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A Phase II Randomized Clinical Trial of the Safety and Efficacy of Intravenous Umbilical Cord Blood Infusion for Treatment of Children with Autism Spectrum Disorder

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Objective To evaluate whether umbilical cord blood (CB) infusion is safe and associated with improved social and communication abilities in children with autism spectrum disorder (ASD).

Study design This prospective, randomized, placebo-controlled, double-blind study included 180 children with ASD, aged 2-7 years, who received a single intravenous autologous (n = 56) or allogeneic (n = 63) CB infusion vs placebo (n = 61) and were evaluated at 6 months postinfusion.

Results CB infusion was safe and well tolerated. Analysis of the entire sample showed no evidence that CB was associated with improvements in the primary outcome, social communication (Vineland Adaptive Behavior Scales-3 [VABS-3] Socialization Domain), or the secondary outcomes, autism symptoms (Pervasive Developmental Disorder Behavior Inventory) and vocabulary (Expressive One-Word Picture Vocabulary Test). There was also no overall evidence of differential effects by type of CB infused. In a subanalysis of children without intellectual disability (ID), allogeneic, but not autologous, CB was associated with improvement in a larger percentage of children on the clinician-rated Clinical Global Impression-Improvement scale, but the OR for improvement was not significant. Children without ID treated with CB showed significant improvements in communication skills (VABS-3 Communication Domain), and exploratory measures including attention to toys and sustained attention (eye-tracking) and increased alpha and beta electroencephalographic power.

Conclusions Overall, a single infusion of CB was not associated with improved socialization skills or reduced autism symptoms. More research is warranted to determine whether CB infusion is an effective treatment for some children with ASD. *(J Pediatr 2020;■:1-10)*.

Autism spectrum disorder (ASD) is characterized by impaired social communication and restricted, repetitive behaviors. Behavioral interventions improve outcomes[1]; however, many individuals with ASD have lifelong impairments. Food and Drug Administration-approved medicines for ASD can improve associated irritability but do not address core autism symptoms. Thus, there is a large unmet need for effective ASD treatments.

The etiology of ASD involves genetic and environmental factors.[2] Atypical glutamatergic and GABAeric synaptic function may contribute to abnormal excitatory/inhibitory balance.[3] Abnormal immune function has been described.[4,5]

AE	Adverse event	ID	Intellectual disability
ASD	Autism spectrum disorder	IV	Intravenous
CB	Cord blood	IND	Investigational new drug
CGI-I	Clinical Global Impression-Improvement	MRI	Magnetic resonance imaging
		NVIQ	Nonverbal IQ
CGI-S	Clinical Global Impression-Severity	PANDAS	Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection
CNS	Central nervous system		
DSM-5	Diagnostic and Statistical Manual, 5th ed. American Psychiatric Association	PDDBI	Pervasive Developmental Disorder Behavior Inventory
EEG	Electroencephalography	SAE	Serious adverse event
EOWPVT	Expressive One-Word Picture Vocabulary Test	SS	Standard Score
		TNC	Total nucleated cell
FDR	False Discovery Rate	VABS-3	Vineland Adaptive Behavior Scales, Third Edition
HLA	human leukocyte antigen		

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Increased plasma cytokine levels, up-regulated genes associated with microglial activation, and localized inflammation and astrocyte activation have all been associated with ASD.[6,7] We hypothesized that an infusion of cord blood (CB) would facilitate neural cell protection/repair, reduce inflammation, and thus improve social communication. The rationale is that CB $CD14^+$ monocytes act through paracrine signaling to modulate brain inflammation and/or immune abnormalities, improving brain function and behavior.[8] In animal models of brain injury and cerebral palsy, xenogeneic human CB improves motor function.[9] Improvements in autistic-like behaviors have been reported in ASD mouse models after administration of bone marrow cells or mesenchymal stromal cells.[10,11] A placebo-controlled, crossover study of autologous CB treatment in children with ASD found no serious adverse events (SAEs) and no behavioral effects.[12] An unblinded, controlled trial of combined human CB mononuclear cells and umbilical cord-derived mesenchymal stem cells found improved ASD symptoms.[13]

We conducted an open-label phase I study with 25 children with ASD, aged 2-6 years, to examine the safety and efficacy of a single intravenous (IV) infusion of autologous CB (ClinicalTrials.gov: NCT02176317). The safety profile observed was good, and improvements in social communication were found by 6 months postinfusion.[14] Children with higher IQ exhibited greater improvement. Children showed improved attention[14] and increased electroencephalography (EEG) alpha and beta power posttreatment,[15] which was notable because ASD has been characterized by reduced EEG alpha power, hypothesized to reflect abnormal GABAergic tone.[16] The present study is a phase II randomized, double-blind study that evaluated the safety and efficacy of autologous and allogeneic CB treatment vs placebo.

Methods

This was a single-site, prospective, randomized, double-blind study of a single IV autologous or allogeneic unrelated CB infusion vs placebo in children aged 2-7 years with ASD. Outcomes were assessed at baseline and at 6 months after the initial infusion. Safety and caregiver reports of the children's behavior were assessed remotely at 12 months postinfusion. Written informed consent was obtained for screening and treatment phases of the trial (ClinicalTrials.gov: NCT02847182), which was approved by the Duke Hospital Institutional Review Board, conducted under IND #15949. The study began September 2016; the last participant was examined for the primary outcome in August 2018. The trial protocol is available from the authors on request.

Participants

One hundred eighty children, aged 2-7 years (mean ± SD, 5.47 ± 1.65) who met DSM-5 criteria for ASD participated. Diagnosis was based on the Autism Diagnostic Observation Schedule-2[17] and Autism Diagnostic Interview, Revised.[18] Participants were screened for a genetic cause of ASD with testing for Fragile X and chromosomal microarray. Inclusion criteria included (1) negative genetic testing, (2) qualified CB unit with a minimum banked total nucleated cell dose of $\geq 2.5 \times 10^7$ cells/kg or ≥4/6 HLA-matched allogeneic unrelated CB unit, (3) stable on medications for ≥2 months, (4) ability to travel to study site twice, (5) English speaking, and (6) normal absolute lymphocyte count (≥1500/μL). Exclusion criteria included (1) known diagnosis of depression, bipolar disorder, schizophrenia, obsessive compulsive disorder, or Tourette syndrome; (2) known genetic syndrome or pathogenic mutation or copy number variation associated with ASD; (3) known CNS infection and/or HIV positivity; (4) known metabolic disorder, mitochondrial dysfunction, seizure disorder, primary immunodeficiency disorder, autoimmune cytopenias, active or prior malignancy treated with chemotherapy, significant sensory impairment, or impaired renal or liver function; (5) current or prior cell therapy, use of IV immunoglobulin or other anti-inflammatory medication (except nonsteroidal anti-inflammatory drugs), and/or immunosuppressive therapy; and (6) child unlikely to be able to complete assessments. Two pilot participants and 2 participants who were found to be ineligible after randomization (**Figure 1**) were excluded from the analysis. Characteristics of randomized participants are shown in **Table I** (available at www.jpeds.com). One hundred thirty participants (72.2%) had an Nonverbal IQ (NVIQ) ≥55, and 50 (27.8%) had an NVIQ <55.

Randomization and Masking

Participants were randomly assigned to either Sequence A—CB at baseline followed by placebo infusion—or Sequence B—placebo infusion at baseline followed by CB infusion. Thus, participants who received placebo were treated with CB after the primary outcome was measured. Participants with a qualified autologous CB unit received autologous cells, and those without a suitable autologous CB unit received cells from a ≥4/6 HLA-matched, allogeneic, unrelated CB donor. Randomization was 2:1 CB:placebo, stratified by age (<5 and ≥5 years), NVIQ (<55 vs ≥55), and CB type. The randomization table was generated by RTI International (Research Triangle Park, Durham, North Carolina). Blinded treatment codes were allocated using Medidata Rave (Medidata Solutions, New York, New York). Only designated, unblinded staff were aware of the participant's randomized treatment assignment. During infusion, the syringe was obscured with a label, the IV tubing was covered with a brown plastic bag, and the catheter insertion site was wrapped with CoFlex tape (Andover Healthcare, Portsmouth, New Hampshire). The placebo (TC199 + 1% DMSO) had a similar appearance and odor as a CB unit.

