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Autologous bone marrow mononuclear cell administration in a large cohort of 1,011 patients with autism spectrum disorder: a retrospective observational study

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Background: This retrospective observational study analyzed the therapeutic efficacy of autologous bone marrow mononuclear cells (BMMNCs) in a large cohort of patients with autism spectrum disorder (ASD).
Methods: Overall, 1,011 patients with ASD who received intrathecal administration of autologous BMMNCs were included. Changes in symptoms and outcome measures—the Indian Scale of Autism Assessment (ISAA) and Childhood Autism Rating Scale (CARS)—were recorded. Brain positron emission tomography computed tomography (PET/CT) was used to objectively assess changes in brain metabolism.
Results: At a mean follow-up of 19.3 months, 90.6% of patients showed improvement after cell therapy. Symptomatic improvements were observed in attention and concentration, command following, eye contact, sitting tolerance, social interaction, hyperactivity, communication, speech, stereotypical behavior, aggressiveness, and self-injurious behavior. Patients who received multiple doses of cell therapy demonstrated better outcomes, and improvements were seen across all age groups and regardless of disease severity. Changes in ISAA and CARS scores were statistically significant ($P<0.05$). Comparative PET/CT scans of 401 patients revealed improved metabolism in the amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, and superior and middle temporal poles, which corresponded to symptomatic improvements. No major adverse events were reported. Nine of the 1,011 patients experienced seizures, four of whom had a prior history. These events were managed with medication, with improvements still observed in the nine patients.
Conclusions: Intrathecal transplantation of autologous BMMNCs, combined with neurorehabilitation, yields positive outcomes for patients with ASD. This approach helps reduce the degree of impairment and improves quality of life.

Keywords: Autism spectrum disorder; Bone marrow; Mononuclear cells; Positron emission tomography computed tomography; Cell therapy

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HIGHLIGHTS

- This study demonstrates the efficacy of autologous bone marrow mononuclear cell transplantation in a large cohort of 1,011 patients, with an average follow-up of 19.3 months.
- Of these, 90.6% showed significant symptomatic improvements.
- Comparative brain positron emission tomography/computed tomography revealed increased glucose uptake and cerebral metabolism in key brain regions associated with autism.

INTRODUCTION

Autism spectrum disorder (ASD), a complex neurodevelopmental condition, affects about 1% of the general population worldwide [1]. It is characterized by impairments in communication, social interaction, and cognition, as well as repetitive behaviors [2]. ASD is often associated with other comorbidities, such as hyperactivity and attention disorders, sleep disorders, anxiety, depression, epilepsy, and abnormal functioning of the digestive system, including chronic constipation and/or diarrhea [3]. The neuropathology of ASD may include cerebral hypoperfusion, atypical neural connectivity, cerebellar alterations such as decreased density of Purkinje cells, cerebral cortex defects, altered density of dendritic spines, and atypical myelination, along with immune dysfunction, increased oxidative stress, inflammation and apoptosis, hypoxia, atypical excitatory-inhibitory signaling, neuronal migration defects, and synaptic dysfunction [4–6].

Conventional treatments for ASD, such as psychological interventions, behavioral therapy, occupational therapy, speech therapy, and pharmacotherapy, have limited efficacy in addressing the core neuropathology [2]. Therefore, new treatments are necessary to address these unmet medical needs. Autologous bone marrow mononuclear cells (BMMNCs) have shown promising therapeutic efficacy in ASD based on their regenerative and restorative properties [7]. They do not possess tumorigenicity and eliminate the risk of immune rejection [8]. BMMNCs migrate to dysfunctional areas and stimulate neurogenesis, angiogenesis, immune modulation, and anti-inflammatory responses via paracrine mechanisms [9]. They also promote endogenous neural stem cell proliferation, supporting brain repair processes [10]. In this study, the efficacy of autologous BMMNCs was analyzed in a large cohort of 1,011 patients with ASD.

