Autologous Cord Blood Infusions Are Safe and Feasible in Young Children with Autism Spectrum Disorder: Results of a Single-Center Phase I Open-Label Trial

GERALDINE DAWSON,[a] JESSICA M. SUN ID,[b] KATHERINE S. DAVLANTIS,[a] MICHAEL MURIAS,[a,c] LAUREN FRANZ,[a] JESSE TROY,[b] RYAN SIMMONS,[b] MAURA SABATOS-DEVITO,[a] REBECCA DURHAM,[b] JOANNE KURTZBERG[b]

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[a]Duke Center for Autism and Brain Development, Duke University Medical Center, [b]Robertson Clinical and Translational Cell Therapy Program, Duke University Medical Center, [c]Duke Institute for Brain Sciences, Duke University Medical Center, Durham, North Carolina, USA

Correspondence: Jessica M. Sun, M.D., Robertson Clinical and Translational Cell Therapy Program, Duke University School of Medicine, DUMC 3850, 2400 Pratt Street, Durham, North Carolina 27705, USA. Telephone: 919-668-1102; Fax: 919-668-7161; e-mail: jessica.sun@duke.edu

ABSTRACT

Despite advances in early diagnosis and behavioral therapies, more effective treatments for children with autism spectrum disorder (ASD) are needed. We hypothesized that umbilical cord blood-derived cell therapies may have potential in alleviating ASD symptoms by modulating inflammatory processes in the brain. Accordingly, we conducted a phase I, open-label trial to assess the safety and feasibility of a single intravenous infusion of autologous umbilical cord blood, as well as sensitivity to change in several ASD assessment tools, to determine suitable endpoints for future trials. Twenty-five children, median age 4.6 years (range 2.26–5.97), with a confirmed diagnosis of ASD and a qualified banked autologous umbilical cord blood unit, were enrolled. Children were evaluated with a battery of behavioral and functional tests immediately prior to cord blood infusion (baseline) and 6 and 12 months later. Assessment of adverse events across the 12-month period indicated that the treatment was safe and well tolerated. Significant improvements in children's behavior were observed on parent-report measures of social communication skills and autism symptoms, clinician ratings of overall autism symptom severity and degree of improvement, standardized measures of expressive vocabulary, and objective eye-tracking measures of children's attention to social stimuli, indicating that these measures may be useful endpoints in future studies. Behavioral improvements were observed during the first 6 months after infusion and were greater in children with higher baseline nonverbal intelligence quotients. These data will serve as the basis for future studies to determine the efficacy of umbilical cord blood infusions in children with ASD. STEM CELLS TRANSLATIONAL MEDICINE 2017;00:000–000

SIGNIFICANCE STATEMENT

This phase I study demonstrates that it is safe and feasible to perform autologous umbilical cord blood infusions in young children with autism spectrum disorder and identifies several promising outcome measures for use in future trials.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social communication and the presence of repetitive behaviors and a restricted range of activities, with onset early in life. ASD is estimated to affect approximately 1 in 68 children in the U.S. [1]. The majority of individuals with ASD are not able to live independently and require lifelong support or accommodations. Accordingly, the lifetime cost of supporting an individual with ASD is estimated to be $1.4 million. The cost is $2.4 million for those who also have an intellectual disability [2].

Treatment approaches for ASD include medication, behavioral therapy, occupational and speech therapies, and specialized educational and vocational support. Early intensive behavioral intervention is associated with substantially improved outcomes [3], but even with such intervention, many individuals with ASD remain significantly impaired. All of the currently available medical treatments, such as psychotropic medications, are intended to ameliorate associated comorbid symptoms, such as irritability, but do not address core autism symptoms. In light of this, there is a large unmet need for more effective treatments targeting core symptoms of ASD.

Both genetic and environmental factors contribute to the etiology of ASD [4–6]. Although the exact pathophysiology is unknown, observations have included abnormal synaptic functioning in areas of the brain [7, 8], white matter abnormalities [9], and neuroinflammation [10]. Pathogenesis of immune pathology in the brains of patients with ASD may be due to overexpression of immune-related gene networks [11], presence of maternal antibodies to fetal brain tissue [12], atypical levels of proinflammatory cytokines (IL-6, TNF-α) in the cerebral spinal fluid [13], and excessive microglial activation leading to aberrant neural connectivity pathways [14, 15]. As such, therapeutic approaches impacting immune modulation or regulation of neural connectivity are logical targets for novel treatments for this population. Preclinical models have shown that umbilical cord blood contains effector cells that, through paracrine signaling, alter brain connectivity and also suppress inflammation [16, 17]. Infusions of autologous cord blood cells have been shown to be safe in patients with cerebral palsy and other acquired brain injuries [18–20]. We hypothesized that infusions of autologous cord blood cells could play an important role in the treatment of ASD and conducted a single center, open-labeled, phase I safety and feasibility trial in young pediatric participants. The study focused on (a) the safety of a single intravenous infusion of autologous umbilical cord blood and (b) the sensitivity to change and feasibility of administration of several different assessment tools in young children with ASD.

