Stem Cell Research & Therapy

RESEARCH

Open Access

Outcomes of autologous bone marrow mononuclear cell administration combined with educational intervention in the treatment of autism spectrum disorder: a randomized, open-label, controlled phase II clinical trial

Liem Thanh Nguyen[1,2*†], Phuong Mai Nguyen[3†], Hoang-Phuong Nguyen[1], Hau Thi Bui[2], Lan Thi Mai Dao[1], Minh Van Pham[4,5], Chi Khanh Hoang[5], Phuong Thi Nguyen[6], Thao Thi Phuong Nguyen[1], Anh Thi Phuong Nguyen[2], Van Thi Hoang[1], Hoa Thi Phuong Bui[2], Ngan Kim Vuong[2] and Doan Van Ngo[2]

Abstract

Background This study evaluated the effectiveness of intrathecal autologous bone marrow mononuclear cell (BMMNC) therapy combined with education compared with education alone for the treatment of autism spectrum disorder (ASD).

Methods Fifty-four children with ASD, aged three to seven years, were randomly assigned to two groups. Fifty patients completed the study (25 patients per group). The cell therapy (CT) group received two BMMNC infusions six months apart along with an educational intervention, while the control group received education only. Efficacy outcomes were assessed at baseline, two, six, and 12 months, based on: (1) changes in ASD severity evaluated through the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), the Childhood Autism Rating Scale (CARS), and the Clinical Global Impression-Severity (CGI-S) scale scores and (2) improvements in social interaction, adaptive behavior, and daily living skills measured by the Vineland Adaptive Behavior Scales (VABS-II) and Clinical Global Impression-Improvement (CGI-I) scale scores.

Results At 12 months, the CT group presented a 48.0% reduction in individuals classified at the most severe DSM-5 level compared with 8.0% in the control group ($p = 0.004$). The CARS scores were significantly lower in the CT group (-5.9 points) than in the control group (-1.5 points) ($p < 0.0001$). Similarly, the CT group exhibited greater improvement

†Liem Thanh Nguyen and Phuong Mai Nguyen authors contributed equally and are considered co-first authors.

*Correspondence:
Liem Thanh Nguyen
liem.nt@vinuni.edu.vn

Full list of author information is available at the end of the article

in CGI-S scores (-1.5 points) than did the control group (-0.1 points) ($p < 0.0001$). The VABS-II scores increased by 8.5 points in the CT group versus 1.4 points in the control group ($p < 0.0001$). Finally, the CGI-I scores improved from 2.8 to 2.0 in the CT group but worsened from 3.0 to 3.5 in the control group ($p < 0.0001$).

Conclusions Intrathecal BMMNC combined with an educational intervention improved disease severity and adaptability more than education alone in children with ASD.

Trial registration clinicaltrials.gov, NCT05307536. Date registered 12 February 2022. http://clinicaltrials.gov/study/NCT05307536.

Keywords Autism spectrum disorder, Bone marrow mononuclear cells, Intrathecal infusion, Randomized phase II clinical trial, Cell therapy

Background

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction, along with restricted and repetitive behaviors. Other medical and psychiatric conditions, such as intellectual disabilities, epilepsy, and attention-deficit/hyperactivity disorders, often coexist with ASD [1]. The prevalence and incidence of ASD vary across regions, time periods, and diagnostic criteria. According to estimates by the Centers for Disease Control and Prevention in 2020, the prevalence of ASD is approximately one in 44 children in the United States [2].

ASD significantly impacts the health and quality of life of children and their entire family. According to Kousha M et al., 72.4% of mothers with autistic children suffer from anxiety, and 49.6% suffer from depression [3]. In addition, ASD places a financial burden on families and society. The total cost for ASD in the United States was $268 billion in 2015 and is expected to rise to $461 billion by 2025 [4].

The exact etiology of ASD remains elusive, but it is believed to arise from the interaction of multiple factors, including abnormal immune activation, genetic abnormalities, brain hypoperfusion, and/or both pre- and postnatal environmental influences [5, 6]. Various studies have demonstrated that immune abnormalities observed in individuals with ASD are manifested by increased cytokine production, decreased cytokine regulation, and the presence of brain-reactive antibodies [7–16]. Genetic factors also contribute significantly to the pathophysiology of ASD, with chromosomal abnormalities and pathogenic genetic mutations identified in up to 25% of cases [17]. Genetic alterations often affect brain development, synaptic function, and neuronal communication [18, 19]. Brain hypoperfusion is commonly observed in individuals with autism and causes hypoxia, resulting in inflammation, oxidative stress, excitotoxicity, increased blood–brain barrier permeability, and apoptosis of neurons. A reduction in cerebral perfusion is believed to be associated with behavioral, emotional, and language impairments [6, 20, 21].

The management of ASD remains a challenge. Educational interventions are currently recognized as an essential treatment method for ASD. Various approaches, including applied behavior analysis, speech and language therapy, occupational therapy, and daily living skills training, are commonly employed to improve ASD symptoms and address the diverse needs of individuals with ASD [22–24]. However, responses to these interventions are limited in many children [23]. To increase the quality of life and alleviate symptoms in children with ASD, innovative and more targeted treatments are needed.

In recent years, cell therapy (CT) has emerged as a promising treatment for neurological conditions, including ASD. Preclinical studies have shown that stem cell therapies can ameliorate core autism-like behaviors by modulating neuroinflammation and promoting neurogenesis. In the BTBR mouse model of idiopathic autism, transplantation of mesenchymal stem cells (MSCs) significantly reduced repetitive stereotypies and improved social interactions, accompanied by increased hippocampal neurogenesis and elevated brain-derived neurotrophic factor levels, indicating enhanced synaptic plasticity [25]. Similarly, in a rat model of ASD induced by prenatal valproic acid, intranasal administration of bone marrow-derived MSCs (or their conditioned medium) restored social and cognitive behaviors while markedly dampening brain inflammation [26]. Treated animals exhibited reduced microglial activation and lower levels of proinflammatory cytokines (IL-1β, IL-6) alongside an increase in the anti-inflammatory cytokine IL-10 in brain tissue [26]. Similarly, human adipose-derived MSCs have been shown to reverse autism-like deficits in VPA-exposed mice, normalizing aberrant repetitive behaviors and social avoidance while restoring neuroimmune balance [27]. The therapeutic effects of stem cell transplantation in these models appear to be driven by paracrine mechanisms, including the secretion of cytokines and growth factors. MSC-derived exosomes, when delivered intranasally, have also been found to reproduce therapeutic outcomes, improving social interaction and reducing repetitive behaviors in ASD mice while mitigating

neuroinflammation and promoting synaptic protein expression [28].

In 2013, Sharma et al. reported that the intrathecal infusion of BMMNCs was safe and effective for treating ASD in humans [29]. In the same year, Lv Y-T et al. demonstrated that children who received cord blood mononuclear cells (CBMNCs) and rehabilitation, or both CBMNCs and umbilical cord MSCs combined with rehabilitation, showed greater improvement in baseline symptoms compared to children receiving rehabilitation alone [30]. The efficacy of CT for ASD treatment was further substantiated by subsequent research conducted by Bansal in 2016 [31], Dawson in 2017 [32], and our phase I/IIa study in 2021 [33]. However, some studies reported no significant difference in symptom improvement between patients who received CT and those who did not [34, 35].

Although the safety and efficacy of intrathecal bone marrow mononuclear cell (BMMNC) administration for the treatment of ASD have been reported in various studies [29, 33, 36], to our knowledge, no randomized controlled trial (RCT) has been conducted to verify the effectiveness of this approach. The goal of this study was to compare the efficacy of the intrathecal administration of autologous BMMNCs combined with educational intervention to that of educational intervention alone for children with ASD.

Materials and methods

Study design and patient population

This study was conducted as a phase II open label, RCT, with fifty-four participants randomized into two groups at a 1:1 ratio: [1] the CT group, which received two rounds of intrathecal autologous BMMNC infusion at six-month intervals combined with an educational intervention, and [2] the control group, which received the educational intervention alone. The study occurred at the Vinmec International Hospital, Hanoi, Vietnam, from December 2021 to December 2023.

Calculation of sample size for specific objectives

Secondary analyses in our prior phase I/IIa study demonstrated an improvement in the mean total Vineland Adaptive Behavior Scale-II (VABS-II) score in children with ASD from a baseline of 52.4 ± 7.0 to 58.0 ± 7.9 at 12 months after autologous BMMNC infusion combined with educational intervention [33]. Based on this data, we used the formula for comparing two means: $N = [(Z\alpha/2 + Z\beta)2\times]/(\mu1\text{-}\mu2)$ to estimate the sample size. With an alpha level of 0.05 and a power of 0.8, the required sample size was calculated to be $N = 25$. Accounting for a predicted 10% dropout rate, the final sample size for each group was set at 27 participants.