Procedures

Allogeneic CB units were obtained from the Carolinas Cord Blood Bank at Duke University, a Food and Drug Administration–licensed public CB bank and a member of the National Cord Blood Inventory of the CW Bill Young Cell Transplantation Program. Autologous units were

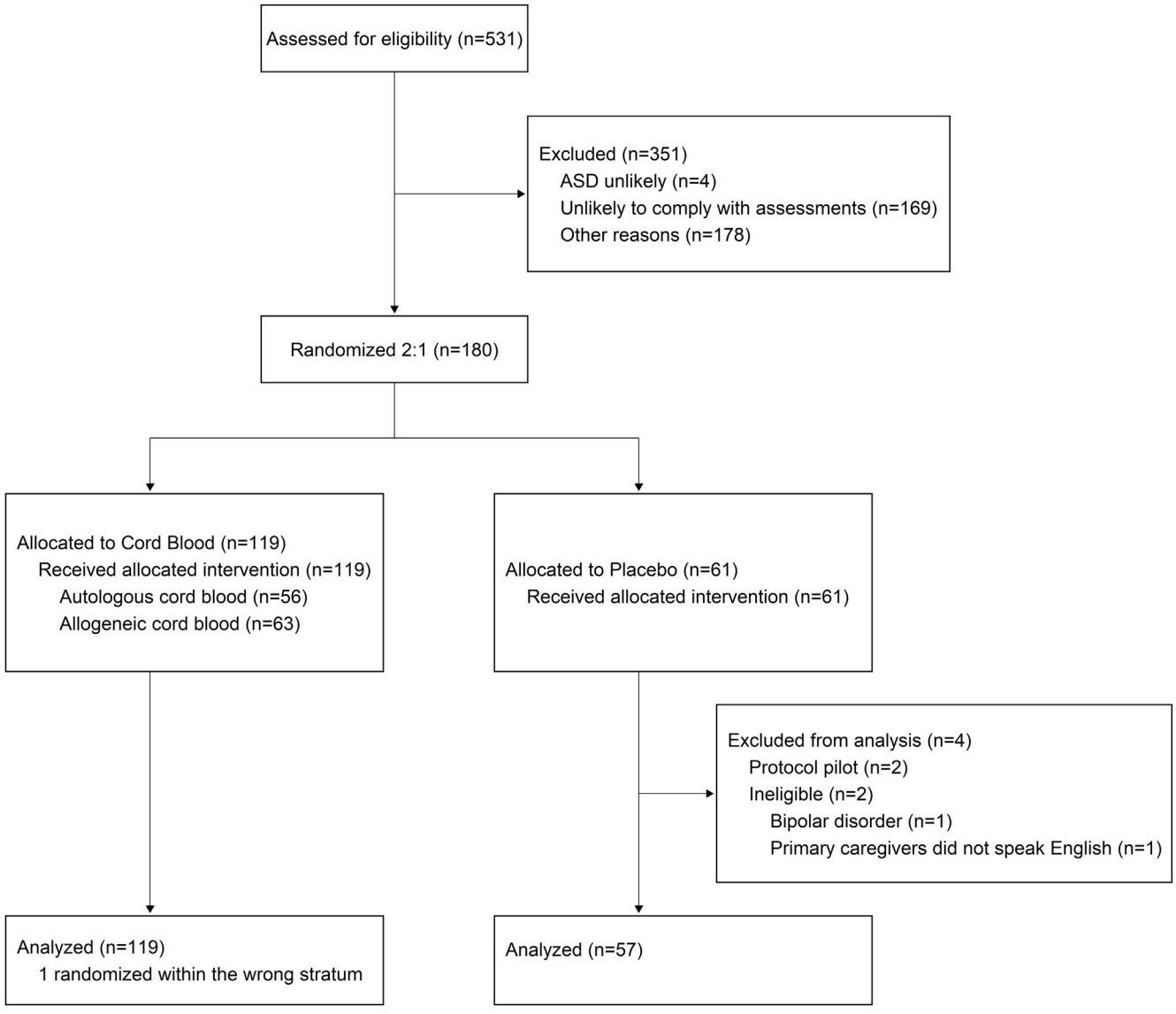

Figure 1. CONSORT diagram of the study.

obtained from the private CB bank used to store the child's CB. All CB units met the following pre-cryopreservation criteria: (1) total nucleated cell count (TNC) count $\geq 2.5 \times 10^7$/kg, (2) sterility cultures performed and negative, (3) negative maternal infectious disease markers tested on the maternal donor or CB product (ie, minimally hepatitis B, hepatitis C, HIV, human T-lymphotropic virus, and syphilis), and (4) test sample available for potency and identity testing. The CB units were shipped to Duke for potency testing.[19] HLA testing was performed on the participant and a sample of the CB unit for identity confirmation for the autologous CB units and to ensure a ≥4/6 HLA loci match for the allogeneic participants. Matching at HLA-A, -B, -C, and -DRB1 was obtained, and matching at 1 or more loci at -A, -B, and -DRB1 was required for allogeneic units. Potency testing was performed on a sample from the CB unit and required at a minimum a post-thaw CD34 viability of 70%. For eligible participants, the cryopreserved CB unit was sent in a dry shipper to Duke Stem Cell Transplant Laboratory and stored under liquid nitrogen until the day of infusion.

CB Infusion

CB units were thawed and washed in dextran 40 + 5% human serum albumin and placed in 1.25 mL/kg dextran 40 + 5% albumin for administration. Thawed CB units were tested for enumeration of TNC count, viable $CD34^+$ cells, percent $CD34^+$ cell viability, colony-forming units, cell viability via Trypan blue, and sterility cultures. Participants received infusions of unfractionated CB mononuclear cells, including cells of the monocyte lineage, which are believed to be the cells with possible therapeutic potential in this heterogeneous

cell product. The infusion was performed following a sedated brain magnetic resonance imaging (MRI). IV access was obtained by a pediatric anesthesiologist before the MRI. Oral and/or nasal midazolam were used before IV placement.

After undergoing MRI, the child was transferred to an outpatient unit for infusion. After premedication with diphenhydramine (0.5 mg/kg IV) and methylprednisolone (0.5 mg/kg IV), and, if the child was awake and able to take oral medications (acetaminophen 10 mg/kg orally), they received either a portion of or the entire CB unit, adjusted to deliver $\geq 2.5 \times 10^7$ cells/kg, via peripheral IV infusion over 2-30 minutes. IV fluids were administered at 1.5 times maintenance for 30 minutes to 2 hours after the CB infusion. Vital signs and pulse oximetry were monitored continuously during the infusion and until the child awoke from sedation.

Safety Evaluation Criteria

The children were observed and monitored during the infusion and assessed in person the day following the infusion. A call was conducted 2 weeks postinfusion. Adverse events (AEs) were documented through a caregiver medical and behavioral questionnaire that queried for new or worsening AEs at 3, 6, 9, and 12 months postinfusion; endorsed items were clarified via a phone call. Information regarding concomitant medications was collected monthly. Verbatim AE terms were mapped onto standard terminology defined by the Common Terminology Criteria for Adverse Events version 4.0 and summarized according to severity and relationship to the intervention as judged by the investigator.

Clinical Assessments

Clinical outcomes were assessed using validated measures that are normed and have adequate test-retest reliability. The Vineland Adaptive Behavior Scales, Third Edition (VABS-3),[20] a well-standardized caregiver interview measuring domains of adaptive functioning, socialization, communication, daily living skills, and motor skills, was administered at baseline, at 6 months, and remotely at 12 months. Reliability with a gold standard rater was maintained at ≥90%. When possible, interviewers and informants were kept consistent. The Pervasive Developmental Disorder Behavior Inventory (PDDBI),[21] a caregiver report assessing social, language, and learning/memory skills and problem behaviors, was administered at baseline, at 6 months, and remotely at 12 months. The Clinical Global Impression-Severity (CGI-S) Scale was rated by clinicians at baseline and 6 months, with separate scores for social communication, restricted and repetitive behaviors, and overall functioning, with 1 indicating "normal" and 7 indicating "extreme impairment." At 6 months, CGI-Improvement (CGI-I) ratings indicated improvement or worsening, ranging from 1, "very much improved", to 7, "very much worse." Participants completed the Expressive One-Word Picture Vocabulary Test-4 (EOWPVT)[22] at baseline and 6 months later.