MATERIALS AND METHODS

Study Design and Overview

This study was a phase I, single-center, open-label trial of a single intravenous infusion of autologous umbilical cord blood in 25 children with ASD. All children were initially enrolled on a screening protocol to obtain medical records and information about their banked cord blood unit. All participants' caregivers completed a prestudy screening interview by phone and provided medical records and videos for review by the study team to determine eligibility for the trial. Children with a confirmed diagnosis of ASD and a qualified banked autologous umbilical cord blood unit were eligible to participate. Written informed consent was obtained for both the screening and the treatment phases of the trial. The trial was approved by the Duke Hospital Institutional Review Board and conducted under IND #15949.

Participants and their caregivers traveled to Duke University three times as part of their participation in the study. At their baseline visit, they were evaluated and received a single intravenous autologous cord blood infusion. At 6 and 12 months post-infusion, participants returned for follow-up clinical assessments. Additional caregiver interviews and questionnaires were collected at 3 and 9 months post-infusion.

Participants

Participants between 2 and 5 years of age who met criteria for a clinical diagnosis of ASD based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [21] were eligible for study inclusion. The DSM-5 diagnosis of ASD was established by expert clinicians and informed by the Autism Diagnostic Observation Schedule (ADOS), Second Edition [22] and the Autism Diagnostic Interview, Revised (ADI-R) [23]. Additional inclusion criteria included (a) a nonverbal intelligence quotient (IQ) of ≥ 35 on the Stanford-Binet Intelligence Scales for Early Childhood, Fifth Edition [24] or Mullen Scales of Early Learning [25], (b) availability of a qualified autologous umbilical cord blood unit, (c) participant

was stable on their current medications for at least 2 months prior to the infusion, (d) ability to travel to Duke University three times (baseline and 6 and 12 months post-baseline), and (e) parents were English speaking. Exclusion criteria included (a) a history of prior cell therapy, (b) use of intravenous immunoglobulin or other anti-inflammatory medications (with the exception of NSAIDs), (c) known genetic (e.g., fragile X) or other significant medical comorbidity, (d) obvious physical dysmorphology suggestive of a genetic syndrome, (e) an uncontrolled seizure disorder, (f) significantly impaired renal or liver function, and (g) clinically significant abnormalities in complete blood count.

Umbilical Cord Blood Units

All participants had to have an available autologous umbilical cord blood unit banked at a family or public cord blood bank. During screening, potential participants' cord blood reports were reviewed to ensure they met the following pre-cryopreservation criteria: (a) total nucleated cell count (TNCC) $\geq 1 \times 10^7$/kg, (b) sterility cultures which were preformed and negative, (c) negative maternal infectious disease markers tested on the maternal donor or cord blood product (minimally including hepatitis B, hepatitis C, human immunodeficiency virus [HIV], human T-lymphotrophic virus [HTLV], and syphilis), and (d) test sample available for additional testing. If the participant and their cord blood unit were likely to be eligible, a sample of the cord blood unit was shipped to Duke for potency testing [26]. Low-resolution HLA testing was performed on both the participant and a sample of the cord blood unit for identity confirmation. If CD45 viability on the test sample was >40% and HLA-identity was confirmed, the cryopreserved cord blood unit was shipped in a dry shipper to Duke Stem Cell Transplant Laboratory, where it was stored under liquid nitrogen until the day of infusion.

Procedures

Autologous Umbilical Cord Blood Infusion

On the day of infusion, the cord blood was thawed and washed in dextran 40 + 5% albumin (DA) and placed in 1.25 ml/kg DA for administration [27]. Thawed cord blood units were tested for enumeration of TNCC, viable CD34+ cells, colony-forming units (CFUs), cell viability via trypan blue, and sterility cultures. The autologous umbilical cord blood infusion was performed following a sedated brain magnetic resonance imaging scan (MRI). Intravenous (IV) access was obtained by a pediatric anesthesiologist. When the MRI was complete, children were admitted to the Duke Children's Health Center Day Hospital, an outpatient treatment center, for their infusion. After premedication with Benadryl (0.5 mg/kg IV), Solu-Medrol (0.5 mg/kg IV), and, if the child was awake and able to take oral medications, Tylenol (10 mg/kg PO), participants received either a portion of or their entire cord blood unit, adjusted to deliver 1–5 $\times$ 10^7 cells per kilogram, via peripheral IV infusion over 2 to 30 minutes. Intravenous fluids were administered at 1.5 times maintenance for 30 minutes to 2 hours after the cord blood infusion. Vital signs and pulse oximetry were monitored continuously during the infusion and until the child awoke from sedation.