Randomization method

A block randomization method (block size of six) was employed to allocate participants to the two arms. The randomization sequence was generated by an independent statistician, external to the study team, using computer-based software (R Studio software version 1.4.1106). Each block of six participants was allocated such that three were assigned to the CT group and three to the control group. The allocation list was sequentially labeled, kept in sealed envelopes, and released to the study physicians only after enrollment to ensure unbiased implementation of the intervention. This approach maintained transparency and validity by minimizing selection bias and preserving the integrity of group assignments (Fig. 1).

Inclusion criteria

The enrolled participants were children of both sexes, aged three to seven years, who were diagnosed with ASD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria [37], with Childhood Autism Rating Scale (CARS) scores ranging from ≥ 30 to < 50 and VABS-II scores of ≥ 50 [38].

Exclusion criteria

The exclusion criteria were epilepsy, hydrocephalus, coagulation disorders, allergies to anesthetic agents, severe health conditions such as cancer or heart, lung, liver, or kidney failure, and active infections. Individuals with Fragile X syndrome were also excluded from this study.

Interventions

Bone marrow aspiration

For each infusion, bone marrow was obtained from the anterior iliac crest via a puncture technique under general anesthesia performed by experienced surgeons in the operating room. The volume collected was determined by the patient's body weight according to our experience from a previous study: 8 ml/kg for patients weighing less than 10 kg and [80 ml+(body weight in kg-10)×7 ml] for patients weighing more than 10 kg, with a maximum limit of 250 ml in total [33].

BMMNC isolation and characterization

Harvested BMMNCs were processed in an ISO-14,644 standard clean room as previously described [33]. In brief, the cells were isolated using density gradient centrifugation with Ficoll-Paque™ PREMIUM density gradient media (Cytiva, Sweden) at 1400xg for 18 min, washed with 1x phosphate-buffered saline solution (Gibco, USA), and resuspended in 10 ml of autologous plasma. The total blood components before and after Ficoll-Paque separation were evaluated by the laboratory department using

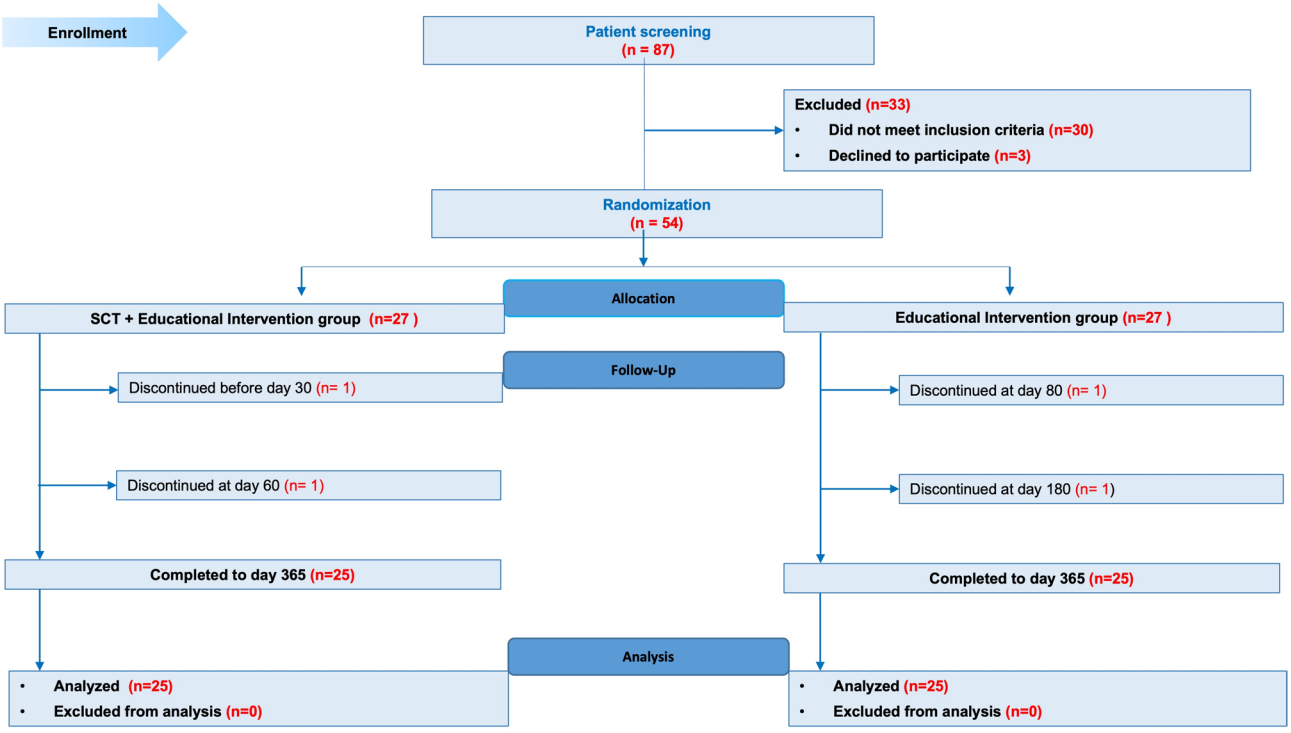

Fig. 1 CONSORT diagram of the study. This flow diagram illustrates the patient enrollment process, randomization, allocation, follow-up, and analysis in the study. A total of 87 patients were screened, resulting in 54 patients who were randomized into two groups. The CT group ($n = 27$) received stem cell therapy (SCT) in combination with an educational intervention. During follow-up, one patient discontinued on day 30, and another discontinued on day 60. The control group received only the educational intervention. During follow-up, one patient discontinued on day 80, and another discontinued on day 180. A total of 25 patients in each group completed the study through day 365 and were included in the final analysis

a whole blood count. The products were tested for bacteria and fungi contamination using the BacT/Alert3D microbial detection system (bioMérieux, Durham, North Carolina). The cells were examined for mycoplasma contamination using the MycoAlert Mycoplasma Detection Kit (Lonza, Switzerland), and the endotoxin levels were measured using the EndoSafe-PTS Kit (Charles River Laboratories, USA) according to the manufacturer's instructions. The cell products had to be negative for mycoplasma and contained endotoxin levels below 0.2 EU/Kg body weight/h for intrathecal administration. The quantification of $CD34^+CD45^+$ cells was performed using a Navios flow cytometer (Beckman Coulter, USA) with Stem-Kit™ Reagents (Beckman Coulter, USA). The number of $CD271^+MSCA\text{-}1^+$ MSCs was determined using an MSC Enumeration Kit (Miltenyi Biotech, Germany) according to the manufacturer's instructions.

BMMNC infusion

The cell products were infused intrathecally via the space between the 4th and 5th lumbar vertebrae using a 22 G spinal puncture needle for 30 min. Each patient received two BMMNC infusions spaced six months apart. After each infusion, patients stayed in the hospital for 48 h for observation and monitoring of any potential adverse events (AEs) or serious adverse events (SAEs).

Educational intervention

All patients in both groups participated in the same educational intervention program for 160 h (two hours per day, five days per week for approximately four months) at a center for educational intervention for children with ASD. The educational intervention was delivered one-on-one (one teacher to one student) and was based on a multidisciplinary approach, including applied behavioral analysis [22], speech therapy, and occupational therapy. Parents were also trained to continue implementing the intervention at home.

Measurements of outcomes

Clinical outcomes

A psychologist, pediatrician, and pediatric psychologist participated in the clinical evaluation at baseline and at two, six, and 12 months. To minimize potential bias from any single assessor, the three specialists collaborated in evaluating efficacy outcomes. ASD severity was categorized according to the DSM-5 criteria into three levels: level one, requiring support; level two, requiring substantial support; and level three, requiring very substantial

support [37]. The CARS assesses 15 domains, yielding a total score between 15 and 60, with higher scores indicating more severe symptoms [39]. The VABS-II provides overall scores and scores across subscales of socialization, communication, daily living, and motor skills, with increased scores correlated with better adaptive behaviors [38]. The CGI-S and CGI-I scales evaluate symptom severity and treatment response, each rated on a 7-point scale, with lower scores reflecting better improvement [40].

Monitoring for AEs and SAEs

AEs and SAEs were recorded during bone marrow collection, BMMNC infusion, during the 48-hour post-infusion observation period, and throughout the follow-up period until the end of the study, according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 4.03 [41]. The relationship between each AE and the intervention was assessed according to the National Cancer Institute guidelines for investigators: adverse event reporting requirements [42].

Laboratory tests

Routine hematological tests and assessments of creatinine, uremia, and liver enzyme levels were conducted at baseline and at six months. Baseline brain magnetic resonance imaging (MRI) and electroencephalography (EEG) were performed using the SIGNA Pioneer 3 Tesla system from GE Healthcare and the NicoletOne EEG system.

Genetic testing was performed for Fragile X syndrome using the AmplideX PCR/CE FMR1 Kit (Asuragen) and AmplideX® PCR/CE FMR1 Reporter Software. Single-nucleotide polymorphism (SNP) array analysis was deployed with the Infinium™ Human CytoSNP 12 (Illumina) with 300 K probes and BlueFuse Multi v4.5 analysis software.

