68.8)
Female	12 (37.5)	9 (29)	11 (35.5)	10 (31.3)
Race – no. (%)				
White	27 (84.4)	28 (90.3)	26 (83.9)	29 (90.6)
Non-white	5 (15.6)	3 (9.7)	5 (16.1)	3 (9.4)
Type of cerebral palsy – no. (%)				
Hypotonic quadraplegia	1 (3.1)	3 (9.7)	2 (6.5)	2 (6.3)
Spastic diplegia	6 (18.8)	6 (19.4)	7 (22.6)	5 (15.6)
Spastic hemiplegia	15 (46.9)	15 (48.4)	12 (38.7)	18 (56.3)
Spastic quadraplegia	10 (31.3)	7 (22.6)	10 (32.3)	7 (21.9)
GMFCS level[a] – no. (%)				
I/II	21 (65.6)	21 (67.7)	18 (58.1)	24 (75.0)
III/IV	11 (34.4)	10 (32.3)	13 (41.9)	8 (25.0)
Baseline GMFM-66 score – mean (SD)	48.9 (16.2)	52.0 (15.7)	48.9 (20.3)	51.9 (10.1)
Cord blood characteristics – median (range) [b]				
Collection volume, ml	66 (4.5–146)	64 (5.7–150)	56 (4.5–146)	83 (20–150)
Pre-cryo TNCC, $\times 10^8$	4.4 (1.1–15.5)	5.1 (1.9–12.6)	2.8 (1.1–10.3)	7.1 (2.9–15.5)
Cell dose infused, $\times 10^7$/kg	2.0 (0.8–4.8)	—	1.5 (0.4–1.9)	3.1 (2.0–5.0)
CD34+ dose infused, $\times 10^5$/kg	0.60 (0.11–3.90)	—	0.40 (0.05–2.00)	0.80 (0.20–3.90)
CFU dose infused, $\times 10^5$/kg	3.91 (0.04–36.21)	—	4.0 (0–36.2)	4.6 (0–20.0)

[a]GMFCS = Gross Motor Function Classification System. Difference between dosing groups is not statistically significant.
[b]All cord blood characteristics except CFU dose are statistically significant between doing groups ($p < .01$) and not statistically different between randomized groups.
Abbreviation: TNCC, total nucleated cell count.

($n = 12$). Of these 12 patients, etiologies and MRI findings were highly variable and included two patients who were born premature, one with a porencephalic cyst, three with white matter abnormalities, five with normal MRIs, and one of unclear etiology. One-third of patients had moderately severe GMFCS levels (3–4) at study entry. Treatment groups were balanced with respect to age, sex, race, type, and severity of CP (Table 2).

ACB units were retrieved from 16 international cord blood banks. All subjects received all infusions as intended. The median precryopreservation TNCC of banked ACB units was 4.9×10^8. To achieve the target TNCC dose of $1–5 \times 10^7$/kg, the entire ACB unit was used in 31 patients. In the other 32 patients for whom the cell dose from the whole ACB unit would have exceeded the dosing range, a portion of the ACB unit was used for infusion and the remainder was cryopreserved and stored for potential future use. Post-thaw, a median of 2×10^7 TNCC/kg were administered (range $0.38–5.03 \times 10^7$/kg), containing a CD34+ dose of 0.5×10^5/kg (range $0.05–4.9 \times 10^5$/kg). ACB unit characteristics are shown in Table 2.

Safety of ACB Infusions

Infusions of thawed ACB and placebo products, both containing DMSO, were well tolerated, and there were no serious adverse events related to the infusions. One patient had transient infusion reactions consisting of hives +/– low-grade fever after both placebo and ACB infusions, successfully treated with additional diphenhydramine. Despite negative precryopreservation cultures, one ACB unit grew β-hemolytic streptococcus from a sample of the thawed unit. That patient was not treated with antibiotics and did well.