Safety Evaluation Criteria

Participants were observed during the infusion and monitored for infusion reactions. Additional adverse events (AE) were identified through phone interviews with participants' parent/guardian at 7–10 days, 3 months, and 9 months after infusion, and in person at the baseline, 6- and 12-month clinic visits. 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.

Clinical Assessments

Multiple assessments were used to determine both feasibility of administration and utility as an endpoint for future phase II and III clinical trials. These included the Vineland Adaptive Behavior Scales-II (VABS-II), Clinical Global Impression Scale (CGI), Pervasive Developmental Disorder Behavior Inventory (PDDBI), Expressive One-Word Picture Vocabulary Test-4 (EOWPVT-4), Behavior Assessment for Children-Social Skills subscale, Aberrant Behavior Checklist, Sensory Experiences Questionnaire, Repetitive Behavior Scale, Intelligence Scales (Mullen Scales of Early Learning or Stanford-Binet), Language Environment Analysis, Preschool Age Psychiatric Assessment, Aberrant Behavior Checklist, ATN GI Symptoms Inventory, and Parenting Stress Index. In addition, three objective biomarkers were collected: Eye Gaze Tracking of Social Stimuli (EGT), EEG, and brain MRI. EEG and brain MRI findings will be reported separately. Outcomes of measures that were chosen a priori as a primary behavioral endpoint (VABS-II Socialization Subscale Standard Score) and as key secondary behavioral endpoints (CGI, PDDBI, EOWPVT) and of the EGT biomarker are included in this report.

The Vineland Adaptive Behavior Scales-II (VABS-II) [28] is a caregiver questionnaire that is used to assess children's adaptive behavior across a wide range of domains. The VABS-II is a well-standardized measure with strong reliability and validity [29–32] which yields an overall composite score, as well as subscale standard scores in the following domains: Socialization, Communication, Daily Living Skills, and Motor Skills. The VABS-II was collected from each participant's primary caregiver at the baseline and 6- and 12-month visits. The Socialization Subscale Score was used to measure improvements in the core ASD symptom of social behavior.

The Clinical Global Impression (CGI) is a commonly used rating scale that measures symptom severity and treatment response or change in behavior between time points. Two versions of the CGI were used: CGI-Severity (CGI-S) and CGI-Improvement (CGI-I). The CGI-S is a 7-point scale indicating the severity of each participant's symptoms of ASD at the time of assessment, relative to the expert rater's past experience with participants who have the same diagnosis. Based on the expert rater's lifetime clinical experience and all available information, each participant was rated as 1: not present (no ASD), 2: ASD symptoms barely evident, 3: mild ASD symptoms, 4: moderate ASD symptoms, 5: moderately severe ASD symptoms, 6: severe ASD symptoms, or 7: very severe ASD symptoms. Each participant was assigned a CGI-S rating at the baseline and 6 and 12 month visits. The CGI-I is a 7-point scale indicating the degree of improvement or worsening of ASD symptoms relative to baseline. Based on all available information, each participant was rated as 1: very much improved, 2: much improved, 3: minimally improved, 4: no change, 5: minimally worse, 6: much worse, or 7: very much worse. Each participant was assigned a CGI-I rating at the 6- and 12-month visits, and each referenced the degree of improvement or worsening relative to baseline. All CGI-S and CGI-I ratings were made by highly experienced clinicians with expertise in ASD.

The PDDBI [33] is a caregiver questionnaire that is designed to measure social behavior, adaptive functioning, and maladaptive functioning in areas typically affected by ASD. The PDDBI was standardized with a sample of caregivers and teachers of children with ASD from a range of racial, ethnic, and socioeconomic backgrounds [34]. The PDDBI was collected from each participant's primary caregiver at the baseline, 6- and 12-month visits, as well as remotely at 3 and 9 months post-baseline.

The EOWPVT-4 [35] is a clinician-administered assessment which measures an individual's ability to match a spoken word with an image of an object, action, or concept. The EOWPVT-4 was administered to each child at the baseline and 6- and 12-month visits.

A task designed to measure visual attention to social versus nonsocial stimuli via EGT was administered. EGT is a technology that enables quantification of gaze patterns of individuals from infancy through adulthood. The EGT hardware [36] uses infrared light-emitting diodes and infrared cameras to measure corneal reflections, which are used to calculate eye gaze direction. During the EGT task, participants watched a 4-minute video of dynamic social stimuli, which includes episodes of an actress presenting bids for joint attention [37]. Using these stimuli, prior studies have found that young children with ASD show decreased attention both to the entire scene and to the actress's face during bids for joint attention. Decreased attention to the entire scene was also correlated with autism symptom severity [37]. The EGT task was presented to each child at the baseline, 6- and 12-month visits.