METHODS

The study protocol was approved by the Central Drugs Standard Control Organization (CDSCO)-registered Institutional Ethics Committee (IEC) of NeuroGen Brain and Spine Institute (NGBSI/IEC/AT-CS-01/2018/ISSUE-01/REVISION-01). The treatment protocol and possible adverse events (AEs) were explained in detail to all parents or caregivers, and written informed consent was obtained prior to the intervention, including consent for publication.

Study Design

This retrospective observational study included patients diagnosed with ASD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria who underwent autologous BMMNC transplantation. A total of 1,011 patients with ASD were enrolled. The primary aim was to evaluate the efficacy of cell transplantation in combination with neurorehabilitation as a treatment for ASD in a large population. Additionally, the study describes various factors influencing the outcome of the intervention. The intervention consisted of intrathecal administration of autologous BMMNCs along with standard neurorehabilitation. Neurorehabilitation included behavioral therapy, psychological intervention, occupational therapy, activities of daily living (ADL) training, physiotherapy, aquatic therapy, speech therapy, special education, and dietary recommendations.

Patient Selection

Patient selection was based on the World Medical Association's Helsinki Declaration for ethical principles in medical research involving human participants [11].

Inclusion Criteria

Both male and female patients who were diagnosed with ASD based on DSM-5 criteria and were over 2 years old were included.

Exclusion Criteria

Patients were excluded if they had acute infections, severe anemia (hemoglobin <8 g/dL), pyrexia, human immuno-

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deficiency virus, hepatitis B, hepatitis C, malignancies, bleeding tendencies, renal failure, severe liver dysfunction, or other acute medical conditions such as respiratory infections. Patients with other comorbid neurological conditions, such as cerebral palsy, intellectual disability, or learning disability, were also excluded.

Intervention

Preintervention assessment

All patients underwent detailed serological, biochemical, and hematological testing before the intervention. They also underwent brain magnetic resonance imaging (MRI), brain positron emission tomography computed tomography (PET/CT), and electroencephalography (EEG). Further evaluation was performed by a multidisciplinary team, including a neurologist, neurosurgeon, pediatrician, physician, psychologist, occupational therapist, physiotherapist, speech therapist, and special educator, to assess the severity of each patient's condition.

Aspiration of bone marrow mononuclear cells

Patients were administered granulocyte colony stimulating factor subcutaneously at 48 hours and 24 hours prior to BMMNC aspiration. The dosage was weight-dependent: patients weighing ≤15 kg received 150 µg, while those >15 kg received 300 µg. On the day of transplantation, bone marrow was aspirated under general anesthesia with sedation in the operating theater under aseptic conditions. Between 80 and 100 mL of bone marrow (depending on the patient's age and body weight) was aspirated from the anterior superior iliac bone using a bone marrow aspiration needle and collected in heparinized tubes. Heparin (Gland Pharma) diluted in normal saline (250 IU/mL) was added to the sterile 50 mL BD Falcon tubes (Corning) in which the bone marrow was collected.

Isolation of bone marrow mononuclear cells

Mononuclear cells (MNCs) were separated from the bone marrow using a density gradient separation method. The bone marrow was diluted 1:1 with normal saline and centrifuged at 440 × *g* for 35 minutes in a swinging bucket rotor without a brake at 20 °C. MNCs were then obtained as a buffy coat and collected using a 3-mL BD pipette. The cells were washed three times with normal saline by centrifuging at 300 × *g* for 15 minutes in a swinging bucket rotor without a brake at 20 °C, then resuspended in 1 mL of normal saline. The viability of the isolated cells was assessed using trypan blue vital dye, which was mixed in a 1:1 proportion and loaded onto a hemocytometer to determine total cell count and viability. More than 1×10^8 MNCs were injected, with a viability exceeding 98%. CD34+ analysis of the samples was also performed using a flow cytometer (Attune NXT; Thermo Fisher Scientific Inc.).