Biomarker Assessments: Eye-Tracking and EEG

Attention to dynamic stimuli was measured at baseline and 6 months via eye-tracking.[23] Participants watched a movie that included specific episodes: "Actress with Dyadic Bid," in which an actress surrounded by stationary silent toys engaged in child-directed speech, and "Actress with Moving Toys," in which an actress looked at toys that moved and made noise. The Dyadic Bid condition assessed attention to social (actress) vs nonsocial (toys) stimuli. Actress with Moving Toys assessed sustained attention to complex, audiovisual stimuli. Children with ASD tend to have shorter look duration to dynamic audiovisual stimuli.[24]

EEG recordings were collected at baseline and at 6 months while the child watched "social" (woman saying nursery rhymes) vs "nonsocial" (brightly colored, sound-making dynamic toys) videos. EEG was recorded from 124 electrodes with reference to Cz using a Hydrocel Geodesic Sensor Netand Net Amps 400 amplifierusing Net Station 4.5.6 (EGI/Phillips, Eugene, Oregon) with a sampling rate of 1000 Hz. EEG data were filtered with a 1- to 100-Hz bandpass filter and a 58- to 62-Hz bandstop filter. Data were decomposed using second-order blind identification implemented in EEGLAB and re-referenced to common average. Fast Fourier transformation was performed on the rectangular windowed time series. Sufficient artifact-free EEG data at both baseline and 6 months were available for 127 participants for the social video and for 137 participants for the toys video.

Other Treatments Received During the Trial

Information was collected from caregivers regarding other supplemental therapies that the children received, including the number of hours of behavioral intervention (in or outside of school). There were no differences between treatment groups in the amount or types of additional therapies or in the total number of behavioral intervention hours ($P = .19$).

Outcomes

All within-participant comparisons were between baseline and 6 months, except for CGI, which was at 6 months. The primary outcome measure was change in VABS-3 Socialization Standard Score (SS). Among the multiple secondary outcome measures (**Table II**) considered key were changes in VABS-3 Communication SS, PDDBI Autism Composite, CGI-S, CGI-I, and EOWPVT-4 raw score. Exploratory biomarker measures were brain connectivity via MRI (not included), eye-tracking, and EEG. Eye-tracking measures were change in the proportion of total time spent looking at actress vs toys during Dyadic Bid and change in sustained attention (average look duration) during Actress with Moving Toys and across both episodes (total viewing time divided by the number of periods of sustained attention). Owing to known age-related changes in absolute EEG power, changes in relative power were analyzed in theta (5-7 Hz), alpha (8-10 Hz), beta1 (11-20 Hz), beta2 (21-30 Hz), and gamma (31-70 Hz) bands from EEGs collected from averaged electrodes from 3 scalp regions of interest (frontal, central, and posterior) and across all brain

Table II. Summary of Prespecified efficacy analyses

Endpoints	Statistical test method	Observed test statistic	Critical value	*P* value	FDR *P* value	Point estimate (95% CI)
Vineland Socialization SS (primary)	ANOVA w/stratification factors	0.55	5.11	.4580	.924	1.02 (−1.67 to 3.71)*
Vineland Socialization SS by treatment and type of CB (primary)	ANOVA w/stratification factors	0.03	5.11	.8720	.924	−0.45 (−5.86 to 4.97)†
Vineland Communication SS	Two-sample *t* test	0.04	1.98	.9650	.965	0.07 (−2.95 to 3.08)*
Vineland Daily Living SS	Two-sample *t* test	−0.24	1.98	.8100	.924	−0.28 (−2.6 to 2.03)*
Vineland Adaptive Behavior Composite SS	Two-sample *t* test	0.27	1.98	.7910	.924	0.26 (−1.66 to 2.18)*
Vineland Motor Skills SS	Two-sample *t* test	0.87	1.99	.3840	.924	1.08 (−1.37 to 3.53)*
PDDBI Autism Composite T score	Two-sample *t* test	−0.35	1.98	.7290	.924	−0.52 (−3.51 to 2.46)*
PDDBI Repetitive, Ritualistic, and Pragmatic Problem Behaviors T score	Two-sample *t* test	−0.22	1.98	.8280	.924	−0.32 (−3.23 to 2.59)*
PDDBI Approach/Withdrawal Problems T score	Two-sample *t* test	−0.39	1.98	.6990	.924	−0.56 (−3.42 to 2.3)*
PDDBI Expressive Social Communication Abilities T score	Two-sample *t* test	0.53	1.98	.6000	.924	0.46 (−1.29 to 2.22)*
PDDBI Receptive/Expressive Social Communication Abilities T score	Two-sample *t* test	0.86	1.98	.3920	.924	0.71 (−0.93 to 2.35)*
CGI Severity Socialization Communication Score	Wilcoxon-Mann-Whitney *U* test	−0.83	1.96	.4050	.924	1.1 (0.81-1.5)‡
CGI Severity Restricted, Repetitive Behaviors Score	Wilcoxon-Mann-Whitney *U* test	0.20	1.96	.8410	.924	0.94 (0.67-1.3)‡
CGI Severity Overall Score	Wilcoxon-Mann-Whitney *U* test	−1.20	1.96	.2290	.924	1.17 (0.85-1.61)‡
CGI Improvement Social Communication Score	Wilcoxon-Mann-Whitney *U* test	0.66	1.96	.5080	.924	0.86 (0.61-1.21)‡
CGI Improvement Restricted, Repetitive Behaviors Score	Wilcoxon-Mann-Whitney *U* test	0.31	1.96	.7540	.924	0.92 (0.65-1.29)‡
CGI Improvement Overall Score	Wilcoxon-Mann-Whitney *U* test	0.39	1.96	.6950	.924	0.9 (0.64-1.28)‡
EOWPVT SS	Two-sample *t* test	−0.65	1.99	.5160	.924	−0.99 (−4 to 2.03)*
Vineland Socialization SS by Treatment and NVIQ	ANOVA	0.03	5.11	.8720	.924	0.49 (−5.47 to 6.45)†

FDR, false discovery rate.
*Change from baseline to 6 months in CB vs placebo.
†Estimate (β) for interaction term from ANOVA.
‡Odds (CB < placebo).

regions.[25] Exploratory clinical measures were changes in VABS-3 Socialization raw score and age equivalent, VABS-3 Communication age equivalent, VABS-3 Daily Living Skills SS and age equivalent, VABS-3 Adaptive Behavior Composite SS, and PDDBI subscale T scores.

Statistical Analyses

Sample size was calculated using PASS version 12.0 (NCSS, LLC., Kaysville, Utah) to support the evaluation of 2 primary hypotheses, with type I error controlled by Bonferroni correction: (1) CB is associated with greater positive changes in Socialization SS relative to placebo, and (2) there would be a differential treatment effect according to the type of CB (autologous or allogeneic). A total of 144 participants randomized to CB or placebo at a 2:1 were required for 80% power to detect a standardized effect size (Cohen *d*)[26] of 0.55, assuming a 2-sided type I error of 2.5%. This equates to a mean difference of 3.7 points in the change score for the VABS-3 Socialization SS comparing randomized groups, assuming high correlation ($r = 0.9$) between baseline and 6-month measures with a SD of 15 at each time point. This sample size was estimated to provide ∼80% power to detect differential treatment effects by type of CB infused (interaction term for randomized group crossed with available CB type [autologous vs allogeneic]) using ANOVA when the differential treatment effect was large (Cohen $f = 0.4$)[26] with 2-sided type I error of 2.5%.