Information about the number of hours that children were involved in behavioral, speech-language, occupational, and other behavioral therapies and educational services the child received was assessed every three months from baseline to 12 months post-baseline via a structured Intervention History Interview with the parent.

Statistical Methods

The analysis relied primarily on descriptive methods, beginning with a summary of the baseline characteristics of the cohort. Box plots were prepared to illustrate the distribution of continuous outcome measures over time. The frequency of ordinal outcome measures at each time point was plotted using bar charts. Statistical significance of change on continuous and ordinal outcomes was assessed using the Wilcoxon signed rank test except for the PDDBI, which was modeled using a fixed effect linear spline with knot at 3 months. This model was chosen over other longitudinal fixed and random effects models using the Akaike Information Criteria. EGT was analyzed using Generalized Estimating Equations (GEE) with logit link, binomial error structure, and exchangeable or unstructured working correlation. The association of baseline age, nonverbal IQ, and infused cell dose with change over time was explored in each analysis using Spearman correlation (r_s). There were not enough females enrolled to explore patterns of change by sex. Finally, we evaluated the potential for false-positive results by applying the Benjamini-Hochberg False Discovery Rate (FDR) procedure to the observed results for the baseline-to-6-month and 6-to-12-month follow-up periods.

Participants

Twenty-five participants (21 males, 4 females), majority white ($n = 22$, 1 Asian, 2 mixed race), were enrolled with a median age of 4.62 years (range 2.26–5.97) and median nonverbal IQ of 65 (range 22–123). The median ADOS comparison score of the participants at study entry was 8.0 (range 6–10), and 72% had moderately severe or severe ASD symptoms (Table 1). All participants completed the baseline and 6-month assessments. Three participants did not complete the 12-month assessment.

Table 1. Baseline characteristics of patients and autologous cord blood units ($n = 25$)

| Patient characteristics	
Sex, no. (%)	
Female	4 (16.0%)
Male	21 (84.0%)
Age, years, median (range)	4.62 (2.26–5.97)
Race, no. (%)	
White	22 (88%)
Other	3 (12%)
Ethnicity, no. (%)	
Hispanic	2 (8%)
Not Hispanic	23 (92%)
ADOS Severity Score, median (range)	8 (6–10)
Nonverbal intelligence quotient, median (range)	65 (22–123)
CGI-S, no. (%)	
Barely evident	4 (16.0%)
Moderate ASD symptoms	3 (12.0%)
Moderately severe ASD	10 (40.0%)
Severe ASD symptoms	8 (32.0%)
Cord blood characteristics, median (range)	
Total cells infused, $\times 10^8$	4.42 (1.53–12.28)
Cell dose infused, $\times 10^6$/kg	25.80 (9.97–80.80)
Viable CD34+ dose infused, $\times 10^5$/kg	0.3 (0.1–4.2)
CFU dose infused, /kg	1,225.50 (85.50–4,620.00)

Abbreviations: ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; CFU, colony-forming units; CGI-S, Clinical Global Impression-Severity; TNCC, total nucleated cell count.

Umbilical Cord Blood Infusions

Autologous umbilical cord blood units were retrieved from two U.S. family cord blood banks and one public bank ($n = 1$). All were stored in dual compartment bags. To achieve the target cell dose of $1–5 \times 10^7$ TNCC/kg, the entire cord blood unit was used in six participants. In 19 participants, the 80% compartment of the cord blood unit was thawed and used for infusion and the remaining 20% portion was maintained in the cryopreserved state and stored, with the parents' permission, for potential future use. All patients completed their cord blood infusion. Characteristics of the thawed cord blood product administered to the patient are shown in Table 1. The median TNCC and viable CD34 cell doses infused were 2.6×10^7/kg (range $1–8 \times 10^7$) and 0.3×10^5/kg (range $0.1–4.2 \times 10^5$), respectively. The median CFUs infused was 1225.5/kg (range 85.5–4620). Although the dosing criteria used in this study were consistent with our previous studies utilizing privately banked cord blood units, these TNCC, CD34, and CFU values are lower than our prior experience [18]. Despite negative pre-cryopreservation sterility cultures reported by the bank, one unit grew coagulase negative staphylococcus from a post-thaw sample at Duke.