Administration of bone marrow mononuclear cells

The isolated BMMNCs were injected immediately using a 25-G spinal needle between the fourth and fifth lumbar vertebrae under general anesthesia with sedation. Simultaneously, 20 mg/kg body weight of methylprednisolone in 100 mL Isolyte P or normal saline was given intravenously to reduce local inflammation and enhance survival of the transplanted cells [12]. Patients were monitored for AEs. In the present study, 543 patients received more than one dose of cell therapy, with the cell therapy protocol repeated as described above for each dose.

Neurorehabilitation

Each patient underwent extensive neurorehabilitation for 4 days after cell transplantation. Rehabilitation protocols were personalized and included applied behavioral analysis, psychological intervention, occupational therapy, sensory integration therapy, ADL training, physiotherapy, aquatic therapy, speech therapy, special education, and dietary recommendations. Patients were also given a home program to continue rehabilitation after discharge.

Follow-up

Patients were followed up at regular intervals (6 months, 12 months, and 18 months). The mean follow-up duration in the study was 19.3 (±12.8) months. At each follow-up, patients underwent detailed neurological evaluation and assessment using the Indian Scale for Assessment of Autism (ISAA) and the Childhood Autism Rating Scale (CARS).

Outcome Measures

The outcome measures used to analyze the effect of the intervention at follow-up were the ISAA [13] and the CARS (Supplementary Table 1) [14]. Symptomatic improvements were also recorded. Brain PET/CT was repeated after 6 months and compared to the preintervention scan to assess the effect of the intervention on brain function. The parents or caregivers of 401 patients consented to a repeat PET/CT scan.

Brain PET/CT Imaging, Processing, and Quantification

Brain PET/CT imaging was performed for all patients as

part of the preintervention protocol. ^{18}F-fluorodeoxyglucose (FDG) brain PET/CT was used to assess brain glucose uptake on a combined PET/CT scanner (Discovery IQ; GE HealthCare). Images were reconstructed using Advantage Workstation (GE HealthCare) software. The percent hypometabolism of different brain regions was analyzed using Oasis Cerquant quantification software (Segami Corporation). The percent hypometabolism of nine brain regions that are typically hypometabolic in autism was determined and analyzed [15]. These regions included the amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, and superior and middle temporal poles. Based on the percent hypometabolism, these regions were graded from 1 to 10 as shown in Supplementary Table 2. Of the 1,011 patients, 401 underwent a repeat PET/CT scan after cell therapy to determine the effect of the intervention on brain metabolism.

Adverse Event Monitoring

All major and minor AEs were monitored throughout the procedure, during the hospital stay, and at follow-up. Long-term AEs were also tracked. AEs were categorized as either procedure-related or cell therapy-related. Procedure-related AEs included events associated with bone marrow aspiration and cell administration via lumbar puncture, such as fever, spinal headache, nausea, vomiting, pain at the site of aspiration or injection, back pain, skin rashes, etc. Cell therapy-related AEs included seizures, increased hyperactivity, and aggressiveness, etc.

Methodology of Analysis

A detailed analysis was conducted to assess the outcomes of the intervention. This included percentage analysis of changes in symptoms and calculation of the mean follow-up period. Subgroup analyses were performed to evaluate the effects of age, severity of illness, and the number of cell therapy doses received. The degree of improvement was estimated using the ISAA score in relation to the number of cell therapy doses.

Statistical Analysis

The Wilcoxon signed-rank test (significance level <0.05) was used to statistically analyze the effects of the intervention on ISAA and CARS scores. This test was also applied to determine statistical significance within each domain of the ISAA. Statistical analyses were conducted for subgroups based on age, severity of illness, and number of cell therapy doses. Changes in percent hypometabolism grades in PET/CT scans before and after intervention were also evaluated using the Wilcoxon signed-rank test ($P<0.05$).