Thirteen cytokines (IFN-gamma, IL-1 beta, IL-2, IL-4, IL-5, IL-6, IL-12p70, TNF alpha, IL-10, IL-17 A, IL-21, IL-1 alpha, and IL-31) in plasma were analyzed at baseline and two months after the first infusion, using a Procarta 45 Plex Kit (Thermo Fisher Scientific) and a Luminex 200 instrument (Luminex Corporation, USA) according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using R Studio software version 1.4.1106. Student's t test was used for normally distributed data, whereas the Mann–Whitney U test was used for non-normally distributed data. Statistically significant differences between subpopulations were identified via the McNemar test, repeated-measures analysis of variance (ANOVA), the chi-square test or Fisher's exact test, and a two-tailed Wilcoxon rank-sum test. Specifically, a comparison between the two groups at baseline was performed using either an independent-samples T-test or the Mann–Whitney U test, depending on the data distribution. Within-group comparisons of continuous variables from baseline to follow-up were conducted using paired t tests or Wilcoxon signed-rank tests, depending on whether the data met parametric assumptions. For multiple comparisons evaluating the effects of BMMNC treatment across various posttreatment assessments, a Bonferroni correction was applied, with statistical significance defined at $p < 0.01$. Mixed-effects models were employed to assess longitudinal changes in CARS and VABS-II scores, reflecting clinical improvement over time. Linear mixed-effects models (LMMs) were applied to analyze these outcome variables across multiple assessment points, comparing postintervention measurements to baseline values. Plots were prepared using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA). A p-value less than 0.05 was considered statistically significant.

Results

A total of 87 individuals were screened for enrollment in the study, 33 (38.0%) of whom were excluded, either because they did not meet the inclusion criteria [30] or declined to participate [3]. The remaining 54 patients were enrolled in the study and randomly assigned to two groups: 27 in the CT group and 27 in the control group. During the study period, two patients from the CT group withdrew from the study on days 30 and 60, and two patients from the control group withdrew on days 80 and 180, all due to family-related issues. At the time of withdrawal, the health status of all patients was stable. Consequently, 25 patients in the CT group and 25 patients in the control group were included in both the safety and efficacy analyses. Among these patients of the CT group, 23 received two BMMNC infusions, whereas two received only one infusion but were followed until study completion (Fig. 1).

Patient characteristics at baseline

The main patient characteristics were presented in Table 1, showing the comparable ages, sex distributions, durations of prior educational intervention, DSM-5 levels, CARS scores, and VABS-II scores of the two groups at baseline.

In the CT group, all 25 patients underwent brain MRI, including conventional MRI, perfusion arterial spin labeling (ASL), and diffusion tensor imaging (DTI). In the control group, only 18 of 25 patients underwent brain MRI (seven patients' parents refused to undergo MRI). None of the patients in either group exhibited major morphological abnormalities on DTI. There were no significant differences in cerebral blood flow reduction between the

Table 1 Patient characteristics at baseline

Characteristic/Measurement	CT group ($n=25$)	Control group ($n=25$)	*P*value[A]
Age of children (yr)	4.7 ± 1.1	4.7 ± 0.9	0.984
Sex (assigned at birth)			0.185
Male	21 (84.0%)	17 (68.0%)	
Female	4 (16.0%)	8 (32.0%)	
Age at ASD[B] diagnosis (months)	20.1 ± 4.8	19.5 ± 4.1	0.812
Duration of educational interventions before the study (months)	29.7 ± 14.1	28.7 ± 15.7	0.808
DSM- 5[C]			0.565
Level 1 – requires support	0 (0.0%)	1 (4.0%)	
Level 2 – requires substantial support	4 (16.0%)	3 (12.0%)	
Level 3 – requires very substantial support	21 (84.0%)	21 (84.0%)	
CARS[D] score	46.5 ± 3.5	45.2 ± 4.8	0.279
VABS-II[E] score	56.8 ± 4.2	57.9 ± 5.8	0.647
CGI-S[F] scale score	6.0 ± 0.7	5.5 ± 1.1	0.037

Data are presented as n (%) or mean (SD) unless specified otherwise. [A]The chi-square or Fisher's exact test, as well as a two-tailed Wilcoxon rank-sum test, were used to identify statistically significant differences. [B]ASD = Autism Spectrum Disorder. [C]DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. [D]CARS = Childhood Autism Rating Scale. [E]VABS-II = Vineland Adaptive Behavior Scale II. [F]CGI-S = Clinical Global Impression-Severity

two groups (Supplementary Table 1). No EEG abnormalities were observed in either group.

SNP array testing was performed for 25 patients in the CT group and 24 in controls (one patient refused to undergo the test). Three patients in the CT group had copy number variants (CNVs) of uncertain significance (VUS). Three control patients contained ASD-related pathogenic CNVs and two patients had VUS. Among those patients, two had deletions (one in each group), and six had duplications (two in the CT group and four in the controls). Three patients (one of the CT group and two of the controls) had the same duplication in chromosome 2q13, which included two candidate genes, *MALL* and *NPHP1* (Supplementary Table 2).

Infused cells

The average counts of BMMNCs, CD34 + cells, and MSCs per kg of body weight were $47.5 \pm 14.7 \times 10^6$/kg, $2.6 \pm 1.5 \times 10^6$/kg, and $2.6 \pm 1.5 \times 10^4$ for the first administration and $37.9 \pm 11.3 \times 10^6$/kg, $2.5 \pm 1.2 \times 10^6$/kg, and $2.2 \pm 1.1 \times 10^4$ for the second infusion, respectively. The average cell viability for the first infusion was 96.2 ± 2.6%, and that of the second infusion was 97.8 ± 1.4% (details in Supplementary Tables 3a and 3b).

Clinical outcomes

The disease severity, adaptability, and treatment response using CARS, VABS-II, CGI-I, and CGI-S scores evaluated at baseline, two, six, and 12 months after the first cell infusion were presented in Table 2.

After 12 months of follow-up, the severity of the disease, according to the DSM-5, was significantly lower in the CT group than in the control group. In the CT group, the percentage of individuals classified at the most severe DSM-5 level decreased by 48.0% after 12 months, whereas it was only 8.0% in the control group ($p=0.004$). The McNemar test indicated a statistically significant improvement within the CT group from baseline to 12 months ($p<0.001$), while no significant change was observed within the control group ($p=0.157$) (Table 3).

Improvements in CARS and VABS-II scores at follow-up points were evaluated with linear mixed-effects models. Autism severity, quantified by CARS, declined more in the CT group than in controls. At 12 months, the model-estimated mean CARS total was 18.2 (95% CI: 16.9–19.5) in the CT group versus 21.0 (95% CI: 19.8–22.2) in the control group. The average decrease per assessment was 1.96 points in the CT group and 0.53 points in the control group, corresponding to an additional reduction of 1.43 points per evaluation for the CT group ($p<0.001$). Hence, while CARS scores declined in both groups, the magnitude of improvement was significantly greater with the CT group (Table 4 and Fig. 2).

A similar pattern emerged for the VABS-II outcomes (Table 5). Baseline Vineland-II scores did not differ between groups ($\beta=1.5$, SE = 1.7, $t=0.88$, $p=0.38$). At follow-up, the CT group improved by 2.81 points per evaluation, whereas the control group improved by 0.45 points, resulting in an additional gain of 2.36 points per evaluation for the CT group ($p<0.001$), confirming a superior rate of adaptive improvement (Table 5).

In VABS-II subscale scores, the mean difference in communication (receptive), daily living skills (personal, domestic, community), social skills (interpersonal relationships), and motor skills (gross and fine) between the groups gradually increased over the follow-up period, resulting in significantly higher scores in the CT group at 12 months. For four other subscales, namely, expressive, written, play and leisure time, and coping skills, the improvement was greater in the CT group than in the

Table 2 Comparison of CARS, VABS-II, CGI-I, and CGI-S scores before and after cell infusion