Safety

The primary endpoint of this open label phase I trial was safety (Fig. 1). As previously reported in our prior safety study of autologous cord blood infusion in children with neurologic disorders [18], autologous cord blood infusion was well-tolerated. There were no serious AEs reported in any participant. A total of 92 AEs were reported in 23 participants (Fig. 1) with a median of three events per participant (range: 1–15). All events were graded as Mild (71 events) or Moderate (21 events). Twelve events (13%) were considered related to the infusion, with the most common being allergic reaction, manifested by urticartia and or/cough occurring on the day of infusion (5 events in 4 participants; all
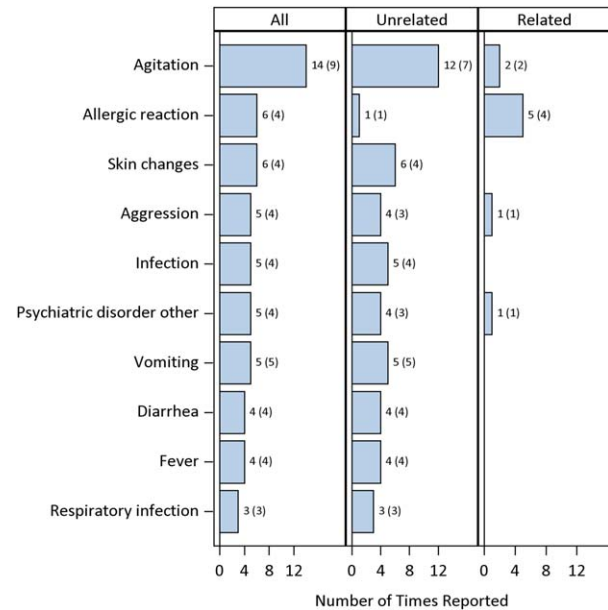
Figure 1. Frequency of adverse events. Number of patients reporting an event is listed in parentheses.

Mild; 2 requiring an additional dose of IV Benadryl). The most common unrelated AEs were agitation, skin changes, and typical childhood infections, reported between 2 days and 1 year post-infusion. There were no infusion-related infections or bloodstream or serious infections noted in any patient.

Behavioral Testing

We also tested the feasibility of administration and described results of several measures typically used to assess behavioral outcomes in children with ASD. Multiple parent-rated and clinician-rated measures were evaluated. Behavioral outcomes correlated with baseline IQ, but not age or cell dose. Changes in behavior were also not correlated with the number of hours of behavioral interventions, speech-language therapy, occupational therapy or educational hours the child received during the tenure of the study. The measures chosen, a primary and key secondary behavioral endpoints (VABS-II, CGI-S, GCI-I, PDDBI, EOWPBT) and the EGT biomarker measure, all demonstrated improvements and are described below.

The VABS-II is parent-report measure that assesses socialization, communication, and adaptive behaviors. Figure 2 shows the distribution of standard scores in all patients (Panel A) and stratified by IQ (Panel B) for the VABS-II Socialization domain in the 24 participants who completed the assessments at all three time points. A statistically significant increase in standard score was observed from baseline to 6 months. This change was stable from 6 to 12 months. Change was positively correlated with nonverbal IQ in the Socialization ($r_s = .57$, 95% CI: 0.20–0.79, $p = .004$) and Adaptive Behavior ($r_s = .42$, 95% CI: 0.01–0.70, $p = .04$) domains, but not in the Communication domain ($r_s = .22$, 95% CI: −0.21 to 0.57, $p = .31$).

The CGI-S and –I are clinician-rated measures used to assess the severity and change in severity of the core symptoms of ASD over time. Figure 3 shows the distribution of CGI-S (Panel A) and CGI-I (Panel B) in 22 participants who were fully evaluable at all time points. In the CGI-S, at baseline, the majority of participants were classified as Moderately Severe (43.5%) or Severe (26.1%),
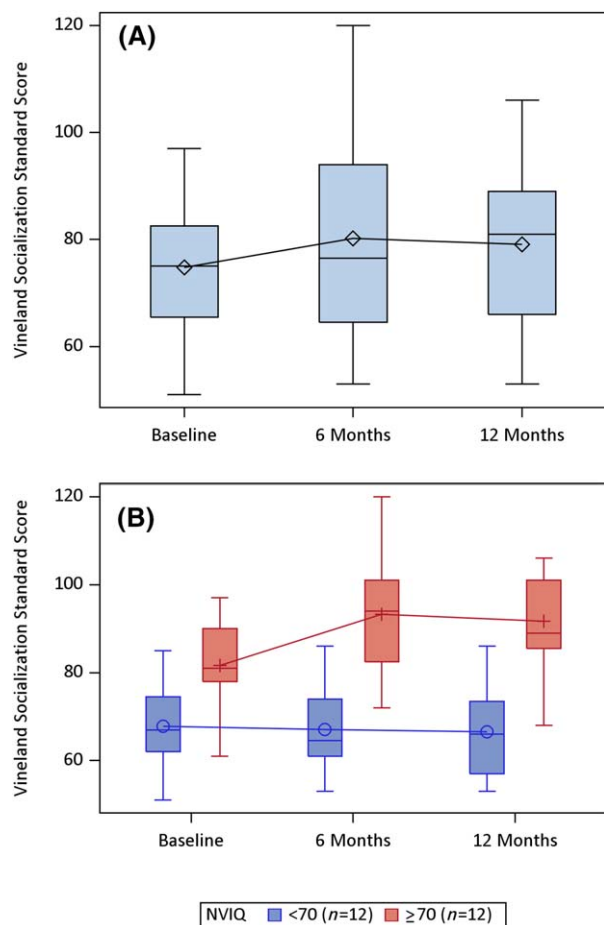
Figure 2. Vineland Adaptive Behavior Scales-II (VABS-II) Socialization Standard Score. **(A)**: Distribution of VABS-II Socialization Standard Score in all participants over time. **(B)**: Distribution of VABS-II Socialization Score stratified by nonverbal intelligence quotient.