RESULTS

A total of 1,011 patients were included in the analysis, comprising 841 males and 170 females (male-to-female ratio 5:1), with an age range of 3 to 31 years (Table 1). The results of the percentage analysis of symptomatic changes at a mean follow-up of 19.3 months are shown in Fig. 1.

Overall, 90.6% (916 of 1,011) of patients demonstrated improvement on at least one outcome measure after cell therapy. Both ISAA and CARS scores displayed positive changes following cell transplantation: 85% of patients showed improvement in ISAA scores, while 85.8% showed improvement in CARS scores (Fig. 2). Statistical analysis using the Wilcoxon signed-rank test (significance level $P<0.05$) demonstrated that improvements in ISAA (Table 2) and CARS (Table 3) scores were statistically significant.

Analysis of Individual Domains of Indian Scale for Assessment of Autism

Changes in the scores of individual ISAA domains before and after the intervention were also analyzed. The ISAA comprises six domains: social relationship and reciprocity, emotional responsiveness, speech-language and communication, behavior patterns, sensory aspects, and cog-

Table 1. Demographic data

Variable	Total (n=1,011)
Sex	
Male	841
Female	170
Age (yr)	
0–5	96
6–10	529
11–15	266
>15	120
Severity based on ISAA	
Mild autism (70–106)	268
Moderate autism (107–153)	728
Severe autism (>153)	15

ISAA, Indian Scale for Assessment of Autism.

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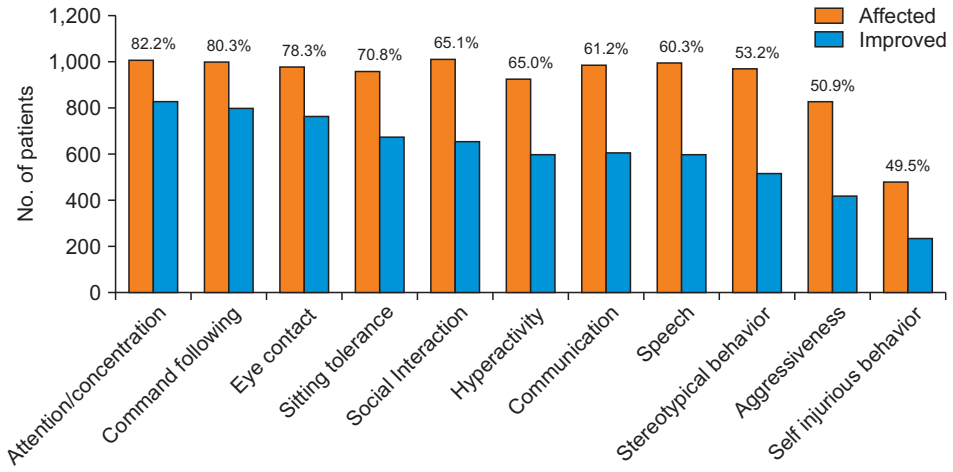

Fig. 1. Symptomatic improvements after cell therapy. The percentages shown on the graph represent that percentage of patients demonstrating symptomatic improvements.

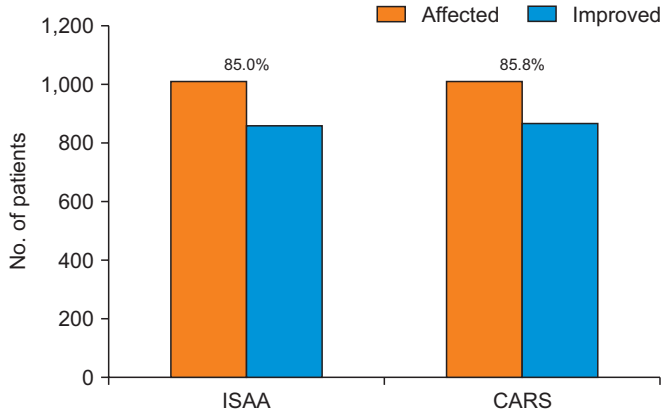

Fig. 2. Improvements in ISAA and CARS scores following cell therapy. The percentages shown on the graph represent that percentage of patients demonstrating improved scores of ISAA and CARS. ISAA, Indian Scale for Assessment of Autism; CARS, Childhood Autism Rating Scale.