A reassessment of our distributional assumptions underlying the sample size calculation was done in a prespecified, blinded interim analysis that examined the primary outcome variance and correlation between repeated measures from 75% of participants. To account for possible dropout, the target accrual was set at 165 participants (110 assigned to the CB arm and 55 assigned to the placebo arm). Accrual was expanded to 180 participants owing to the enrollment of an unexpectedly high number of participants with NVIQ <55. (Lower IQ was associated with reduced improvement in our previous open-label trial.[14]) One participant assigned to the CB arm had an NVIQ of 58 but was incorrectly randomized within the NVIQ <55 stratum. The modified intention-to-treat population comprised 176 participants, including 119 assigned to the CB arm (56 with autologous CB and 63 with allogeneic CB) and 57 assigned to the placebo arm. The prespecified efficacy analysis used ANOVA to compare the 6-month change in VABS Socialization SS between the CB and placebo arms, with adjustment for the randomization stratification factors. ANOVA analyses of type of CB (autologous or allogeneic) were used with an interaction term, randomized treatment (CB or placebo) crossed with type of CB. These analyses were conducted in 176 of the 180 participants (**Figure 1**).

The Benjamini-Hochberg false discovery rate (FDR) method was used to evaluate differences between randomized groups on primary and secondary efficacy outcome measures. The analysis plan included prespecified exploratory analysis of treatment effect modified by IQ, which was included in the FDR adjustment. The safety analyses included all 180 participants. The severity of AEs was classified using Common Terminology Criteria for Adverse Events version 4. Infusion reactions were considered related to the study

product. Other events were coded as related based on investigator judgment and summarized according to MedDRA system organ class and preferred term. Post hoc analyses of the primary and secondary outcome measures by participant IQ based on the presence or absence of ID (<70 vs ≥70) were conducted. ANCOVA models were fit in which the 6-month score was regressed on the baseline value and other covariates, as appropriate. Logistic regression was used to compare the odds of improvement (1, 2, or 3) on the CGI-I associated with CB (autologous, allogeneic, or both combined) vs placebo after adjustment for covariates.

Eye-tracking and EEG biomarker measures were analyzed via ANCOVA. EEG data were log-transformed to correct for skewness. Biomarker measures were also analyzed separately for participants without ID (IQ ≥70). We report *P* values for prespecified primary and secondary analyses for which type I error control was considered at design. Results of all prespecified and post hoc exploratory analyses are reported using descriptive statistics and confidence intervals or graphical displays. Type I error was not controlled for in exploratory analyses.

Results

A total of 531 participants were screened, of whom 180 were enrolled and randomized between September 13, 2016, and February 26, 2018 (**Figure 1** presents a CONSORT diagram of the trial). Given the influence of participant IQ on the degree of improvement in our open-label trial,[14] we intended to enroll a minimum of 144 participants with NVIQ ≥55 and to be able to conduct post hoc analyses with participants with IQ ≥70. During screening, NVIQ was estimated through a remote review of records, and participants were eligible regardless of actual tested NVIQ once they arrived at the study site. This strategy resulted in 101 participants rather than the projected 144 meeting the higher IQ threshold. This possibly compromised the analysis of the primary endpoint, which was based on the assumption of a larger sample of participants without moderate to severe ID.

Baseline characteristics were well-balanced between the randomized groups (**Table I**). However, the prevalence of ID was not balanced between participants receiving autologous CB (35.7%) and those receiving allogeneic CB (53.4%). Furthermore, cell dose was lower with autologous CB (median, 26.88×10^6/kg; range, 15.14-57.57 $\times 10^6$/kg) compared with allogeneic CB (median, 38.45×10^6/kg; range, 20.68-64.16 $\times 10^6$/kg), as expected (**Table III**; available at www.jpeds.com).

Primary and Key Secondary Clinical Outcomes

Results revealed a large expectancy effect for the primary and secondary outcome measures, which were generally greatest in younger children with ID. There was no significant effect of treatment with CB vs placebo for the primary trial outcome; the mean 6-month change in VABS-3 Socialization SS was 3.13 ± 8.76 in the CB group and 1.98 ± 8.41 in the placebo group ($F_{1,171} = 0.55$; $P = .458$, adjusted for randomization strata), with no differential treatment effect based on NVIQ (<55 vs ≥55) ($\beta_{interaction} = 0.49$; 95% CI, −5.47 to 6.45) (**Table II**). There was no evidence of treatment effects by the type of CB infused (autologous or allogeneic) (test of interaction: $F_{1,170} = 0.03$; $P = .872$). There were no significant main effects of treatment group for the key secondary outcome measures comparing the 2 groups (**Table II**).

There was a significant interaction between treatment group and NVIQ for the Communication SS in ANCOVA models (**Tables IV** and **V**; available at www.jpeds.com). Participants with NVIQ ≥70 treated with either type of CB had a 6-month Communication Score that was 5.45 points higher than those treated with placebo (95% CI, −0.08 to 10.23). The equivalent interaction between NVIQ and treatment in the model for the Socialization SS was 1.09 points (95% CI, −4.32 to 6.50).

Improvement based on the CGI-I in the entire cohort was high: 53.4% in all participants, 50.1% in the placebo arm, and 54.7% in the CB arm. The treatment group difference was not modified by ID using a logistic regression model. The OR for improvement comparing CB with placebo for those with NVIQ <70 was 1.03 (95% CI, 0.37-2.82). The same OR for participants with NVIQ ≥70 was 1.43 (95% CI, 0.62-3.33) (test of interaction: $\chi^2_{df=1} = 0.247$). The results of a descriptive analysis of CGI-I by NVIQ and type of CB infused are shown in **Table VI** (available at www.jpeds.com). There was a large between-group difference in the percentage of participants showing improvement (76.9% in the CB arm vs 57.1% in the placebo arm) in participants with NVIQ ≥70 (**Figure 2**); however, there was uncertainty in this estimate (OR, 2.36; 95% CI, 0.80-6.96).

Infused dose was not associated with the primary and secondary outcomes, with the exception of the CGI. The allogeneic cohort received a higher TNC dose compared with the autologous cohort (3.8×10^7/kg vs 2.7×10^7/kg) making it impossible to determine whether the effect in the allogeneic group was due to the higher cell dose or to CB type.

Exploratory Clinical Outcomes

Tables IV and **V** present a descriptive analysis of all VABS SSs according to NVIQ and type of CB infused. The mean change in each domain of the VABS was generally higher in participants with NVIQ ≥70 than in those with NVIQ <70. Differences between each type of CB compared with placebo were larger in the higher NVIQ group, with a slight advantage for autologous vs allogeneic CB. Intersubject variability was high across all VABS domains.

Eye-Tracking

Data were available for 172 participants from the modified intention-to-treat population. The reasons for missing eye tracking data are shown in **Table VII** (available at www.jpeds.com). The dependent variable was the proportion of total viewing time during which the participant viewed the