and the remaining participants had Moderate or Barely Evident ASD symptoms (13.6% each). At 6 months, the proportion of participants with Moderately Severe and Severe symptoms decreased (22.7% each), with the remaining participants classified as Moderate (31.8%), Mild (13.6%), or Barely Evident (9.1%). Figure 3B shows the distribution of CGI-I at 6 and 12 months. The improvement measured at each of these time points is relative to baseline. At 6 months, 9 participants (40.9%) had not exhibited any change, whereas 2 (9.1%) were Minimally Improved, 8 (36.4%) were Much Improved and 3 (13.6%) were Very Much Improved ($p < .001$). The CGI-I at 12 months was similar ($p = .001$), although 2 participants (13.6%) were rated as Minimally Worse than baseline, whereas no participants were in this category at the 6-month assessment. The CGI-I at 12 months was associated with nonverbal IQ, but not age or infused cell dose (not shown).

The PDDBI—Autism Composite T-Score is a parent reported measure assessing ASD symptoms. The PDDBI was administered at baseline, 3, 6, 9, and 12 months (Table 2). The Autism Composite T-Score declined over time, suggesting an improvement in ASD symptoms. The majority of the change occurred in the interval from baseline to 3 months, with a predicted mean decline of 7.52 points (95% CI: −12.38, −2.67; $p = .004$). There was no significant change from 3 to 12 months (mean: 0.72, 95% CI: −1.14, 2.57; $p = .43$).
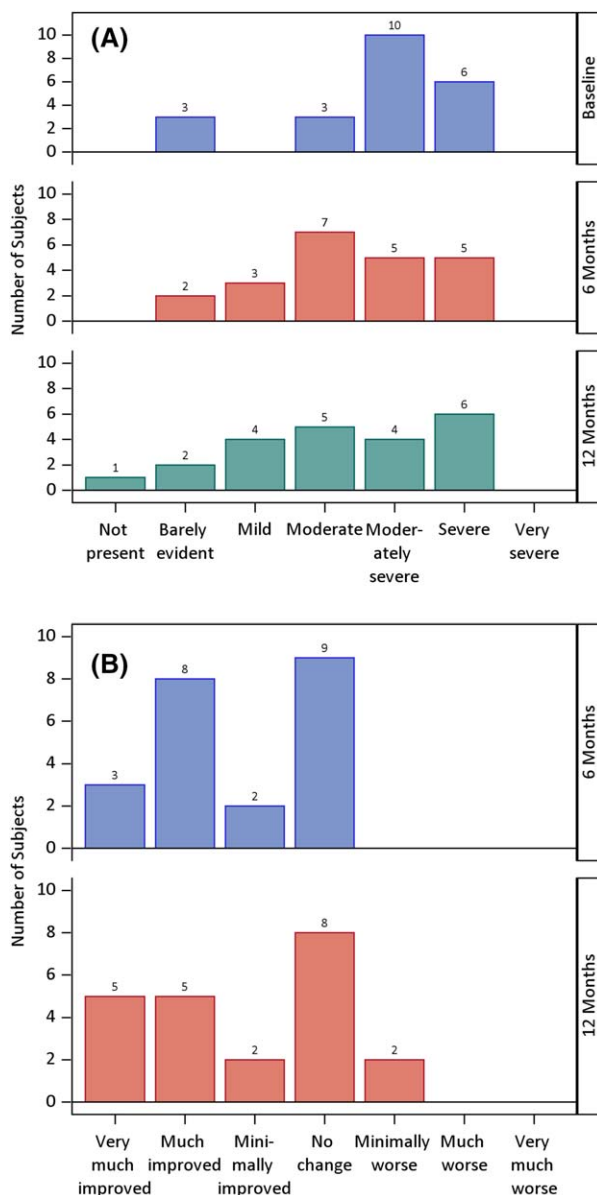
Figure 3. Global Impression Scale (GCI). **(A)**: CGI-Severity over time. **(B)**: CGI-Improvement over baseline as assessed at 6 and 12 months.

The EOWPVT is a clinician-administered assessment of the ability to match a spoken word with a picture. The EOWPVT raw score improved in 57% of patients between baseline and 6 months and in 68% of patients between 6-12 months (Table 2). Change in the EOWPVT raw score was associated with nonverbal IQ (baseline to 6 months: $r_S = .59$, 95% CI: 0.23, 0.80, $p = .002$; 6–12 months: $r_S = .55$, 95% CI: 0.15, 0.78; $p = .009$), but not age or infused cell dose ($p > .05$ for both).