Table 2. Wilcoxon signed-rank test results for ISAA total scores and individual domains

Variable	Pre-mean score	Post-mean score	P-value
ISAA	116.93	107.17	<0.05
Category			<0.05
Social relationship and reciprocity	35.28	32.74	
Emotional responsiveness	14.03	12.43	
Speech-language and communication	23.57	22.19	
Behavior patterns	18.55	16.6	
Sensory aspects	15.09	13.72	
Cognitive components	10.41	9.46	

ISAA, Indian Scale for Assessment of Autism.
*P<0.05

Table 3. Wilcoxon signed-rank test results for CARS scores

Scale	Pre-mean score	Post-mean score	P-value
CARS	38.24	35.73	<0.05

CARS, Childhood Autism Rating Scale.
*P<0.05

nitive components. Scores in each ISAA domain showed statistically significant improvements after cell therapy (Table 2).

Factors Affecting Outcome of Intervention

Subgroup analyses were performed based on age, severity of illness (as measured by ISAA), and the number of doses of cell therapy.

Age

Age-wise analyses were performed for both ISAA and CARS. All age groups displayed statistically significant improvement (Tables 4 and 5, Fig. 3).

Severity of illness (ISAA)

Patients were categorized by severity based on ISAA scores: 70–106 (mild), 107–153 (moderate), and >153 (severe autism). All groups showed statistically significant improvements. However, only 15 patients were classified as having severe autism. The results are presented in Table 4.

Number of Cell Therapy Doses Received

Percentage analysis based on ISAA and CARS scores

Patients were categorized into three groups based on the number of BMMNC doses received: one dose, two dos-

Table 4. Wilcoxon signed-rank test results for subgroup analysis of ISAA scores

Variable	Affected	Improved	Percent improvement (%)	P-value
Age (yr)				<0.05
0–5	96	83	86.5	
6–10	529	460	87.0	
11–15	266	227	85.3	
>15	120	89	74.2	
Severity on ISAA				<0.05
Mild	268	223	83.2	
Moderate	728	623	85.6	
Severe	15	13	86.7	
Cell therapy doses received				<0.05
1	467	334	71.5	
2	429	414	96.5	
>2	115	111	96.5	

ISAA, Indian Scale for Assessment of Autism.
*P<0.05

Table 5. Wilcoxon signed-rank test results for subgroup analysis of CARS scores

Variable	Affected	Improved	Percent improvement (%)	P-value
Age (yr)				<0.05
0–5	96	86	89.6	
6–10	529	461	87.1	
11–15	266	224	84.2	
>15	120	96	80.0	
Cell therapy doses received				<0.05
1	467	334	71.5	
2	429	419	97.7	
>2	115	114	99.1	

CARS, Childhood Autism Rating Scale.
*P<0.05

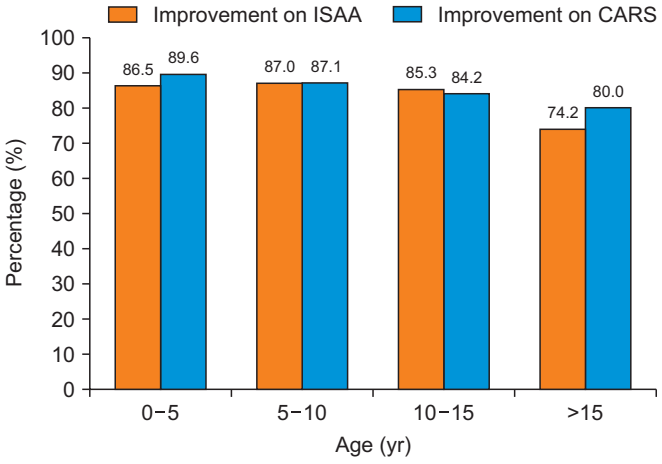

Fig. 3. Age-wise analysis of ISAA and CARS outcomes after cell therapy. ISAA, Indian Scale for Assessment of Autism; CARS, Childhood Autism Rating Scale.