GMFM-66 Results

Change in GMFM-66 score from baseline to 1-year was the primary endpoint. The observed mean change in GMFM-66 score was 7.5 points (SD 6.8) in the ACB group and 6.9 points (SD 5.5) in the placebo group ($t_{df} = 61, = 0.36$, $p = .72$, Fig. 2). Of note, both groups improved more than expected based on patients' age and GMFCS level at study entry [16]. However, subjects randomized to ACB who were treated with TNCC doses above the median precryopreservation or infused doses of 3×10^7/kg and 1.98×10^7/kg, respectively, demonstrated statistically significant, clinically meaningful improvement in GMFM-66 change scores, above MCIDs, compared with subjects who received lower cell doses ($p < .01$ for precryopreservation dose, $p = .05$ for infused dose) or placebo ($p = .02$ for precryopreservation dose) (Fig. 2B). Cell

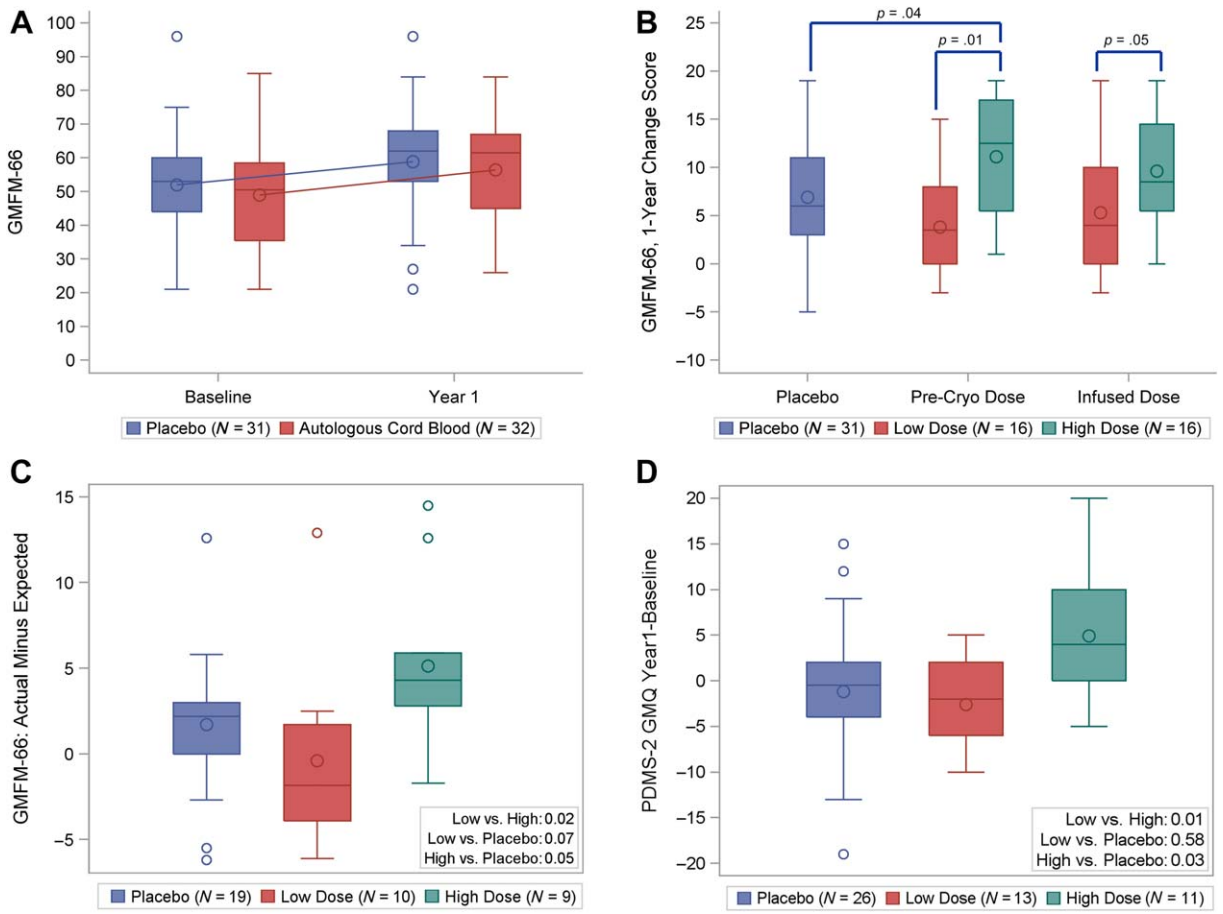

Figure 2. GMFM-66 scores from baseline to year 1 by randomized treatment assignment and cell dose. **(A):** Distribution of GMFM-66 score at baseline and 1 year in patients randomized to placebo and autologous cord blood. Lines connect the group means (circles) over time. **(B):** GMFM-66 change scores based on median cell doses (Precryopreservation doses: Low, $<3 \times 10^7$/kg, $N = 16$ vs. High, $\geq 3 \times 10^7$/kg, $N = 16$; Infused doses: Low, $<1.98 \times 10^7$/kg, $N = 16$ vs. High: $\geq 1.98 \times 10^7$/kg, $N = 16$). **(C):** One year Observed-Expected GMFM-66 scores in patients ≥ 2 years of age at baseline based on infused cell dose (Low, $N = 10$; High, $N = 9$; Placebo, $N = 19$). **(D):** PDMS-2 gross motor quotient change scores based on infused cell dose (Low, $N = 13$; High, $N = 11$; Placebo, $N = 25$). Abbreviation: GMFM-66, Gross Motor Function Measure-66.

doses were not associated with baseline age or type/severity of CP (Table 2). In the placebo group, change from baseline to 1 year was not associated with the precryopreservation cell dose available in the subjects' ACB unit (not shown). CD34 cell doses were not associated with motor improvement.