Eye-Gaze Tracking is a computerized test where the participants' eye movements are tracked by a computer while the subject views a naturalistic, dynamic social stimulus (actress making bids for engagement) surrounded by various nonsocial stimuli on a monitor. Attention was measured toward four targets (actress's eyes, mouth, face, and upper body) and separate GEE models were fit for each target. Each model included 21 participants who were measured at baseline, 6, and 12 months. These models

Table 2. Summary of behavioral assessments

		Baseline to 6 months		6 to 12 months	
Outcome measure	No. of patients evaluated	Change score median (Range)	p value	Change score median (Range)	p value[a]
VABS Socialization Standard Score	24	2.0 (−8, 30)	.016	0 (−19, 9)	.602
VABS Communication Standard Score	24	4.5 (−8, 20)	.002	0.0 (−13, 13)	.459
VABS Adaptive Behavior Composite Standard Score	24	3 (−3, 24)	.007	0 (−12, 8)	.687
VABS Motor Standard Score	24	0 (−10, 7)	.788	0 (−14, 16)	.991
VABS Daily Living Standard Score	24	1 (−9, 34)	.457	0 (−16, 16)	.999
EOWPVT Raw Score	22	4 (−1, 24)	.001	5.5 (−12, 16)	.001
PDDBI Autism Composite T-Score[b]	25	7.52 (−12.38, −2.67)	.004	0.72 (−1.14, 2.57)	.430

[a]p values are from the Wilcoxon signed rank test or spline model for PDD-BI.
[b]The PDD-BI was collected at baseline, 3, and 6 months. Change scores are the predicted mean (and 95% confidence interval) from a linear spline model with knot at 3 months.
Abbreviations: EOWPVT, Expressive One-Word Picture Vocabulary Test-4; VABS, Vineland Adaptive Behavior Scales-II; PDDBI, Pervasive Developmental Disorder Behavior Inventory.

Table 3. Summary of eye tracking studies ($n = 21$)

Target	Odds ratio (95% CI)[a]	p value
Eyes	1.20 (1.00, 1.43)	.048
Actress	1.02 (0.92, 1.12)	.716
Mouth	0.93 (0.81, 1.06)	.270
Face	1.02 (0.91, 1.14)	.800

[a]Odds ratios are estimated using Generalized Estimating Equations (one model for each target) and reflect the average trend in the cohort between successive 6-month follow-up periods (baseline to 6 months, and 6 to 12 months).

showed a 20% increase in odds of gazing at the actress' eyes over time (OR = 1.20, 95% CI: 1.00, 1.43, $p = .048$). There were no significant changes in gaze at the other three targets (Table 3). Examination of the relation between eye-tracking and the VABS-II socialization standard score revealed that a 7-point change in VABS-II socialization standard score was associated with a 14% increase in odds of gazing at the actress (OR = 1.14, 95% CI: 1.07,1.21; $p < .001$).

Adjustment for Multiple Testing

Given the large number of behavioral tests in this study, we explored the possibility of false positive results among the nine behavioral outcome measures by applying the FDR method to the first and second 6-month follow-up periods separately (Table 4). All of the outcome measures that showed significant results during the first 6 months of follow-up remained statistically significant after FDR adjustment of p values. During the period from 6 to 12 months, the EOWPVT and CGI-I remained statistically significant after application of the FDR procedure.

DISCUSSION

In this phase I open-label study, we evaluated the safety and feasibility of a single intravenous infusion of autologous umbilical cord blood in young children with ASD. We also described changes in various behavioral and functional outcome measures to determine which would be best suited for use as endpoints in future cell therapy trials. Assessments of AEs over the 12 months post-infusion indicated that the cord blood infusion was safe and well tolerated. All related events were considered expected and resolved without sequelae. The most common unrelated AEs were agitation, skin changes, and typical childhood infections. Agitation, in particular, had not been a common side effect in our

prior studies of autologous cord blood infusions in children with other acquired neurologic conditions, and thus may be specific to children with ASD. In this study, participants underwent sedation for an MRI immediately prior to their cord blood infusion. The increased incidence of agitation may thus reflect the challenges of waking from sedation and having an IV and pulse oximeter in place for a child with ASD, and as such may be related to their underlying condition.

Significant improvements in behavior were found across a wide range of outcome measures in this study. These included improvements in parent-reported measures including the VABS-II Socialization, Communication, and Adaptive Behavior Scores and the PDDBI, clinician assessments including the CGI-S, CGI-I, and EOWPVT, and objective eye gaze tracking measurements. Most of the observed behavioral changes occurred during the first 6 months and were sustained between 6 and 12 months post-infusion. A robust finding was that children's nonverbal IQ was correlated with change for the majority of outcomes measures, with higher nonverbal IQ being associated with greater improvements in behavior.