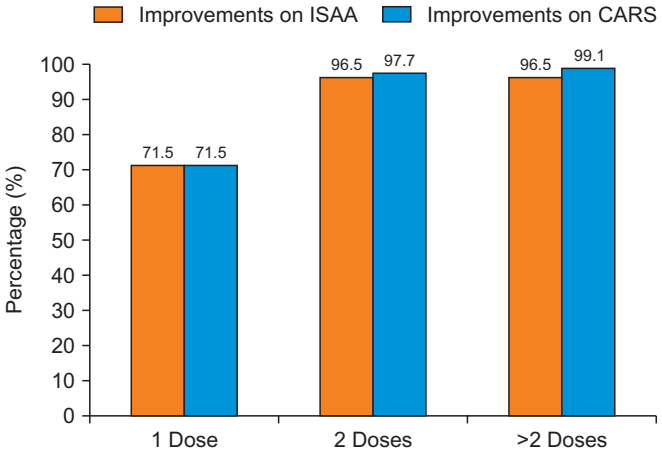

Fig. 4. Analysis of ISAA and CARS outcomes based on the number of cell therapy doses received. ISAA, Indian Scale for Assessment of Autism; CARS, Childhood Autism Rating Scale.

es, and more than two doses. Patients who underwent cell therapy two or more times demonstrated better outcomes, with more than 96% showing improvement, compared to those who underwent cell transplantation only once (Tables 4 and 5, Fig. 4).

Correlation of degree of improvement on ISAA with number of cell therapy doses

The relationship between the number of cell therapy doses and the degree of improvement in ISAA scores was analyzed using the Wilcoxon signed-rank test. All groups showed statistically significant improvements (P<0.05) in ISAA scores after cell therapy (Table 6). The greatest change in mean ISAA score before and after intervention was observed in patients who received two or more doses.

Comparative Analysis of Brain FDG PET Scans

Brain FDG PET scans performed before and after intervention were compared to assess metabolic changes in the brain following BMMNC administration. ^{18}F-FDG PET scans show glucose uptake in different brain regions and reflect the efficacy of treatment in patients with ASD

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Table 6. Analysis of the degree of improvement in ISAA scores based on the number of cell therapy doses received

Cell therapy doses received	Number	Pre-mean ISAA score	Post-mean ISAA score	Mean difference	P-value
1	467	117.93	113.38	4.55	<0.05
2	429	116.64	103.59	13.05	<0.05
>2	115	113.89	95.25	18.64	<0.05