To examine the effect of cell dose and to adjust response for the natural history of expected gains based on baseline GMFCS levels and GMFM-66 scores of each subject, we used published percentiles [16] to compare the actual GMFM-66 score change to the predicted change. The difference between the observed 1-year GMFM-66 score and the predicted 1-year GMFM-66 score was then calculated. Since percentile values are only available for children ≥ 2 years, this analysis included the 38 patients who were ≥ 2 years old at study entry. There was no significant difference in the median observed-expected difference in GMFM-66 scores at 1 year in patients randomized to ACB ($n = 19$; 1.7; IQR -2.5 to 4.5) versus placebo ($n = 19$; 2.2; IQR 0.0 to 3.0; $p = .99$). However, in an exploratory analysis, subjects who received a TNCC $\geq 2 \times 10^7$/kg ($n = 9$) improved a median of 4.3 points (IQR 2.8–5.9) *greater* than expected, and this change was statistically significantly different from that observed in subjects who received $<2 \times 10^7$/kg ($n = 10$; median -1.9, IQR -3.9 to 1.7; $p = .02$) or placebo ($n = 19$; median 2.2, IQR 0.0 to 3.0; $p = .05$, Fig.2C), with improvement above the MCID of large effect size.

We then used the 2-year data to further explore the effect of cell dose by comparing the difference between observed and expected GMFM-66 scores 1 year after ACB infusion in all subjects who were ≥ 2 years old when they were treated ($n = 46$), regardless of when the infusion was given (baseline or 1 year). In this analysis, the dose relationship from the primary analysis was confirmed: subjects who received $\geq 2 \times 10^7$ cells per kg ($n = 23$) improved a median 3.6 points (IQR -0.4 to 4.5) *greater* than expected, whereas subjects who received $<2 \times 10^7$/kg did not improve beyond expectation (median -1.1, IQR -3.7 to 1.3, $p = .003$, Fig. 3A).