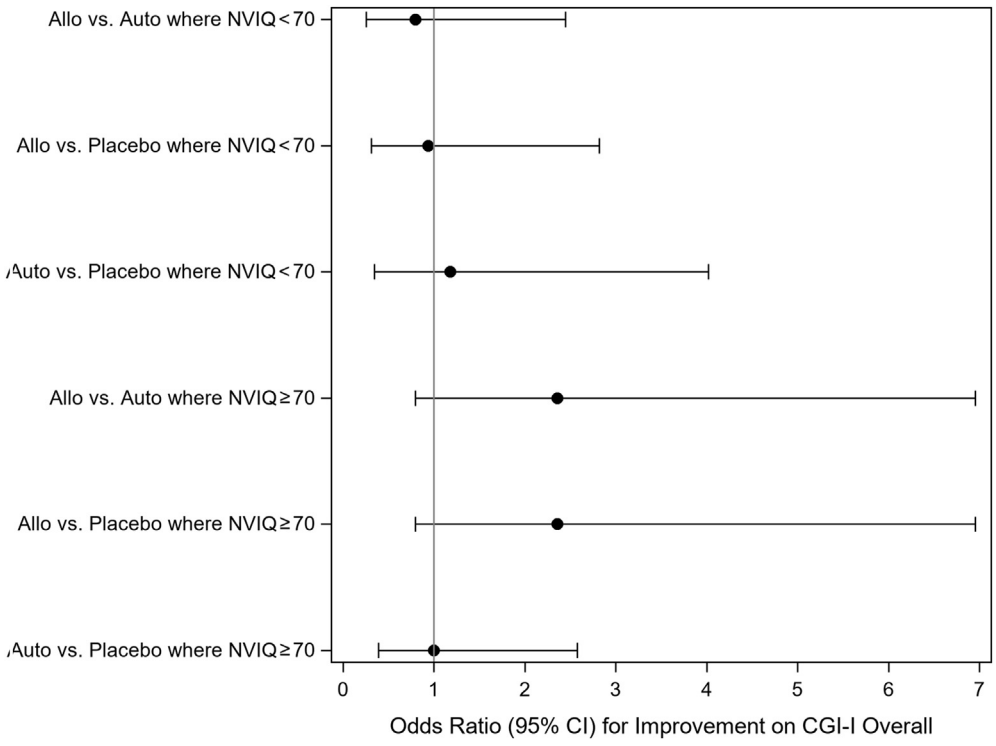

Figure 2. Results of logistic regression comparing odds of improvement on the Clinical GIS-I by type of CB and NVIQ.

region of interest. The baseline and 6-month measures were included in the outcome vector with the randomized group, time point, and group-by-time interaction as covariates. Participants had greater odds of gazing at the toys during the Dyadic Bid episode 6 months after treatment with CB compared with placebo (OR, 1.43; 95% CI, 1.15-1.78).

The mean look duration during the Actress with Moving Toys and Dyadic Bid episodes, and the average of the 2 episodes, were analyzed using ANCOVA-type models for normally distributed outcomes that included a 3-way interaction: group-by-time-by-NVIQ. During the Actress with Moving Toys episode, participants treated with CB had a significantly longer sustained attention (mean look duration) compared with placebo, especially those participants with NVIQ ≥70 (**Figure 3** and **Table VIII**; available at www.jpeds.com). Change in mean look duration over time during the Actress with Moving Toys episode was highly variable among participants with lower NVIQ. Averaged across the 2 episodes, there were divergent trends in mean look duration for participants treated with CB compared with placebo for participants with NVIQ ≥70 (**Figure 4**; available at www.jpeds.com).

EEG

Our analyses revealed a main effect between treatment groups, with participants receiving CB exhibiting significantly lower beta2 $\text{power}_{\text{posterior/social}}$ ($P = .026$). However, there was also a significant NVIQ-by-treatment group interaction ($P = .035$). Our results indicated that the subgroup of participants with lower NVIQ who received CB exhibited significant reductions in beta2 $\text{power}_{\text{posterior/social}}$ ($P = .009$). There were no additional significant effects of CB treatment for EEG power for analyses conducted with the whole group.

When participants with NVIQ ≥70 were analyzed separately, the results indicated that participants without ID who were treated with CB exhibited significantly increased relative alpha $\text{power}_{\text{posterior/toys}}$ ($P = .02$) and significantly increased relative beta1 $\text{power}_{\text{all brain regions/social}}$ ($P = .02$) compared with the placebo group (**Figure 5**). No main effects were found for theta or gamma power.

AEs

SAEs in the first 12 months and infusion reactions and psychiatric AEs in the first 6 months are summarized in **Table IX** (available at www.jpeds.com). There were 6 SAEs reported in 6 unique participants, including 3 in the placebo arm (viral gastroenteritis, dehydration, and aggression), 1 in the autologous CB cohort (concussion), and 2 in the allogenic CB cohort (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection [PANDAS] and dehydration). None of these events was related to the study product, and all resolved with no sequelae except for concussion and PANDAS, which were still being treated at the end of the study. No deaths, graft-versus-host-disease, alloimmunization, or product-related infections were reported. A total of 535 non-serious AEs were reported during the 6-month period (485 mild, 45 moderate, and 5 severe). Most were typical

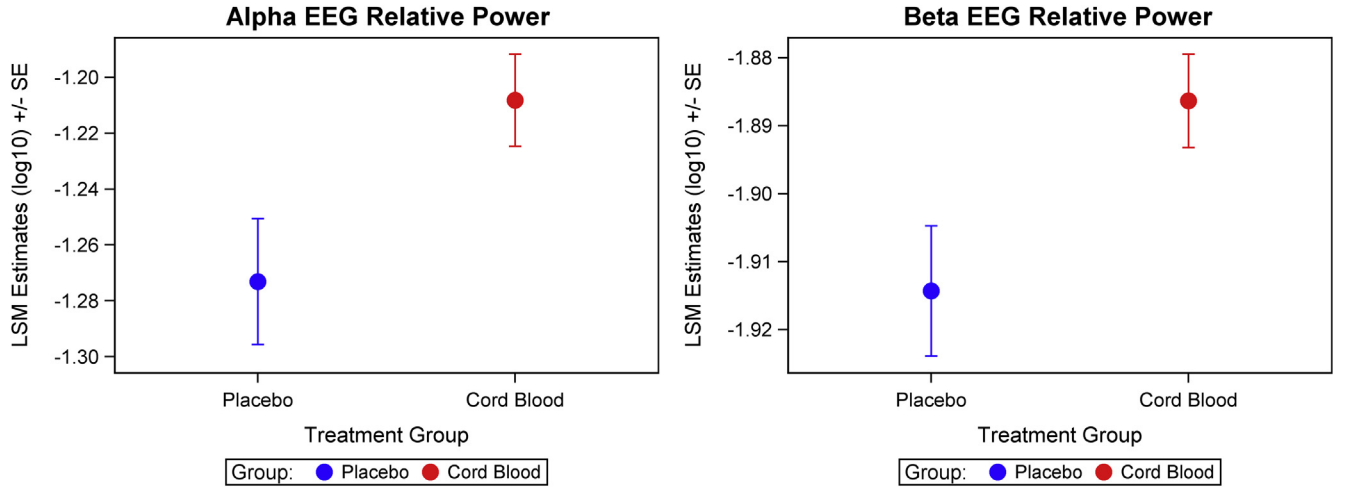

Figure 5. Relative EEG alpha and beta power outcomes at 6 months for participants with NVIQ ≥70. Relative EEG spectral power at the 6-month outcome based on ANCOVA where the 6-month scores shown in the graph were regressed on the baseline value. (Left) Relative alpha EEG power (posterior region, toys video). (Right) Relative beta 1 EEG power (all brain regions, social video).

childhood infections or surgical procedures (eg, ear tube implantation, tonsillectomy) and were expected. The overall frequency of events was similar in the 2 arms, 81.5% in the CB arm and 83.6% in the placebo arm. Infusion reactions occurred in 4 participants in the placebo group (6.6% of participants; all mild) and in 12 participants in the CB group (10%: 5 mild, 3 moderate, and 4 severe). The frequency of infusion reactions was higher in the allogeneic CB cohort (14.3%; 9 of 63) compared with the autologous CB cohort (5.4%; 3 of 56), and all severe infusion reactions, characterized by bronchospasm and/or facial flushing and swelling, occurred in participants receiving allogeneic CB. One participant developed donor-specific HLA antibodies, detected at the 6-month visit before their second infusion. Worsening of symptoms associated with ASD were coded under the MedDRA Psychiatric Disorders system organ class. These types of events occurred in 29 participants (47.5%) in the placebo group and 55 (46.2%) in the CB group.

Discussion

In this double-blind, randomized, placebo-controlled study, we evaluated the safety and efficacy of a single infusion of autologous or allogeneic CB vs placebo for the treatment of ASD. Our results indicate that the CB infusion was safe and well-tolerated. Primary analyses indicated that a single infusion of CB was not associated with improvements in social communication skills, autism symptoms, vocabulary, or overall functioning. Secondary analyses suggested that in children with ASD without ID, CB treatment was associated with improved communication skills, increased sustained attention, and changes in brain activity, characterized by increases in EEG alpha and beta power.