ISAA, Indian Scale for Assessment of Autism.
*P<0.05

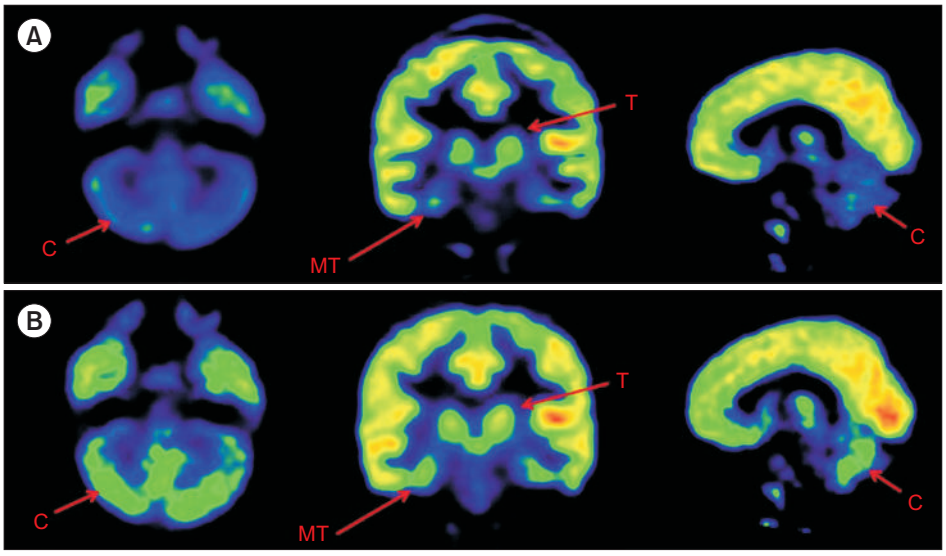

Fig. 5. Representative comparative brain fluorodeoxyglucose positron emission tomography scans of patients with autism spectrum disorder before and after cell therapy. (A) Arrow-marked blue areas indicate hypometabolism before therapy. (B) Arrow-marked green areas indicate improved metabolism after cell therapy. C, cerebellum; MT, medial temporal cortex; T, thalamus.

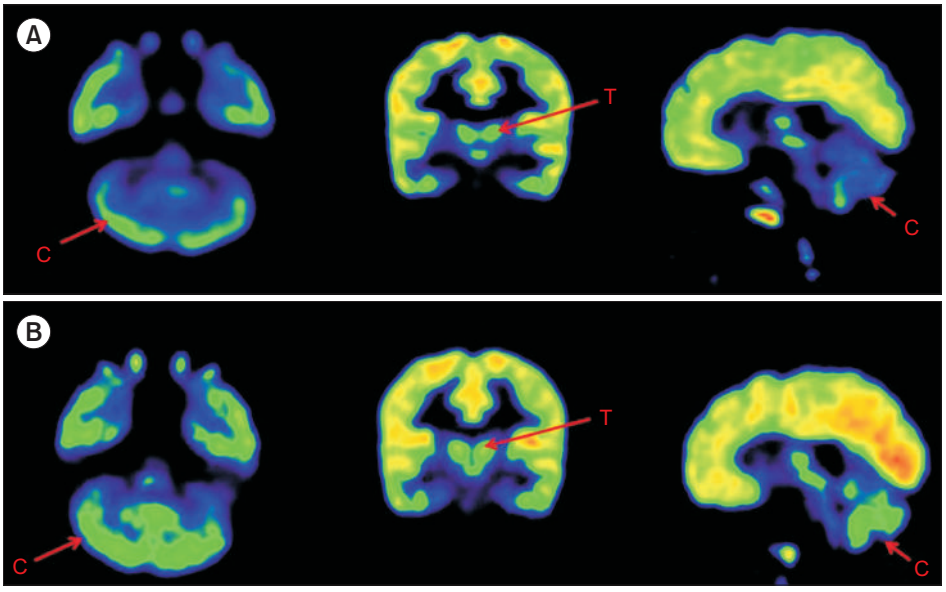

Fig. 6. Representative comparative brain fluorodeoxyglucose positron emission tomography scans of patients with autism spectrum disorder before and after cell therapy. (A) Arrow-marked blue areas indicate hypometabolism before therapy. (B) Arrow-marked green areas indicate improved metabolism after cell therapy. C, cerebellum; T, thalamus.

through changes in metabolic activity [16]. Comparative PET/CT scans were performed in 401 patients. Improved metabolism, evidenced by a reduction in percent hypometabolism, was observed in regions including the amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, and

superior and middle temporal poles (Figs. 5 and 6). Grading of these nine regions showed statistically significant improvement after cell therapy, as demonstrated by the Wilcoxon signed-rank test ($P<0.05$).