PDMS-2 Results

At 1 year post initial treatment (ACB vs. placebo), 50 patients were eligible for analysis of the PDMS-2 Gross Motor Quotient, which assesses gross motor skills in young children from birth to 72 months of age. Of note, eight subjects excluded from analysis of observed-expected GMFM-66 scores due to age (<2 years) were included in the PDMS analysis. The median change from baseline did not differ significantly between randomized groups

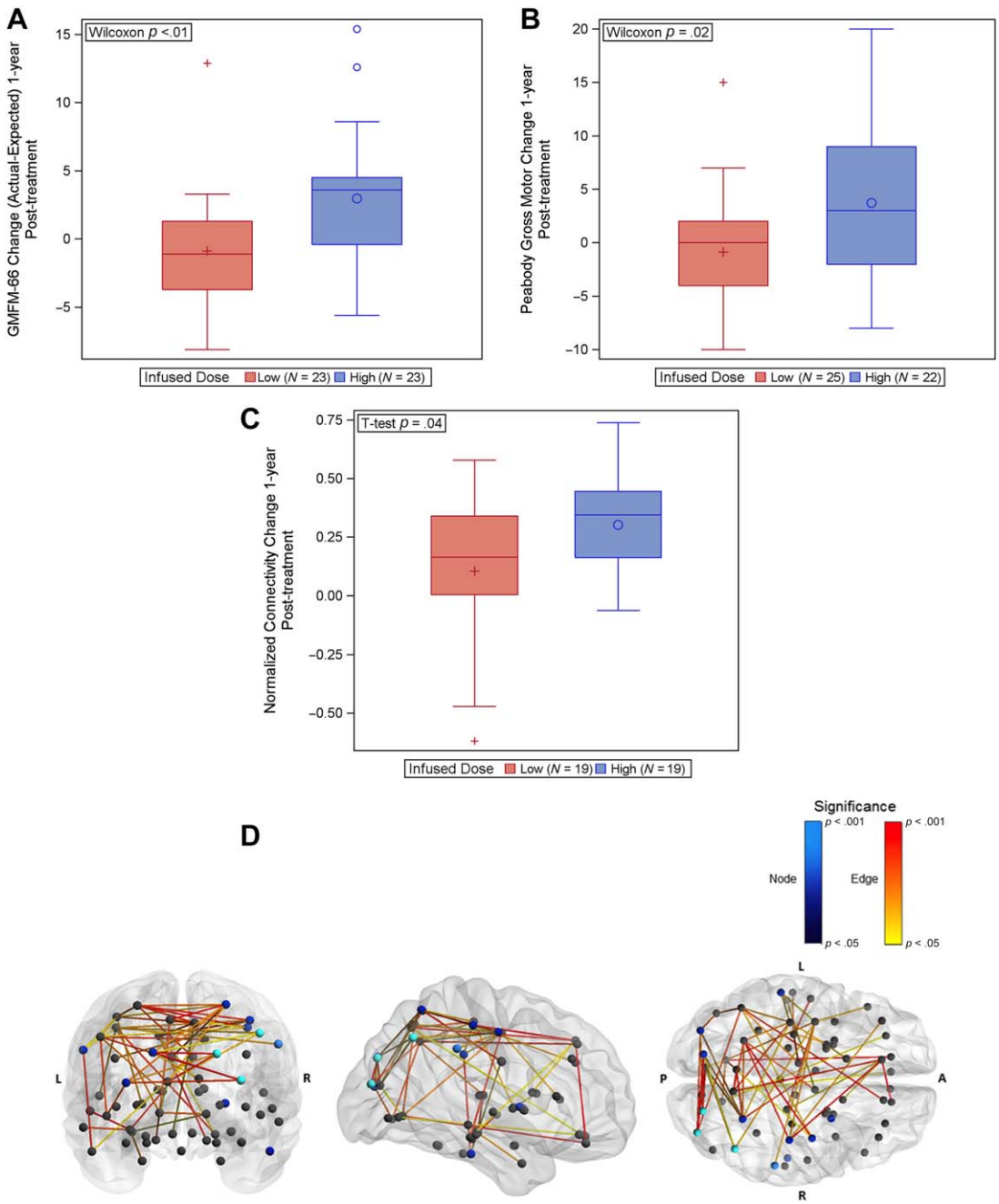

Figure 3. Gross motor function and brain connectivity 1 year after autologous cord blood treatment by cell dose. High dose $\geq 2 \times 10^7$/kg, low dose $< 2 \times 10^7$/kg. **(A):** Observed-Expected GMFM-66 scores 1 year after treatment in patients ≥ 2 years of age at the time of ACB infusion. **(B):** Peabody Developmental Motor Scales-2 gross motor change scores 1 year after treatment. **(C):** Change in normalized whole brain connectivity 1 year after treatment. **(D):** Connectome representation. The nodes and edges included are those that demonstrated significantly increased improvement in children receiving high doses compared with those receiving low doses, as indicated by the color chart, with insignificant nodes shown in gray. Representative nodes in the sensorimotor network with significant changes correlated with improvement in GMFM-66 scores include the pre- and post-central gyri, basal ganglia, and brain stem. Abbreviation: GMFM-66, Gross Motor Function Measure-66.

(ACB 1.0, IQR −4.5 to 4.5 vs. placebo −0.5, IQR −4.0 to 2.0; $p = .39$); but analysis of the treated group by infused cell dose confirmed the observation from the GMFM-66 analysis, with greater improvement in the high dose group (Fig. 2D). The dose finding was consistent in the 2-year analysis, when all subjects treated with ACB were analyzed by infused TNCC ($>/<2 \times 10^7$/kg); subjects receiving high doses showed statistically significant improvement compared with subjects receiving lower doses (median [IQR]: high-dose 3.0 [–2.0 to 9.0] vs. low-dose 0 [–4.0 to 2.0], $p = .02$, Fig. 3B).

Imaging Results

We explored relationships between motor response, total brain connectivity, and cell dose. Accurate anatomical image

parcellation could not be obtained in approximately one-third of subjects due to injury that distorted normal brain morphology, leaving 23 treated and 15 placebo patients with evaluable connectivity data ($n = 38$). Data from 17 of these subjects has been reported previously [17]. There were no statistically significant differences in CP type, GMFCS level, or age between patients with and without analyzable images.