Time-point	CT[A] group (*n* = 25)	Control group (*n* = 25)	*P* value[G]	Between-group comparisons	
				Difference[H] between CT - control [95% CI[F]]	F & *P* value[I]
CARS[B] total score					
Baseline (T0)	46.5 (± 3.5)	45.2 (± 4.8)	0.254		F = 29.13
2 months (T1)	44.6 (± 4.2)	44.7 (± 5.0)	0.891	-1.4 [-2.4; -0.8]	*P* < 0.0001
6 months (T2)	42.7 (± 4.5)	44.0 (± 5.1)	0.370	-2.6 [-3.7; -1.7]	
12 months (T3)	40.6 (± 4.9)	43.7 (± 5.5)	**0.046**	-4.4 [-5.9; -3.0]	
VABS-II[E] total score					
Baseline (T0)	56.9 (± 4.2)	57.9 (± 5.8)	0.471		F = 23.47
2 months (T1)	60.9 (± 5.2)	59.1 (± 7.0)	0.316	2.8 [0.9; 4.7]	*P* < 0.0001
6 months (T2)	63.3 (± 5.9)	59.0 (± 1.4)	**0.034**	5.0 [2.8; 7.1]	
12 months (T3)	65.4 (± 6.2)	59.3 (± 6.7)	**0.001**	7.2 [4.8; 9.6]	
CGI-S[C] scale score					
Baseline (T0)	6.1 (± 0.7)	5.5 (± 1.1)	**0.025**	-0.2 [-0.6; 0.0]	F = 30.65
2 months (T1)	5.6 (± 0.9)	5.2 (± 1.2)	0.295	-0.7 [-1.1; -0.4]	*P* < 0.0001
6 months (T2)	5.0 (± 1.1)	5.1 (± 1.1)	0.703	-1.4 [-1.7; -1.1]	
12 months (T3)	4.6 (± 1.1)	5.4 (± 1.4)	**0.025**	-0.2 [-0.6; 0.0]	
CGI-I[D] scale score					
Baseline (T0)					F = 22.42
2 months (T1)	2.8 (± 0.6)	3.0 (± 0.7)	0.496		*P* < 0.0001
6 months (T2)	2.2 (± 0.7)	2.7 (± 0.6)	**0.020**	-0.3 [-0.7; 0.1]	
12 months (T3)	2.0 (± 0.8)	3.5 (± 0.7)	**< 0.0001**	-1.3 [-1.9; -0.9]	

Data are presented as mean and standard deviation (SD). [A]CT=cell therapy. [B]CARS=Childhood Autism Rating Scale. [C]CGI-S = Clinical Global Impression-Severity. [D]CGI-I = Clinical Global Impression-Improvement. [E]VABS-II = Vineland Adaptive Behavior Scale-II. [F]CI=confidence interval. [G]Independent-sample t-test was used to compare the difference between the two groups at each time point. [H]Difference between CT - control = [T1, T2, T3 -T0 of the CT group] - [T1, T2, T3 -T0 of the control group]. [I]Repeated-measures analysis of variance was used to calculate the time*group interaction

Table 3 Comparison of disease severity changes by DSM-5[A] classification across four assessment time points

Level 3 – requires very substantial support	Time points				*P* value[C]	*P* value[D]
	Baseline	2 months	6 months	12 months		
CT[B] group (*n* = 25)	21 (84.0%)	20 (80.0%)	16 (64.0%)	9 (36.0%)	< 0.001	0.004
Control group (*n* = 25)	21 (84.0%)	21 (84.0%)	20 (80.0%)	19 (76.0%)	0.157	

Data is n (%). [A]DSM-V = DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. [B]CT=cell therapy

[C]The McNemar test was performed to assess changes within each group from baseline to 12 months

[D]Chi-square test was performed to compare the proportion of Level 3 severity between CTB and Control groups at 12 months

Table 4 Mixed-effects analysis of the CARS scores

Fixed effects	Estimate ± SE[A]	t value	*p* value
(Intercept)	51.32 ± 1.81	28.23	< 0.0001
Group	-2.79 ± 1.15	-2.43	0.019
Time	-3.39 ± 0.35	-9.71	< 0.0001
Group: time	1.43 ± 0.22	6.46	< 0.0001
Correlation of fixed effects:			
	(Intr)	**Group**	**time**
Group	-0.95		
Time	0.012	-0.011	
Group: time	-0.011	0.012	-0.95

[A]Data is Estimate ± SE (standard error)

control group, but the differences did not reach statistical significance (Table 6*)*.

In the CT group, the CGI-S score decreased by 1.4 points, from 6.1 points at baseline to 4.6 points after 12 months, whereas it decreased by only 0.1 points in the control group ($p < 0.001$). The CGI-I score for global improvement decreased from 2.8 points two months after the cell infusion to 2.0 points at 12 months in the CT group. Conversely, the CGI-I score increased from 3.0 points at two months to 3.5 points at 12 months in the control group ($p < 0.001$) (Table 2*)*.

Notable improvements in clinical manifestations were observed in the CT group, which surpassed those of the control group. A significant difference between the groups was found for 16 items (Table 7*)*.

In the remaining domains, although improvements in the CT group were greater, the difference did not reach statistical significance (Table 8*)*.

AE and SAEs

In the CT group, all patients underwent bone marrow aspiration, BMMNC infusions, and follow-up without experiencing any SAEs. 19 AEs were considered to be

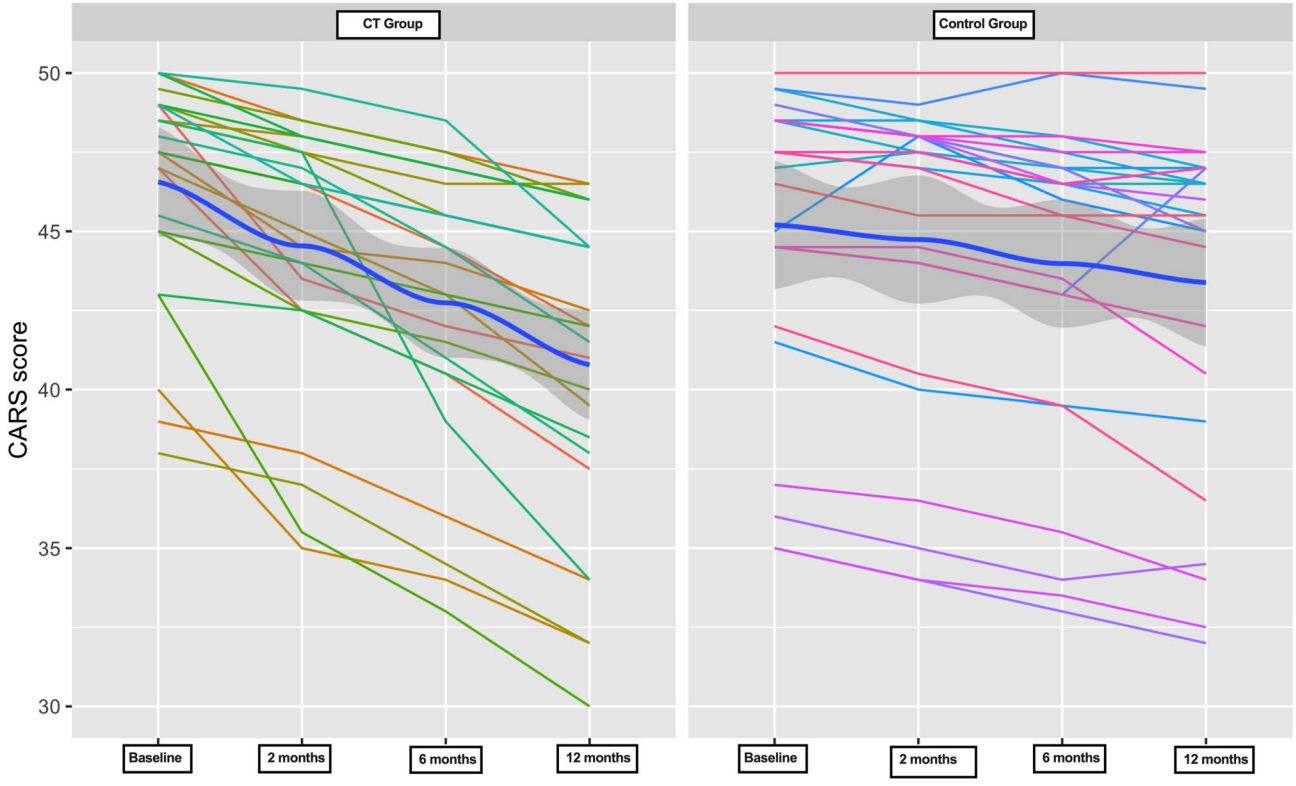

Fig. 2 The CARS score decreased significantly more in the CT group than in the control group. This figure shows the trajectories of the CARS scores for individual participants in the CT group and the control group over four time points: baseline, two, six months, and 12 months. Each line represents an individual participant's CARS score over time. The shaded areas represent the mean trend and variability within each group. The CARS score decreased significantly more in the CT group than in the control group, indicating greater improvement in autism-related symptoms for participants in the CT group

Table 5 Mixed-effects analysis of the VABS-II

Fixed effects	**Estimate ± SE[A]**	**t value**	***p* value**
(Intercept)	51.38 ± 2.37	21.68	< 0.001
Group	3.22 ± 1.50	2.15	0.037
Time	5.17 ± 0.59	8.71	< 0.001
Group: time	-2.36 ± 0.38	-6.30	< 0.001
Correlation of fixed effects:			
	(Intr)	**Group**	**time**
Group	-0.95		
Time	0.22	0.21	
Group: time	0.21	-0.22	-0.95

[A]Data is Estimate ± SE (standard error)

related to the intervention, including 4 Grade 1 (mild) events and 15 Grade 2 (moderate) events; notably, no Grade ≥ 3 events were observed. These AEs primarily included minor pain at the procedure site, minor lower back pain, and headaches *(details in* Table 9 and Supplementary Table 4). These AEs resolved spontaneously or with medication.