We encountered challenges with the study design in 2 areas. First, the expectancy effect was substantially higher than expected for the primary and key secondary endpoints. This was most prominent in outcome measures based on caregiver report and in younger children with more severe ID. Second, our ability to detect a treatment effect might have been adversely influenced by the fact that the projected target accrual of participants without severe to moderate ID was not achieved.

For the sample as a whole, there was no significant effect of CB treatment on the primary outcome or evidence of differential effects by the type of CB infused. However, the mean 6-month change in VABS Socialization SS was larger in the CB group compared with the placebo group (3.13 vs 1.98) and met the threshold for a minimally clinically important difference.[27] There were also no significant overall treatment group differences in secondary efficacy outcome measures, including autism symptoms (PDDBI), clinician-rated CGI-I, and expressive vocabulary. However, for children without ID, clinician CGI ratings indicated that children treated with allogeneic CB, but not those treated with autologous CB, showed improvement over placebo. Children without ID also showed significantly greater improvement in a prespecified secondary outcome measure of VABS Communication SS when treated with CB compared with placebo.

Based on eye-tracking, at 6 months post-infusion, participants who received CB were more likely to spend time looking at the toys during the Actress with Dyadic Bid episode compared with those in the placebo group. This is in contrast to earlier findings in which CB treatment was associated with increased attention to the actress. It is possible the CB treatment increased the attention to stimuli that the children found interesting (toys) but did not affect a preference for social vs nonsocial stimuli. Participants without ID (≥70) treated with CB exhibited a significant increase in sustained

attention (mean look duration) during the Actress with Moving Toys episode compared with the placebo group. Studies have shown that children with ASD exhibit shorter look durations when looking at complex, dynamic audiovisual stimuli, which has been hypothesized to reflect overarousal.[24] The finding of increased look duration during the most stimulating episode suggests that CB treatment might enhance attention to complex, arousing stimuli in children with ASD without ID.

Children with ID treated with CB showed significant decreases in EEG beta power. Abnormally increased peak beta power has been found in children with Dup15q syndrome, which is characterized by ASD associated with ID.[28] In contrast, participants without ID treated with CB treatment exhibited increased posterior EEG alpha power and beta1 power across all brain regions. The findings in participants without ID parallel to a large extent those found in our open-label trial in which increases in EEG alpha and beta power posttreatment were observed.[15] An atypical pattern of significantly reduced alpha power is a consistent finding in studies of EEG brain activity in individuals with ASD.[16] In the present study, the finding of increased look duration to complex dynamic stimuli and increased EEG alpha power suggests that CB treatment might affect level of arousal. In a variety of conditions involving synaptopathies, it has been hypothesized that proinflammatory cytokines may affect the excitatory/inhibitory balance, leading to impaired synaptic function.[29] The finding of increased beta power could possibly be interpreted in terms of the association between increased beta oscillations and top-down control of attention.[30] We can speculate that these biomarkers signal early signs of efficacy in the present study.

In conclusion, this study illustrates the challenges of performing randomized, placebo-controlled studies in young children with ASD. The high expectancy effect in the placebo arm and the larger-than-anticipated number of participants with ID might have compromised study results. Lessons learned must inform the design and primary endpoint selection of future studies, which will need to consider the moderating influence of cognitive ability, effects of expectancy, and substantial variability across participants in clinical change over time. The results of the present study do not currently support the use of CB as a treatment for autism outside a formal or expanded access IND-sponsored clinical trial. Future research is warranted to determine whether CB is an effective treatment for autism. ■

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Data Statement

Data sharing statement available at www.jpeds.com.

References

1. Rogers SJ, Estes A, Lord C, Munson J, Rocha M, Winter J, et al. A multisite randomized controlled two-phase trial of the Early Start Denver Model compared to treatment as usual. J Am Acad Child Adolesc Psychiatry 2019;58:853-65.
2. de la Torre-Ubieta L, Won H, Stein JL, Geschwind DH. Advancing the understanding of autism disease mechanisms through genetics. Nat Med 2016;22:345-61.
3. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, et al. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 2011;477:171-8.
4. Takano T. Role of microglia in autism: recent advances. Dev Neurosci 2015;37:195-202.
5. Zantomio D, Chana G, Laskaris L, Testa R, Everall I, Pantelis C, et al. Convergent evidence for mGluR5 in synaptic and neuroinflammatory pathways implicated in ASD. Neurosci Biobehav Rev 2015;52:172-7.
6. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment. Neurotoxicol Teratol 2013;36:67-81.
7. Schork AJ, Won H, Appadurai V, Nudel R, Gandal M, Delaneau O, et al. A genome-wide association study of shared risk across psychiatric disorders implicates gene regulation during fetal neurodevelopment. Nat Neurosci 2019;22:353-61.
8. Saha A, Patel S, Xu L, Scotland P, Schwartzman J, Filiano AJ, et al. Human umbilical cord blood monocytes, but not adult blood monocytes, rescue brain cells from hypoxic-ischemic injury: mechanistic and therapeutic implications. PLoS One 2019;14:e0218906.
9. Drobyshevsky A, Cotten CM, Shi Z, Luo K, Jiang R, Derrick M, et al. Human umbilical cord blood cells ameliorate motor deficits in rabbits in a cerebral palsy model. Dev Neurosci 2015;37:349-62.
10. Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SB, Guyenet PG, et al. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. Nature 2012;484:105-9.
11. Segal-Gavish H, Karvat G, Barak N, Barzilay R, Ganz J, Edry L, et al. Mesenchymal stem cell transplantation promotes neurogenesis and ameliorates autism related behaviors in BTBR mice. Autism Res 2016;9:17-32.
12. Chez M, Lepage C, Parise C, Dang-Chu A, Hankins A, Carroll M. Safety and observations from a placebo-controlled, crossover study to assess use of autologous umbilical cord blood stem cells to improve symptoms in children with autism. Stem Cells Transl Med 2018;7:333-41.
13. Lv YT, Zhang Y, Liu M, Qiuwaxi JN, Ashwood P, Cho SC, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. J Transl Med 2013;11:196.
14. Dawson G, Sun JM, Davlantis KS, Murias M, Franz L, Troy J, et al. 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. Stem Cells Transl Med 2017;6:1332-9.
15. Murias M, Major S, Compton S, Buttinger J, Sun JM, Kurtzberg J, et al. Electrophysiological biomarkers predict clinical improvement in an open-label trial assessing efficacy of autologous umbilical cord blood for treatment of autism. Stem Cells Transl Med 2018;7:783-91.
16. Wang J, Barstein J, Ethridge LE, Mosconi MW, Takarae Y, Sweeney JA. Resting state EEG abnormalities in autism spectrum disorders. J Neurodev Disord 2013;5:24.

17. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. Autism diagnostic observation schedule (ADOS-2). 2nd ed. Torrance (CA): Western Psychological Services; 2012.
18. Rutter M, LeCouteur A, Lord C. Autism diagnostic interview-revised (ADI-R). Torrance (CA): Western Psychological Services; 2003.
19. Shoulars K, Noldner P, Troy JD, Cheatham L, Parrish A, Page K, et al. Development and validation of a rapid, aldehyde dehydrogenase bright-based cord blood potency assay. Blood 2016;127:2346-54.
20. Sparrow SS, Cicchetti DV, Saulnier CA. Vineland adaptive behavior scales. 3rd ed. (Vineland-III): Pearson; 2016.
21. Cohen IL, Sudhalter V. PDD behavior inventory (PDDBI) parent rating form. Lutz (FL): Psychological Assessment Resources; 2005.
22. Martin NA, Brownell R. Expressive one-word picture vocabulary test (EOWPVT-4). 4th ed. Novato (CA): Academic Therapy Publications; 2018.
23. Tobii Technology. Tobii TX300 eye tracker. Revision 2. Danderyd, Sweden: Tobii Technology AB; 2014.
24. Isaev D, Major S, Murias M, Carpenter KLH, Carlson D, Sapiro G, et al. Relative average look duration and its association with neurophysiological activity in young children with autism spectrum disorder. Sci Rep 2020;10:1912.
25. McEvoy K, Hasenstab K, Senturk D, Sanders A, Jeste SS. Physiologic artifacts in resting state oscillations in young children: methodological considerations for noisy data. Brain Imaging Behav 2015;9:104-14.
26. Cohen J. A power primer. Psychol Bull 1992;112:155-9.
27. Chatham CH, Taylor KI, Charman T, Liogier D'ardhuy X, Eule E, Fedele A, et al. Adaptive behavior in autism: minimal clinically important differences on the Vineland-II. Autism Res 2018;11:270-83.
28. Frohlich J, Reiter LT, Saravanapandian V, DiStefano C, Huberty S, Hyde C, et al. Mechanisms underlying the EEG biomarker in Dup15q syndrome. Mol Autism 2019;10:29.
29. Pozzi D, Menna E, Canzi A, Desiato G, Mantovani C, Matteoli M. The communication between the immune and nervous systems: the role of IL-1β in synaptopathies. Front Mol Neurosci 2018;11:111.
30. Stoll FM, Wilson CRE, Faraut MCM, Vezoli J, Knoblauch K, Procyk E. The effects of cognitive control and time on frontal beta oscillations. Cereb Cortex 2016;26:1715-32.