There was a moderate correlation between change in GMFM-66 score and total connectivity 1 year after baseline in all analyzable subjects ($n = 38$, Spearman $r = 0.53$; 95%CI: 0.25, 0.73; $p < .001$). In this cohort, total connectivity change was not related to baseline GMFCS level, typography of CP, or sex, but was inversely correlated with age (Spearman $r = -0.52$; 95%CI: -0.72, -0.23; $p = .001$). In the 2-year analysis when all evaluable subjects were examined by cell dose, patients who received $\geq 2 \times 10^7$ TNCC/kg ($n = 19$) demonstrated a statistically significant greater increase in normalized whole brain connectivity 1 year after treatment than children who received lower doses ($n = 19$; $p = .04$, Fig. 3C). In the sensorimotor network, nodes with significant increases in connectivity that correlated with improvement in GMFM-66 scores included the pre- and post-central gyri, basal ganglia, and brain stem.

Discussion

We tested whether a single IV infusion of ACB could improve GMFM-66 change scores in young children with CP. We observed that children who received higher cell doses ($\geq 2 \times 10^7$/kg infused) demonstrated superior gains in both whole brain connectivity and motor function 1 year after infusion of ACB. Based on preclinical models, we hypothesized that ACB acts through homologous paracrine signaling, rather than cellular integration or engraftment of infused cells.

There are some limitations of this study. The first is the small sample size and heterogeneity of patients within groups. Also, in this study cohort, both randomized groups including placebo patients experienced gains in motor function above those predicted by the GMFM-66. This may reflect the impact of developmental therapies and other interventions in the highly motivated and resourced families who participated in this study. Nonetheless, the median change in GMFM-66 score in the high-dose group (8.5, IQR 5.5–14.5), exceeded that of the low-dose (4, IQR 0.0–10.0) and placebo (6, IQR 3.0–11.0) groups by more than established MCIDs [13], indicating a statistically significant and clinically meaningful difference between dosing groups. These responses were not correlated with age or type, etiology, or severity of CP.