Cytokine assessment before and after cell therapy infusion

Plasma cytokine levels at baseline and two months after BMMNC administration are presented in Supplementary Table 5. 11 out of 13 cytokines were detected and no significant difference was observed between before and after infusion.

Discussion

Overall, our study shows a favorable benefit of intrathecal BMMNC infusion combined with educational intervention versus education alone for children with ASD. In terms of safety, we observed no SAEs related to CT during bone marrow harvest, cell infusion, or post discharge. The incidence of AEs was considered to be related to the intervention with CT was low (10.9%), and all were mild, spontaneously resolved, or easily managed. These findings are consistent with our prior Phase I/IIa study [33] as well as several other reports in the literature. Sharma et al. reported no SAEs among 254 ASD patients who received intrathecal BMMNC infusions [43]. Similarly, Sharifzadeh et al. confirmed the safety of this approach, with no SAEs occurring among 14 patients who received 28 intrathecal injections of BMMNCs [34]. The safety of intrathecal cell administration into the spinal cord cavity has also been demonstrated in children with other diseases. A study conducted by Sharma et al. in 2015 involving the intrathecal administration of BMMNCs to 40 children with cerebral palsy revealed that the procedure was safe, with no mortality or severe complications [44].

Table 6 Comparison of VABS-II scores before and after BMMNC infusion at follow-ups

Domain score	Time point[A]	CT[B] group ($n=25$)	Control group ($n=25$)	*P* value[C]	Difference[F] between CT - control [95% CI[F]]	F & *P* value[E]
COMMUNICATION						
Receptive	T0	13.4 (±5.8)	16.3 (±6.9)	0.117		F = 5.57
	T1	17.7 (±5.9)	19.6 (±6.6)	0.304	1.0 [-1.0; 3.1]	*P* = 0.005
	T2	20.0 (±5.2)	21.0 (±6.7)	0.559	1.9 [-0.2; 3.9]	
	T3	22.7 (±5.7)	22.2 (±6.7)	0.768	3.4 [0.9; 5.9]	
Expressive	T0	22.4 (±12.0)	24.2 (±12.1)	0.608		F = 1.39
	T1	28.4 (±15.6)	27.3 (±15.0)	0.804	2.9 [-0.8; 6.5]	*P* = 0.254
	T2	31.6 (±17.3)	29.6 (±15.9)	0.672	3.8 [-0.2; 7.8]	
	T3	35.5 (±20.4)	32 (±17.3)	0.519	5.3 [-0.5; 11.0]	
Written	T0	3.2 (±5.3)	2.8 (±4.5)	0.796		F = 1.39
	T1	4.3 (±6.2)	3.2 (±5.9)	0.516	0.7 [-0.7; 2.2]	*P* = 0.314
	T2	4.8 (±6.3)	3.6 (±5.9)	0.519	0.8 [-0.6; 2.1]	
	T3	6.5 (±7.4)	4.6 (±6.7)	0.352	1.5 [-0.5; 3.5]	
DAILY LIVING SKILLS						
Personal	T0	31.8 (±9.9)	30.3 (±10.1)	0.585		F = 4.90
	T1	37.6 (±10.8)	33.5 (±10.4)	0.185	2.6 [-0.5; 5.5]	*P* = 0.009
	T2	42.7 (±11.5)	36.8 (±11.0)	0.068	4.4 [0.6; 8.2]	
	T3	47.2 (±10.3)	39.6 (±11.8)	**0.020**	6.1 [2.2; 9.8]	
Domestic	T0	2.5 (±2.8)	2.2 (±2.7)	0.684		F = 8.21
	T1	4.6 (±3.6)	3.7 (±3.1)	0.316	0.6 [-0.8; 2.0]	*P* < 0.0001
	T2	6.5 (±4.2)	4.5 (±3.2)	**0.070**	1.7 [0.0; 3.3]	
	T3	8.7 (±4.7)	5.6 (±3.8)	**0.014**	2.8 [0.9; 4.6]	
Community	T0	4.2 (±3.8)	5.0 (±3.6)	0.495		F = 9.09
	T1	7.0 (±5.8)	5.7 (±5.6)	0.444	2.1 [0.0; 3.9]	*P* < 0.0001
	T2	8.6 (±6.2)	6.6 (±4.7)	0.190	2.8 [0.9; 4.7]	
	T3	11.2 (±6.5)	7.5 (±4.6)	**0.022**	4.5 [2.4; 6.5]	
SOCIAL SKILLS						
Interpersonal Relationships	T0	19.9 (±3.5)	20.3 (±3.8)	0.672		F = 6.65
	T1	23.4 (±4.0)	22.6 (±6.8)	0.578	1.2 [-1.7; 4.4]	*P* = 0.002
	T2	25.5 (±4.6)	23.2 (±6.0)	0.131	2.7 [0.0; 5.5]	
	T3	28.6 (±5.8)	24.6 (±6.6)	**0.029**	4.4 [1.1; 7.7]	
Play and Leisure Time	T0	11.5 (±5.7)	12.0 (±4.5)	0.764		F = 2.13
	T1	16.5 (±6.0)	14.0 (±5.3)	0.127	3.0 [0.4; 5.4]	*P* = 0.124
	T2	18.8 (±6.1)	16.0 (±5.3)	0.084	3.3 [0.5; 6.1]	
	T3	21.8 (±6.3)	17.7 (±5.5)	**0.016**	4.6 [1.8; 7.3]	
Coping Skills	T0	3.4 (±3.7)	3.3 (±2.0)	0.962		F = 2.94
	T1	4.4 (±4.0)	3.9 (±2.3)	0.607	0.4 [-0.5; 1.4]	*P* = 0.057
	T2	5.4 (±4.0)	4.3 (±1.7)	0.234	1.0 [-1.5; 2.1]	
	T3	8.0 (±4.4)	6.2 (±2.9)	0.096	1.7 [0.2; 3.4]	
MOTOR SKILLS						
Gross	T0	60.1 (±7.2)	61.0 (±6.3)	0.618		F = 5.67
	T1	64.0 (±7.1)	62.9 (±6.0)	0.552	2.0 [-0.7; 4.8]	*P* = 0.004
	T2	67.4 (±6.1)	64.8 (±5.6)	0.071	3.5 [0.9; 6.2]	
	T3	70.9 (±5.5)	66.6 (±5.7)	**0.009**	5.3 [2.5; 8.1]	
Fine	T0	28.7 (±10.0)	26.0 (±8.3)	0.292		F = 10.52
	T1	31.5 (±10.8)	28.8 (±6.8)	0.284	0.0 [-2.7; 2.7]	*P* < 0.0001
	T2	36.3 (±12.2)	31.0 (±6.5)	0.054	2.6 [-0.8; 5.9]	
	T3	42.3 (±12.1)	33.8 (±6.8)	**0.004**	5.6 [2.4; 9.0]	

Data are presented as mean and standard deviation (SD). [A]Time-point: T0: Baseline, T1: 2 months, T2: 6 months, T3: 12 months. [B]CT=cell therapy. [C]Independent-sample t-test was used to compare the difference between the two groups at each time point. [D]CI=confidence interval. [E]Repeated-measures analysis of variance was used to calculate the time*group interaction. [F]Difference between CT - control = [T1, T2, T3 -T0 of the CT group] - [T1, T2, T3 -T0 of the control group]

Table 7 Comparison of clinical manifestations before and after BMMNC infusion between groups

Domain	CT[A] group ($n=25$)	Control group ($n=25$)	*P* value[B]
Social interaction: None or little capable of social interaction			
Before infusion	8 (32.0%)	1 (4.0%)	**0.023**
After 12 months	0 (0.0%)	0 (0.0%)	
Interact with family members: Not capable of interacting with family members			
Before infusion	21 (84.0%)	21 (84.0%)	**0.038**
After 12 months	1 (4.0%)	9 (36.0%)	
Play with peers: Not capable or little play with peers			
Before infusion	20 (80.0%)	18 (72.0%)	**0.038**
After 12 months	1 (4.0%)	9 (36.0%)	
Expression of emotions: No or few appropriate emotions			
Before infusion	24 (96.0%)	24 (96.0%)	**0.002**
After 12 months	5 (20.0%)	17 (68.0%)	
Time for eye contact maintenance: Under 10 s			
Before infusion	22 (95.6%)	18 (94.7%)	**<0.0001**
After 12 months	6 (24.0%)	18 (75.0%)	
Language comprehension: No or little understanding of words			
Before infusion	24 (96.0%)	24 (96.0%)	**<0.0001**
After 12 months	1 (4.0%)	12 (48.0%)	
Stereotyped/repetitive language:			
Before infusion	19 (76.0%)	15 (60.0%)	**0.046**
After 12 months	13 (52.0%)	16 (64.0%)	
Concentration ability: Unable to concentrate/concentrate for under 5 min			
Before infusion	19 (76.0%)	11 (44.0%)	**0.010**
After 12 months	0 (0.0%)	2 (8.0%)	
Body language gestures: No or few body language gestures			
Before infusion	25 (100.0%)	24 (96.0%)	**<0.0001**
After 12 months	5 (20.0%)	17 (68.0%)	
Self-feeding and drinking: Unable to self-feed			
Before infusion	25 (100.0%)	24 (96.0%)	**0.009**
After 12 months	10 (40.0%)	19 (76.0%)	
Toileting skills: Incapable of independently using the restroom			
Before infusion	25 (100.0%)	23 (92.0%)	**0.005**
After 12 months	15 (60.0%)	22 (88.0%)	
Hyperactivity: Yes			
Before infusion	21 (84.0%)	19 (76.0%)	**0.018**
After 12 months	11 (44.0%)	17 (68.0%)	
Rigid habits: Yes		›	
Before infusion	19 (76.0%)	15 (60.0%)	**<0.0001**
After 12 months	6 (24.0%)	14 (56.0%)	
Sensory impairments: Yes			
Before infusion	21 (84.0%)	20 (80.0%)	**0.002**
After 12 months	13 (52.0%)	21 (84.0%)	
Sleep problems: Yes			
Before infusion	9 (36.0%)	12 (48.0%)	**0.022**
After 12 months	4 (16.0%)	13 (52.0%)	
Responsive when called by name: No response or response without gestures (such as turning back, making eye contact, smiling)			
Before infusion	23 (92.0%)	22 (88.0%)	**0.008**
After 12 months	0 (0.0%)	8 (32.0%)	