Of note, the majority of participants in this study were white, reflecting the demographic in the U.S. likely to have the resources and to choose to bank their baby's umbilical cord blood in a private bank. However, as ASD occurs in children of all demographic backgrounds, if cord blood therapy is effective then access would be limited to families with resources for private banking if only autologous cells are used. Accordingly, our next study will test the best available donor (autologous or allogeneic) versus placebo to lay the groundwork to extend access to this therapy for all affected children, if found to be effective.

While these results provide some promise for future work with cord blood-derived therapies in ASD, it is important to note the limitations of this study. As an uncontrolled open-label study, it is not possible to determine whether the observed behavioral changes were due to the treatment or reflect the natural course of development during the preschool period. A recent longitudinal study of Swedish children ages 1.5 to 3 years with ASD demonstrated a mean improvement of 3.0 points on the VABS Communication scale and 1.0 point on the VABS Socialization scale over a 2-year period [38], versus 4.5 points and 2.0 points, respectively, over a 6-month time period in the cord blood-treated patients in this study. However, potential cultural variations as well as different timing of assessments make it difficult to directly compare

Table 4. Raw and corrected *p* values for tests of the null hypothesis of No change over time in behavioral outcomes

	Baseline to 6 months		6 to 12 months	
Outcome measure	Raw *p* value	FDR *p* value	Raw *p* value	FDR *p* value
EOWPVT Raw Score	.0001	.0009	.0011	.0059
CGI-I	.0010	.0045	.0013	.0059
VABS Communication Standard Score	.0020	.0060	.4590	.8262
PDDBI Autism Composite T-Score[a]	.0040	.0090	.4300	.8262
VABS Adaptive Behavior Composite	.0070	.0126	.6870	.8833
VABS Socialization Standard Score	.0160	.0240	.6020	.8833
CGI-S	.0220	.0283	.3750	.8262
VABS Daily Living Standard score	.4600	.5175	.9999	.9999
VABS Motor Function Standard Score	.7880	.7880	.9907	.9999

[a]*p* values for the PDDBI are for baseline to 3 months and 3 to 12 months.
Abbreviations: CGI-I/S, Clinical Global Impression—Improvement/Severity; EOWPVT, Expressive One-Word Picture Vocabulary Test-4; FDR, False Discovery Rate; PDDBI, Pervasive Developmental Disorder Behavior Inventory; VABS, Vineland Adaptive Behavior Scales-II.

these two groups. Additionally, the small sample size in this study makes it difficult to fully assess the contributions of confounding variables. While we did not find a correlation between behavioral changes and age, amount of behavioral intervention services or infused cell dose, the limited sample size and restricted age and dosing ranges may reduce the ability to detect such associations.

Each outcome measure described above showed sensitivity to change, indicating its potential usefulness in larger trials. Furthermore, attrition due to noncompliance on these measures was minimal, suggesting that these measures are feasible with children with ASD in this age range. Using the results of this study as a guide, we selected the VABS Socialization Standard Score as the primary outcome measure for our next study with administration by trained clinicians to reduce parental expectancy effects. This score provides a validated measure of core social behaviors relevant to autism, showed sensitivity to change over a 6-month period of time in the current study, and is feasible in a larger clinical trial. In keeping with recent draft guidance on design of autism clinical trials from the European Medicines Agency [39], we recognize that a single therapy may not improve all autism symptoms and that global functional improvement is thus an important component of efficacy assessment in autism. Therefore, we have also included the clinician-rated CGI and additional measures as secondary endpoints in our next study, a phase II, double-blind randomized clinical trial designed to formally evaluate the efficacy of umbilical cord blood infusion in improving core symptoms of ASD.

CONCLUSION

We demonstrated in an open label, phase I trial that intravenous infusion of autologous umbilical cord blood in young children with ASD is safe and feasible. We describe significant improvements in behavior observed in the first 6 months post-infusion and sustained at 12 months. Higher baseline nonverbal IQ was associated with a greater degree of improvement. We identified outcome measures that are feasible, sensitive to change, and developmentally-appropriate, and thus are suitable for use in future clinical trials to test the efficacy of cord blood therapy for the treatment of young children with ASD.

AUTHOR CONTRIBUTIONS

G.D., J.M.S., and J.K.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; K.S.D.: conception and design, collection and/or assembly of data, manuscript writing; M.M.: conception and design, collection and/or assembly of data, data analysis and interpretation; L.F.: collection and/or assembly of data, manuscript writing; J.T.: data analysis and interpretation, manuscript writing; R.S.: data analysis and interpretation. M.S.V.: collection and/or assembly of data. R.D.: conception and design.

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