The observation that children receiving cell doses $\geq 2 \times 10^7$/kg had a response is important. As a therapeutic dose had not been established in this setting, we targeted a TNCC 1–5 $\times 10^7$/kg based on safety data for this range in children undergoing allogeneic cord blood transplantation. However, in additional analyses, we observed that children with CP demonstrated statistically significant improvement in gross motor function on two well-validated measures (GMFM-66, PDMS) when ACB was administered at doses (3×10^7/kg precryopreservation and 2×10^7/kg infused) that are above the threshold for hematopoietic reconstitution in allogeneic transplantation [18]. This finding is consistent with two other randomized trials of cell therapy in children with CP, both conducted in Korea using allogeneic cord blood [19, 20]. One study demonstrated greater improvement on select motor and cognitive scales six months after treatment in patients who received a precryopreservation TNCC $\geq 3 \times 10^7$/kg + erythropoietin versus those who received erythropoietin alone or placebo. They also noted a dose correlation, with higher doses associated with greater improvement [19]. The second study showed similar results in 36 children treated with cord blood or placebo [20].

Important relationships were also detected via whole brain connectome analysis of MRI/DTI data, an objective measure of whole brain connectivity including the motor network, suggesting that improvements in motor function result from increased or new connectivity induced by paracrine signaling of ACB cells. We confirmed that increased total brain connectivity is correlated with increased motor improvement, as reported by our previous studies [17]. Furthermore, we also showed that compared with children who received a low cell dose, children who received a dose $\geq 2 \times 10^7$/kg demonstrated a greater increase in both normalized total brain connectivity and changes in the sensorimotor network, including the pre- and post-central gyri, deep gray matter, and brain stem (Fig. 3) 1 year post-treatment with ACB. Although the primary manifestation of CP is motor in nature, it is well known that CP can affect multiple aspects of development and in many cases is a whole-brain disorder. Thus, whole brain connectivity, along with graphical analysis of the brain connectome including the motor network, may serve as a biomarker of treatment response and may help elucidate the mechanism of action of ACB therapy in this patient population.

In the course of conducting this study, we were contacted by hundreds of families of children with CP who did not have a qualified ACB unit available. If umbilical cord blood is indeed established as a source of cells for restorative therapies, a method to provide treatment to such individuals will be necessary. As the hypothesized mechanism of action—that cells contained in ACB act on endogenous cells in the brain via paracrine signaling to enhance brain connectivity and thus function—does not require engraftment or integration of infused cells, it is feasible that donor cord blood cells may be equally efficacious. Therefore, we plan to study the safety and efficacy of allogeneic cell sources in future studies.

Conclusion

Results of this trial suggest that when dosed $\geq 2 \times 10^7$ cells per kg, an IV infusion of ACB improves whole brain connectivity and motor function in young children with CP. These findings have important implications for the treatment of children with CP and should be further explored in future studies. As it is not feasible for every child to have an available ACB unit, studies of allogeneic cord blood should also be pursued.

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Author Contributions

J.M.S. and J.K.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of the manuscript; A.W.S.: collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of the manuscript; L.E.C.: collection and/or assembly of data, final approval of the manuscript; M.A.M.: conception and design, collection and/or assembly of data, final approval of the manuscript; K.E.G.: conception and design, collection and/or assembly of data; R.S.: data analysis and interpretation, final approval of the manuscript; R.G. and G.W.: conception and design, collection and/or assembly of data, final approval of the manuscript; J.P., C.M., and B.W-P.: collection and/or assembly of data, final approval of the manuscript; L.W.C., S.W., and B.B.: data analysis and interpretation, final approval of the manuscript; J.T.: data analysis and interpretation, manuscript writing, final approval of the manuscript.

Disclosure of Potential Conflicts of Interest

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