Data is n (%). [A]CT=cell therapy. [B]Chi-square test comparing before infusion and after 12 months in both groups ($p<0.05$)

In 2017, Liu et al. reported the outcomes of the intrathecal injection of bone marrow MSCs or BMMNCs in 70 children with spastic cerebral palsy, with each patient receiving four intrathecal injections of the cells. The researchers observed no SAEs, and all minor AEs were alleviated or resolved either with changes in position or medication [36]. Similarly, in our studies of intrathecal BMMNC administration for 30 patients with cerebral palsy due to oxygen deprivation, we did not observe any SAEs [45]. In addition to the intrathecal route, cell administration via the intravenous route has also been used in other studies for ASD [35]. While intravenous infusion is generally considered safer than intrathecal delivery, its drawback is that fewer cells may effectively reach the brain due to retention in the lungs and other organs [46].

Our study is the first RCT of this intrathecal BMMNC combined with an educational intervention to our knowledge. At 12 months of follow-up, we observed statistically significant improvements across all measured domains in the CT group compared to the control group. Disease severity was markedly lower in the CT group than in both the baseline and the control groups. Specifically, the mean CARS score in the CT group decreased by 5.9 points, from 46.5 points at baseline to 40.6 points after 12 months, whereas in the control group, it decreased by only 1.5 points, from 45.2 points to 43.7 points ($p<0.0001$). Furthermore, the positive changes in disease severity, as assessed by educational interventionists using the CGI scale, were significantly more pronounced in the CT group than in the control group ($p<0.0001$). The CGI scale score was reduced by 1.5 points in the CT group compared with 0.1 points in the control group ($p<0.0001$). The disparity in the required support level between the groups was also significant. The CT group showed a notable reduction in the proportion of patients requiring very substantial support over time, whereas minimal change was observed in the control group ($p=0.004$).

The current research also revealed that improvements in individuals' ability to perform daily activities and motor function, as manifested by VABS-II scores, were significantly greater in the CT group than in the control group. Specifically, significant differences were observed in the receptive subdomain of communication ($p=0.005$), the daily living skills domain ($p=0.009$), the interpersonal

Table 8 Comparison of clinical manifestations before and after BMMNC infusion between groups *(continued)*

Domain	CT[A] group ($n=25$)	Control group ($n=25$)	*P* value[B]
Show affection to relatives: No or little expression			
Before infusion	5 (20.0%)	1 (4.0%)	0.189
After 12 months	0 (0.0%)	0 (0.0%)	
Treating others as tools: Sometimes or frequently pulling hands			
Before infusion	25 (100.0%)	24 (96.0%)	1.000
After 12 months	20 (80.0%)	19 (76.0%)	
Pointing when expressing needs: No or little pointing of fingers			
Before infusion	25 (100.0%)	24 (96.0%)	0.148
After 12 months	12 (48.0%)	17 (68.0%)	
Eye contact: **No eye contact**			
Before infusion	2 (8.0%)	6 (24.0%)	0.417
After 12 months	0 (0.0%)	1 (4.0%)	
Engaging in imaginative play, **role-playing**: Does not know how to play, either simply, or diversely			
Before infusion	25 (100.0%)	24 (96.0%)	0.189
After 12 months	20 (80.0%)	23 (92.0%)	
Expressive language: No or little expressive language			
Before infusion	2 (8.0%)	6 (24.0%)	1.000
After 12 months	0 (0.0%)	5 (20.0%)	
Level of language expression: Express single words or double words/phrases			
Before infusion	19 (82.6%)	14 (73.7%)	0.821
After 12 months	14 (56.0%)	10 (50.0%)	
Initiating and sustaining conversations: No			
Before infusion	25 (100.0%)	24 (96.0%)	0.110
After 12 months	21 (84.0%)	24 (96.0%)	
Self-injury behavior: Yes			
Before infusion	4 (16.0%)	1 (4.0%)	0.112
After 12 months	2 (8.0%)	3 (12.0%)	
Digestive status: Digestive disorders (constipation, diarrhea, abdominal pain)			
Before infusion	6 (24.0%)	6 (24.0%)	1.000
After 12 months	2 (8.0%)	3 (12.0%)	
Learning ability: Need support to integrate into school (with help from teachers)			
Before infusion	23 (92.0%)	23 (92.0%)	0.725
After 12 months	17 (68.0%)	19 (76.0%)	
Stereotypic/repetitive behaviors: Yes			
Before infusion	23 (92.0%)	23 (92.0%)	0.345
After 12 months	14 (56.0%)	18 (72.0%)	
Restricted interests: Yes			
Before infusion	25 (100.0%)	24 (96.0%)	0.062
After 12 months	14 (56.0%)	20 (80.0%)	
Picky eating behaviors: Yes			
Before infusion	15 (60.0%)	16 (64.0%)	0.377
After 12 months	4 (16.0%)	9 (36.0%)	

Data is n (%). [A]CT=cell therapy. [B]Chi-square test comparing before infusion and after 12 months in both groups ($p<0.05$)

relationships subdomain of social skills ($p=0.002$), and the motor function domain ($p=0.004$). Moreover, analysis of ASD manifestations before and after the CT intervention revealed greater improvement in the CT group than in the control group across various manifestations, such as interaction with family members, play with peers, expression of emotions, language comprehension, sensory impairments, and sleep problems.

The results of the present study align with those of other studies in which the intrathecal administration of BMMNCs was used to treat ASD. In a study by Sharma et al. involving 254 patients with an average follow-up of 7.7 months postintervention, a significant reduction in CARS scores was observed in 95.3% of the patients. Symptom improvement in ASD patients ranges from 53.3 to 86.5% for various manifestations [43]. Similarly, in a 2020 study by Villarreal-Martinez and colleagues, the intrathecal administration of autologous BMMNCs resulted in positive changes among autistic children. Compared with preintervention levels, the severity of the disease decreased, with a mean reduction in the CARS score of 7.61 ± 3.3 points [47].

While the aforementioned studies utilizing BMMNCs revealed significant improvements in children with ASD, the efficacy of CT using other cell types, such as CBMNCs or bone marrow MSCs, has not been consistently observed in other studies [34, 48]. In a placebo-controlled crossover study using CBMNCs, Chez et al. reported trends toward improvement in the CT group, particularly regarding socialization, compared to the control group; however, the differences were not statistically significant [48]. Importantly, the follow-up period in this study was relatively short, lasting only six months, with a total nuclear cell dose of 16.16×10^6 cells/kg of body weight administered in a single infusion. In contrast, we performed two infusions with higher total nuclear cell doses of $47.5 \pm 14.7 \times 10^6$ cells/kg of body weight for the first infusion and $37.9 \pm 11.3 \times 10^6$ cells/kg of body weight for the second infusion.

Additionally, a follow-up period of six months may not be sufficient to observe a difference in improvement between the groups. Our previous research revealed that this improvement is proportional to the follow-up time [33]. This finding is consistent with our current study, where the difference in VABS-II and CARS scores between the groups became more pronounced at 12 months after the first cell infusion than at two and six months.

Another randomized crossover study was conducted by Sharifzadeh et al., using bone marrow-derived MSCs infused intrathecally [34]. The researchers observed trends toward improvement, particularly in socialization, but the differences between the groups were not statistically significant. Patient age may have influenced

Table 9 AEs and SAEs occurring during the study

AE/SAE	CT group	Control group	Note
Serious Adverse Events (SAE)	**0**	**0**	
Adverse Events (AE)	**173**	**59**	
Unrelated to the CT intervention	69 (39.9%)	59 (100%)	Non-allergic rhinitis, tonsillitis, cold urticaria, leg pain, poor appetite, COVID-19 infection
Unlikely related to the CT intervention	52 (30.1%)	0	Facial erythema, pale skin, poor appetite, fussing, fatigue.
Possibly related to the CT intervention	33 (19.1%)	0	Mild fever, nausea, vomiting, diarrhea.
Probably related to the CT intervention	19 (10.9%)	0	Pain at the procedure site, low back pain, headache.
Definitely related to the CT intervention	0	0	NA

the outcomes of this study, as the average age of patients in the CT group was 10.36 ± 2.53 years, which is greater than that reported in other studies [33, 35, 48].