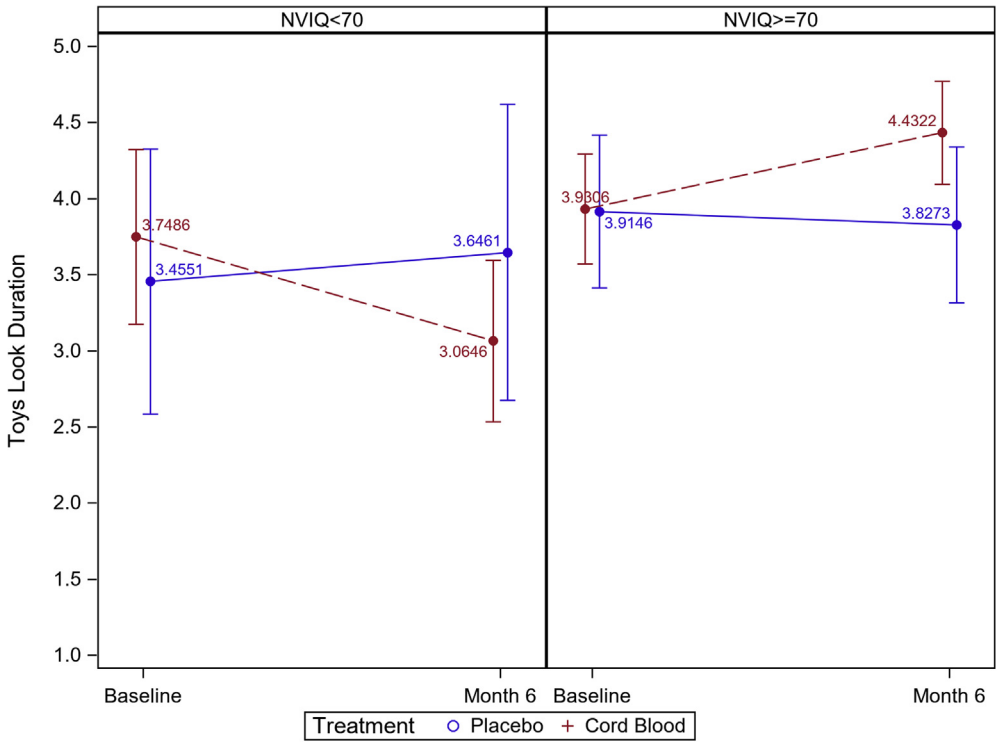

Figure 3. Mean look duration during Actress with Moving Toys ("toys") by treatment and NVIQ. Plots show the mean look duration and 95% CI for the mean at baseline and month 6 by assigned treatment and baseline NVIQ.

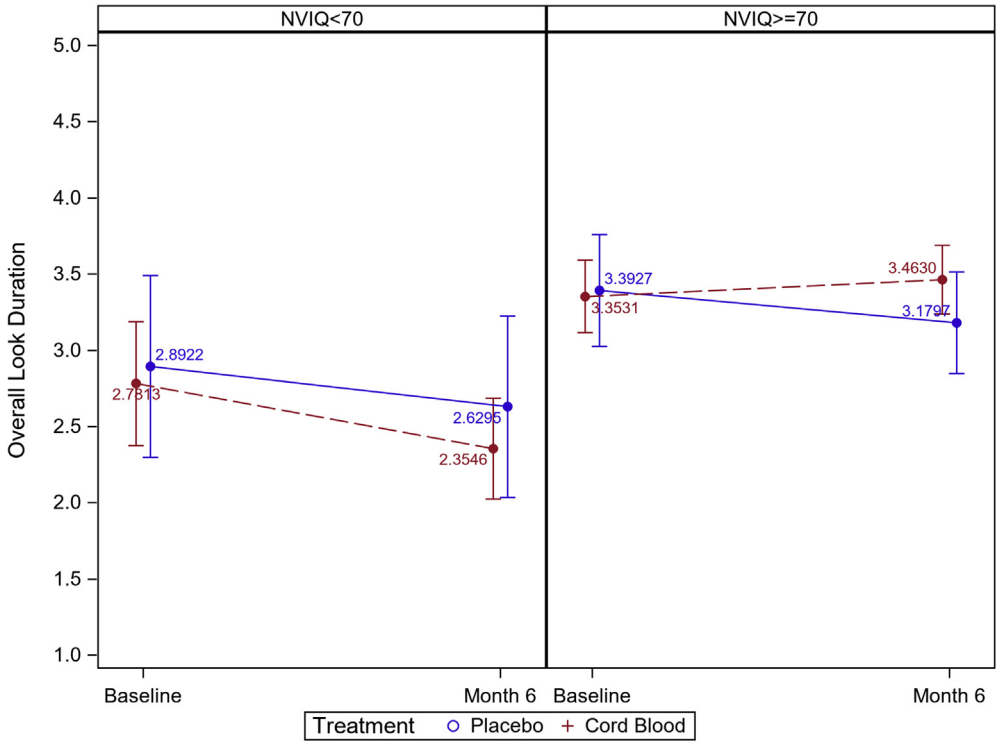

Figure 4. Mean look duration averaged over Actress with Moving Toys and Actress with Dyadic Bid by treatment and NVIQ. Plots show the mean look duration and 95% CI for the mean at baseline and month 6 by assigned treatment and baseline NVIQ.

Table I. Demographic characteristics of sample for efficacy analysis

	Randomized group		CB	
Characteristics	**CB (N = 119)**	**Placebo (N = 61)**	**Autologous (N = 56)**	**Allogeneic (N = 63)**
Sex, N (%)				
Female	21 (17.6)	16 (26.2)	9 (16.1)	12 (19.0)
Male	98 (82.4)	45 (73.8)	47 (83.9)	51 (81.0)
Age, y, median (range)	5.30 (2.39-8.00)	5.24 (2.31-8.13)	5.09 (2.74-7.99)	5.33 (2.39-8.00)
Race, n (%)				
Nonwhite	24 (20.2)	17 (27.9)	13 (23.2)	11 (17.5)
White	95 (79.8)	44 (72.1)	43 (76.8)	52 (82.5)
Ethnicity, n (%)				
Hispanic	26 (21.8)	6 (9.8)	5 (8.9)	21 (33.3)
Non-Hispanic	93 (78.2)	55 (90.2)	51 (91.1)	42 (66.7)
Full-scale IQ, median (range)	67.00 (30.00-115.00)	70.00 (31.00-122.00)	76.50 (37.00-110.00)	62.00 (30.00-115.00)
NVIQ, n (%)				
<55*	32 (26.9)	18 (29.5)	10 (17.9)	22 (34.9)
<70†	53 (44.5)	24 (39.3)	20 (35.7)	33 (52.4)
ADOS severity, median (range)	19.00 (3.00-27.00)	20.00 (7.00-28.00)	18.00 (3.00-26.00)	20.00 (7.00-27.00)

ADOS, Autism Diagnostic Observation Schedule.
*Randomization strata. Note that 1 individual with NVIQ 58 was incorrectly randomized to <55 strata.
†Threshold for ID.