In addition to cell dose, patient age, and post-transplant follow-up duration, the type of infused cells may also influence the outcomes of cell therapy. Chez utilized cord blood [42], and Sharifzadeh employed bone marrow-derived MSCs [34], while we and others have used BMMNCs [33, 37]. BMMNCs contain a heterogeneous population of progenitor cells and stem cells, including hematopoietic stem cells, mesenchymal stem cells, endothelial progenitor cells, and a very small number of embryonic-like cells [49]. In mice with stroke, injected CD34 + cells promote neovascularization in the ischemic region, creating a favorable environment for neuronal regrowth and facilitating the process of neuronal regeneration [50]. Furthermore, MSCsexert proangiogenic effects via the secretion of various protein factors, including HGF, FGF-2, and VEGF, which are believed to play crucial roles in promoting angiogenesis in ischemic tissue [51]. Endothelial progenitor cells (EPCs), which are components of BMMNCs, also play important roles in forming new blood vessels and can, therefore, increase blood flow to brain areas in autistic children. Intravenous injection of EPCs induced local angiogenesis in the ischemic brain and prolonged the lifespan of SHR-SP mice [52]. Each cell population plays a distinct role in therapy, however they can interact with one another, potentially resulting in additive, synergistic effects [53, 54].

The question of whether all children with ASD should be treated with cell therapy combined with rehabilitation remains unanswered. In a randomized, placebo-controlled, double-blind study with 180 children conducted by Dawson et al. in 2020, the researchers revealed that only children without intellectual disabilities exhibited significant improvements in communication skills, attention to toys, sustained attention, and increased alpha and beta electroencephalographic power after CT [35]. Our previous study also revealed that children with a CARS score ≤ 49 at baseline showed better improvement than those with CARS scores > 49 [33]. These findings suggest that cell therapy may not be appropriate for children with intellectual disabilities or CARS score > 49.

On brain MRI, we observed no significant differences in brain hypoperfusion between the groups. Follow-up MRI could not be performed due to a lack of parental consent. There was no difference in the improvement of children with CNVs compared to those without CNVs in both groups. The three CT patients with VUS showed increases in CARS scores from 5 to 7.5 points, similar to the average improvement of 5.9 points observed in the CT group. Two patients with VUS in the control group showed CARS score improvements ranging from 0.5 to 2 points, three control patients with pathogenic CNVs exhibited improvements from 0 to 3 points, corresponding to the average improvement of 1.5 points in the control cohort.

In the present study, we observed that the plasma levels of analyzed cytokines (including INF-γ, IL-1β, IL-2, IL-6, TNF-α, and IL-17) did not change significantly two months after cell infusion compared with baseline. However, Maric et al. reported that reduced concentrations of pro-inflammatory cytokines in cerebrospinal fluid (CSF) correlated with better patient outcomes following treatment with BM aspirate concentrates [55]. Although CSF may provide a more precise measurement for follow-up analysis, it is an invasive procedure associated with a greater risk of side effects, and its use must be carefully considered in future studies.

Limitations

Our study has several limitations. Although we employed a randomized study design, blinding was unfortunately not implemented and investigator bias may not be excluded. Furthermore, MRI after cell therapy to assess brain perfusion or cytokine analyses of CSF were not conducted.

Conclusions

The findings from our study suggest that autologous BMMNC administration, in combination with educational intervention, is safe and may reduce disease

severity, enhance adaptive functioning, and improve clinical symptoms in children with ASD.

Abbreviations

BMMNC	Bone marrow mononuclear cell
BMMNCs	Bone marrow mononuclear cells
MSCs	Mesenchymal stem cells
ASD	Autism spectrum disorder
CT	Cell therapy
DSM-5	Diagnostic and statistical manual of mental disorders, fifth edition
CARS	Childhood autism rating scale
CGI-S	Clinical global impression severity scale
CGI-I	Clinical global impression-improvement
VABS-II	Vineland adaptive behavior scale
AEs	Adverse events
SAEs	Serious adverse events
MRI	Magnetic resonance imaging
EEG	Electroencephalography
ANOVA	Analysis of variance
ASL	Arterial spin labeling
DTI	Diffusion tensor imaging
SNP	Single-nucleotide polymorphism
CNVs	Copy number variants
VUS	Variants of uncertain significance
EPCs	Endothelial progenitor cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04404-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Acknowledgements
The authors acknowledge Vingroup Joint Stock Company for providing financial support for our project. The authors declare that they have not used AI-generated work in this manuscript.

Author contributions
Study conception or design: LTN, PMN, MVP, PTN, VTH. Data acquisition, analysis, or interpretation: LTN, PHN, HTB, LTMĐ, MVP, CKH, PTN, TTPN, ATPN, VTH, HTPB, NKV, and DVN. Drafted the manuscript or critically revised the manuscript: LTN, PMN, PHN, TTPN, and VTH. All the authors critically revised the manuscript and approved the final version of the manuscript. The LTN and PMN agreed to be accountable for all aspects of the work and ensured that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Funding
This trial was exclusively funded by Vingroup Joint Stock Company, grant number ISC.19.50. The funding agency did not have a role in the study design, data collection, management, analysis, interpretation, writing of the report, or the decision to submit the report for publication.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the National Ethics Committee of the Vietnam Ministry of Health, with approval number 120/CN-HDDD. Title of the approved project: "Autologous Bone Marrow Mononuclear Cell Transplantation Combined with Educational Intervention for Autism Spectrum Disorders (ASD): A Phase II Randomized Controlled Trial"; Name of the institutional approval committee: The National Ethics Committee of the Vietnam Ministry of Health (Hanoi, Vietnam); Approval number: 120/CN-HDDD; Date of approval: November 05, 2021. The patients' guardians or legally authorized representatives provided written informed consent for their participation in the study and the use of samples. The consent explicitly included permission to use patient identifiers, such as age and sex, for publication purposes. All treatments, including clinical examinations, laboratory tests, brain MRI, EEG, genetic tests, cell collection, cell processing, cell administration, hospital stay fees, and educational interventions, were provided free of charge to the participants and their families.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Vinmec Research Institute of Stem Cell and Gene Technology (VRISG), College of Health Sciences, VinUniversity, Vinhomes Ocean Park, Gia Lam, Hanoi 100000, Vietnam
[2]Vinmec Times City International Hospital, Vinmec Health Care System, 458 Minh Khai Street, Hai Ba Trung, Hanoi 100000, Vietnam
[3]Department of Pediatrics, Faculty of Medicine Haiphong, Hai Phong University of Medicine and Pharmacy, Hanoi 100000, Vietnam
[4]Rehabilitation Department, Ha Noi Medical University, 1 Ton That Tung Road, Dong Da, Hanoi 100000, Vietnam
[5]Ha Noi Rehabilitation Hospital, 35 Le Van Thiem, Thanh Xuan, Hanoi 100000, Vietnam
[6]Child Integration Education Center, 52/2 Yen Lac, Hai Ba Trung, Hanoi 100000, Vietnam

Received: 19 December 2024 / Accepted: 19 May 2025
Published online: 30 May 2025

References

1. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. J Am Acad Child Adolesc Psychiatry. 2008;47(8):921–9.
2. Baio J. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, united States, 2014. MMWR Surveillance Summaries. 2018;67.
3. Kousha M, Attar HA, Shoar Z. Anxiety, depression, and quality of life in Iranian mothers of children with autism spectrum disorder. J Child Health Care. 2016;20(3):405–14.
4. Leigh JP, Du J. Brief report: forecasting the economic burden of autism in 2015 and 2025 in the united States. J Autism Dev Disord. 2015;45:4135–9.
5. Enstrom AM, Van de Water JA, Ashwood P. Autoimmunity in autism. Curr Opin Invest Drugs (London England: 2000). 2009;10(5):463.
6. Bjørklund G, Kern J, Urbina M, Saad K, El–Houfey A, Geier D, et al. Cerebral hypoperfusion in autism spectrum disorder. Acta Neurobiol Exp. 2018;78(1):21–9.
7. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to Myelin basic protein in children with autistic behavior. Brain Behav Immun. 1993;7(1):97–103.
8. Singh VK. Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. J Neuroimmunol. 1996;66(1–2):143–5.
9. Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and Gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. J Clin Immunol. 2004;24:664–73.

10. Ashwood P, Wakefield AJ. Immune activation of peripheral blood and mucosal CD3 + lymphocyte cytokine profiles in children with autism and Gastrointestinal symptoms. J Neuroimmunol. 2006;173(1–2):126–34.
11. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci. 2007;27(40):10695–702.
12. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Annals Neurology: Official J Am Neurol Association Child Neurol Soc. 2005;57(1):67–81.
13. Wills S, Cabanlit M, Bennett J, Ashwood P, Amaral D, Van De Water J. Autoantibodies in autism spectrum disorders (ASD). Ann N Y Acad Sci. 2007;1107(1):79–91.
14. Singer HS, Morris CM, Gause CD, Gillin PK, Crawford S, Zimmerman AW. Antibodies against fetal brain in Sera of mothers with autistic children. J Neuroimmunol. 2008;194(1–2):165–72.
15. Cabanlit M, Wills S, Goines P, Ashwood P, Van De Water J. Brain-specific autoantibodies in the plasma of subjects with autistic spectrum disorder. Ann N Y Acad Sci. 2007;1107(1):92–103.
16. Warren RP, Yonk J, Burger R, Odell D, Warren W. DR-positive T cells in autism: association with decreased plasma levels of the complement C4B protein. Neuropsychobiology. 1995;31(2):53–7.
17. Huguet G, Ey E, Bourgeron T. The genetic landscapes of autism spectrum disorders. Annu Rev Genom Hum Genet. 2013;14(1):191–213.
18. Hughes H, Moreno R, Ashwood P. Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). Brain, behavior, and immunity. 2023;108:245– 54.
19. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Ercument Cicek A, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature. 2014;515(7526):209–15.
20. Fumagalli S, Perego C, Pischiutta F, Zanier ER, De Simoni M-G. The ischemic environment drives microglia and macrophage function. Front Neurol. 2015;6:81.
21. Ohnishi T, Matsuda H, Hashimoto T, Kunihiro T, Nishikawa M, Uema T, et al. Abnormal regional cerebral blood flow in childhood autism. Brain. 2000;123(9):1838–44.
22. Baer DM, Wolf MM, Risley TR. Some still-current dimensions of applied behavior analysis. J Appl Behav Anal. 1987;20(4):313–27.
23. Dawson G, Rogers S, Munson J, Smith M, Winter J, Greenson J, et al. Randomized, controlled trial of an intervention for toddlers with autism: the early start Denver model. Pediatrics. 2010;125(1):e17–23.
24. Virues-Ortega J, Julio FM, Pastor-Barriuso R. The TEACCH program for children and adults with autism: A meta-analysis of intervention studies. Clin Psychol Rev. 2013;33(8):940–53.
25. 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(1):17–32.
26. Noshadian M, Ragerdi Kashani I, Asadi-Golshan R, Zarini D, Ghafari N, Zahedi E, et al. Benefits of bone marrow mesenchymal stem cells compared to their conditioned medium in valproic acid-induced autism in rats. Mol Biol Rep. 2024;51(1):353.
27. Ha S, Park H, Mahmood U, Ra JC, Suh YH, Chang KA. Human adipose-derived stem cells ameliorate repetitive behavior, social deficit and anxiety in a VPA-induced autism mouse model. Behav Brain Res. 2017;317:479–84.
28. Wong R. Neuroinflammation in autism spectrum disorders: potential target for mesenchymal stem cell-based therapy. Egypt J Neurol Psychiatry Neurosurg. 2022;58.
29. Sharma A, Gokulchandran N, Sane H, Nagrajan A, Paranjape A, Kulkarni P, et al. Autologous bone marrow mononuclear cell therapy for autism: an open label proof of concept study. Stem Cells Int. 2013;2013(1):623875.
30. Lv Y-T, Zhang Y, Liu M, Qiuwaxi J, Cho SC, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. J Translational Med. 2013;11:1–10.
31. Bansal H, Singh L, Verma P, Agrawal A, Leon J, Sundell IB et al. A short study report on bone marrow aspirate concentrate cell therapy in ten South Asian Indian patients with autism. J Stem Cells. 2016;11(1).
32. 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 Translational Med. 2017;6(5):1332–9.
33. Nguyen Thanh L, Nguyen H-P, Ngo MD, Bui VA, Dam PT, Bui HTP, et al. Outcomes of bone marrow mononuclear cell transplantation combined with interventional education for autism spectrum disorder. Stem Cells Translational Med. 2021;10(1):14–26.
34. Sharifzadeh N, Ghasemi A, Tavakol Afshari J, Moharari F, Soltanifar A, Talaei A, et al. Intrathecal autologous bone marrow stem cell therapy in children with autism: a randomized controlled trial. Asia-Pacific Psychiatry. 2021;13(2):e12445.
35. Dawson G, Sun JM, Baker J, Carpenter K, Compton S, Deaver M, et al. 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. J Pediatr. 2020;222:164–73. e5.
36. Liu X, Fu X, Dai G, Wang X, Zhang Z, Cheng H, et al. Comparative analysis of curative effect of bone marrow mesenchymal stem cell and bone marrow mononuclear cell transplantation for spastic cerebral palsy. J Translational Med. 2017;15:1–9.
37. Edition F. Diagnostic and statistical manual of mental disorders. Am Psychiatric Assoc. 2013;21(21):591–643.
38. Perry A, Flanagan HE, Dunn Geier J, Freeman NL. Brief report: the vineland adaptive behavior scales in young children with autism spectrum disorders at different cognitive levels. J Autism Dev Disord. 2009;39:1066–78.
39. Parkhurst J, Kawa JM. (2018). Childhood autism rating scales. In: Kreutzer JS, DeLuca J, Caplan B, editors. Encyclopedia of Clinical Neuropsychology. Springer, Cham. https://doi.org/10.1007/978-3-319-57111-9_1530.
40. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry (edgmont). 2007;4(7):28.
41. Institute NC. Common terminology criteria for adverse events (CTCAE), version 4.03. National Cancer Institute, National Institutes of Health 2009.
42. Institute NC. NCI guidelines for investigators: adverse event reporting requirements for DCTD (CTEP and CIP) and DCP INDs and ides. National Cancer Institute Bethesda, MD 2013.
43. Sharma AK, Gokulchandran N, Kulkarni PP, Sane HM, Sharma R, Jose A, et al. Cell transplantation as a novel therapeutic strategy for autism spectrum disorders: a clinical study. Am J Stem Cells. 2020;9(5):89.
44. Sharma A, Sane H, Gokulchandran N, Kulkarni P, Gandhi S, Sundaram J, et al. A clinical study of autologous bone marrow mononuclear cells for cerebral palsy patients: a new frontier. Stem Cells Int. 2015;2015(1):905874.
45. Nguyen LT, Nguyen AT, Vu CD, Ngo DV, Bui AV. Outcomes of autologous bone marrow mononuclear cells for cerebral palsy: an open label uncontrolled clinical trial. BMC Pediatr. 2017;17:1–6.
46. Eggenhofer E, Benseler V, Kroemer A, Popp F, Geissler E, Schlitt H, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012;3:297.
47. Villarreal-Martinez L, Martínez-Garza LE, Rodriguez-Sanchez IP, Alvarez-Villalobos N, Guzman-Gallardo F, Pope-Salazar S, et al. Correlation between CD133 + Stem cells and clinical improvement in patients with autism spectrum disorders treated with intrathecal bone Marrow-derived mononuclear cells. Innovations Clin Neurosci. 2022;19(4–6):78.
48. 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 Translational Med. 2018;7(4):333–41.
49. Nguyen QT, Thanh LN, Hoang VT, Phan TT, Heke M, Hoang DM. Bone Marrow-Derived mononuclear cells in the treatment of neurological diseases: knowns and unknowns. Cell Mol Neurobiol. 2023;43(7):3211–50.
50. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, et al. Administration of CD34 + cells after stroke enhances neurogenesis via angiogenesisin a mouse model. J Clin Investig. 2004;114(3):330–8.
51. Mohamad Yusoff F, Higashi Y. Mesenchymal stem/stromal cells for therapeutic angiogenesis. Cells. 2023;12(17):2162.
52. Peng C, Dong X-H, Liu J-L, Tao Y-L, Xu C-F, Wang L-P, et al. A preventive injection of endothelial progenitor cells prolongs lifespan in stroke-prone spontaneously hypertensive rats. Clin Sci. 2018;132(16):1797–810.
53. Vahidy FS, Rahbar MH, Zhu H, Rowan PJ, Bambhroliya AB, Savitz SI. Systematic review and meta-analysis of bone marrow–derived mononuclear cells in animal models of ischemic stroke. Stroke. 2016;47(6):1632–9.
54. Yang B, Parsha K, Schaar K, Xi X, Aronowski J, Savitz SI. Various cell populations within the mononuclear fraction of bone marrow contribute to the beneficial effects of autologous bone marrow cell therapy in a rodent stroke model. Translational Stroke Res. 2016;7(4):322–30.

55. Maric DM, Vojvodic D, Maric DL, Velikic G, Radomir M, Sokolovac I, et al. Cytokine dynamics in autism: analysis of BMAC therapy outcomes. Int J Mol Sci. 2023;24(20):15080.

Publisher's note