Table III. CB characteristics

Characteristics	**Total (N = 119)**	**Autologous CB (N = 56)**	**Allogeneic CB (N = 63)**
TNCs, $\times 10^6$, median (range)	730.50 (278.69-1455.50)	583.23 (278.69-1283.80)	883.00 (502.60-1455.50)
TNCs, $\times 10^6$/kg infused, median (range)	35.39 (15.14-64.16)	26.88 (15.14-57.57)	38.45 (20.68-64.16)
$CD34^+$ cells, $\times 10^6$, median (range)	1.08 (0.13-6.56)	0.70 (0.13-4.30)	1.53 (0.13-6.56)
$CD34^+$ cells, $\times 10^6$/kg infused, median (range)	0.05 (0.01-0.29)	0.03 (0.01-0.27)	0.07 (0.01-0.29)
CFU, $\times 10^5$, median (range)	43.11 (0.00-1455.60)	23.53 (0.00-111.70)	63.85 (0.00-1455.60)
CFU, $\times 10^5$/kg infused, median (range)	2.26 (0.00-61.94)	1.15 (0.00-5.53)	2.83 (0.00-61.94)
Viability, %, median (range)	95.00 (74.00-100.00)	95.50 (75.00-100.00)	95.00 (74.00-100.00)
Sterility, N (%)			
No growth	119 (100.0)	56 (100.0)	63 (100.0)

CFU, colony-forming unit.

Table IV. VABS-3: Subgroup analyses by NVIQ and type of CB

		Mean (SD) change in VABS-3 SS, baseline to 6 months					
NVIQ	Treatment	Socialization	Communication	Mean of socialization and communication	Daily living	Motor skills	Adaptive behavior composite
<70	Allogeneic (N = 33)	3 (6.4)	3.76 (11.61)	3.38 (8.33)	2.3 (7.44)	2.36 (5.98)	2.91 (7.03)
	Autologous (N = 20)	1.3 (9.77)	1.15 (7.03)	1.23 (7.53)	0.2 (5.45)	2.7 (5.94)	0.6 (5.36)
	Placebo (N = 22)	1.82 (8.08)	7.09 (11.5)	4.45 (8.48)	2.09 (9.28)	0.14 (8.83)	3.45 (7.9)
≥70	Allogeneic (N = 30)	3.07 (7.76)	2.87 (6.48)	2.97 (6.06)	1.67 (6)	−0.53 (6.24)	2.07 (4.27)
	Autologous (N = 36)	4.31 (10.76)	3.03 (9.08)	3.67 (8.61)	3.69 (7.67)	0.69 (7.07)	3.03 (6.31)
	Placebo (N = 35)	2.09 (8.73)	0.11 (7.29)	1.1 (6.02)	2.74 (6.08)	0.09 (7.9)	1.23 (4.46)

Table V. ANCOVA for Vineland communication and socialization SSs

Parameters	Communication, estimate, mean (SE)	Socialization, estimate, mean (SE)	Mean of communication and socialization, estimate (SE)
Intercept*	69.01 (1.96)	63.85 (1.81)	67.2 (1.66)
Baseline†	0.78 (0.05)	0.82 (0.05)	0.86 (0.05)
CB	−3.37 (2.16)	0.26 (2.13)	−1.7 (1.87)
NVIQ ≥70	−0.63 (2.66)	2.39 (2.36)	−0.5 (2.22)
CB × NVIQ ≥70	5.45 (2.82)	1.09 (2.76)	3.59 (2.43)

Each model is a linear regression of the 6-month score on the baseline value, randomized treatment assignment, NVIQ, and the interaction between treatment assignment and NVIQ.
*Reference category (placebo).
†Interpreted as the effect of a 1-unit increase from the average baseline value for each outcome.

Table VI. CGI-I at 6 months by NVIQ and type of CB

		Improvement (1, 2, or 3) on CGI-I at 6 months, n (%)		
NVIQ	Treatment	Overall	Restrictive and repetitive behaviors	Socialization
<70	Allogeneic (N = 33)	13 (39.39)	10 (30.3)	11 (33.33)
	Autologous (N = 20)	9 (45)	5 (25)	8 (40)
	Placebo (N = 22)	9 (40.91)	7 (31.82)	9 (40.91)
≥70	Allogeneic (N = 29)	22 (75.86)	17 (58.62)	20 (68.97)
	Autologous (N = 35)	20 (57.14)	15 (42.86)	17 (48.57)
	Placebo (N = 35)	20 (57.14)	14 (40)	16 (45.71)

Table VII. Reasons for missing eye-tracking data

Group	Dropped out	Low percent tracked	Non-compliance	Poor calibration	Stood up	Track status froze	Tracked caregiver's eyes
CB, n (%)	2 (8.33)	1 (4.17)	7 (29.17)	11 (45.83)	1 (4.17)	1 (4.17)	1 (4.17)
Placebo, n (%)	0	0	3 (37.50)	5 (62.50)	0	0	0
Total	2	1	10	16	1	1	1

Table VIII. Parameter estimates (group-by-time interaction) and 95% CIs for look duration models

		Model 2		
Look duration, s	Model 1	NVIQ <70	NVIQ ≥70	Model 3
Actress with dyadic bid	0.11 (−0.26 to 0.49)	−0.001 (−0.65 to 0.65)	0.17 (−0.28 to 0.62)	0.17 (−0.62 to 0.96)
Actress with moving toys	0.20 (−0.43 to 0.84)	−0.88 (−2.11 to 0.34)	0.59 (−0.14 to 1.32)	1.46 (0.04 to 2.89)
Average	0.15 (−0.29 to 0.60)	0.07 (−0.71 to 0.85)	0.28 (−0.26 to 0.80)	0.83 (−0.20 to 1.85)

Model 1: Difference between CB and placebo at 6 months without regard to NVIQ.
Model 2: Difference between CB and placebo separately for NVIQ <70 and NVIQ ≥70.
Model 3: Treatment effect (CB vs placebo) in NVIQ ≥70 minus the treatment effect in NVIQ <70.
Mean look duration at the media during the actress with moving toys episode, and during the dyadic bid episode, as well as the average of the 2 (the average look duration), were analyzed using ANCOVA-type models for normally distributed outcomes where the baseline and 6-month measures were part of the outcome vector and the covariates included the treatment group, time, and the group-by-time interaction. Models that included NVIQ (<70 vs ≥70) were also fit for each look duration outcome. These models included a 3-way interaction: group-by-time-by-NVIQ. Because of the complexity of these models we chose to represent the results graphically in the main text. Parameter estimates (for the group-by-time interaction terms) and 95% CIs from these models are shown in **Table IX**. Model 1 shows that on average, look durations increased more over time in CB compared with placebo. Model 2 suggests this effect is stronger in individuals with NVIQ ≥70. Model 3 quantifies the difference in treatment effect comparing NVIQ ≥70 vs NVIQ <70.

Table IX. Safety summary: Number (and percent) of participants experiencing SAEs in the first 12 months and infusion reactions and psychiatric non-serious AEs in the first 6 months

Events	CTCAE severity	Placebo (N = 61), n (%)	Autologous CB (N = 56), n (%)	Allogeneic CB (N = 63), n (%)
SAEs	Moderate	3 (4.9)	1 (1.8)	2 (3.2)
Infusion reactions*	Mild	4 (6.6)	2 (3.6)	3 (4.8)
	Moderate	0	1 (1.8)	2 (3.2)
	Severe	0	0	4 (6.3)
Psychiatric nonserious AEs†	Mild	27 (44.3)	22 (39.3)	30 (47.6)
	Moderate	2 (3.3)	0	3 (4.8)
	Severe	0	0	0

*All infusion reactions are related to the study product.
†The maximum severity event is selected for participants who had more than 1 nonserious AE.