DISCUSSION

Cell therapy has gained considerable importance and has been shown to be beneficial in the treatment of various neurological conditions, including ASD, cerebral palsy, and intellectual disability [17–20]. ASD is characterized by a complex, multifaceted underlying neuropathophysiology that may be ameliorated by cell therapy.

Multiple mechanisms contribute to the pathophysiology of autism. Mitochondrial dysfunction is observed in ASD, with damage to mitochondrial complexes leading to increased production of reactive oxygen species and oxidative stress [21]. Elevated oxidative stress, in turn, contributes to neuronal damage in patients with ASD [22]. ASD is also marked by immune system dysregulation. Patients with autism exhibit increased microglial and astroglial activation. Both microglia and astroglia modulate the immune system by releasing proinflammatory cytokines such as interleukin (IL)-17. Increased activation of these neuroglial cells alters the brain's cytokine profile, heightening the neuroinflammatory process and leading to neuronal and synaptic dysfunction [7,9]. Altered activation of microglia is also associated with ASD behavioral phenotypes, including impaired social interaction, cognitive dysfunction, and anxiety [23]. Higher levels of IL-17 induce production of IL-1β, tumor necrosis factor alpha (TNF-α), and matrix metalloproteinase-9, causing neurotoxicity and increased neuronal apoptosis [24]. Structural and functional MRI studies have shown that individuals with ASD exhibit atypical brain connectivity, gray matter volume, and brain activation [25]. These patients also display an imbalance in excitatory and inhibitory (E/I) neural connections, with hypofunctioning of inhibitory GABAergic neurotransmission leading to a higher E/I ratio [26]. Atypical brain networks and reduced inhibitory control may further contribute to the behavioral and social dysfunctions seen in autism [25,26]. Patients with ASD often have constricted blood vessels, resulting in reduced cerebral blood flow and subsequent brain damage. Diminished blood circulation also limits oxygen delivery, leading to hypoxia [27]. Reduced blood flow has been observed in brain single-photon emission computed tomography studies of patients with autism [28]. Previous brain PET/CT studies have shown that children with ASD have relatively lower metabolism in the amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, and superior and middle temporal poles [15].

BMMNCs are a heterogeneous mixture of mesenchymal stem cells (MSCs), hematopoietic cells, monocytes, macrophages, stromal cells, very small embryonic-like stem cells, progenitor cells, hemangioblasts, and endothelial progenitor cells. As a result, they offer cumulative benefits compared to individual subfractions alone [29,30]. Upon administration, these cells recognize and home to sites of injury or damage, where they facilitate repair and regeneration [31].

In ASD, BMMNCs act through multiple mechanisms, including paracrine effects, neuroprotection, immunomodulation, anti-inflammatory effects, angiogenesis, and synaptogenesis. These cells also stimulate endogenous neural stem cell proliferation, promoting neurogenesis and promoting functional recovery [10]. BMMNCs secrete a variety of growth factors, such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor, and nerve growth factor, which contribute to neuroprotection and neuroplasticity [7,32,33]. These neurotrophins modulate synaptic and structural plasticity, thereby supporting learning and memory [34]. A study in rat models of cerebral infarction demonstrated that BMMNCs activate PI3K/AKT/NRF2 pathways, which directly stimulate cellular defense mechanisms, increase biliverdin expression, and elevate superoxide dismutase and glutathione peroxidase levels that are known to reduce oxidative stress. This process inhibits apoptosis and leads to improvements in various neurological functions [32]. Reduction of oxidative stress can ameliorate ASD symptoms such as communication difficulties, repetitive behaviors, irritability, and sensorimotor dysfunction [35]. BMMNCs also induce hypoxia-inducible factor 1-alpha, which increases VEGF uptake by cells, thus promoting angiogenesis [36]. In addition, these cells serve as immunomodulators by suppressing the production of proinflammatory cytokines such as TNF-α and increasing anti-inflammatory cytokines such as IL-10, reducing inflammation and alleviating symptoms associated with ASD [7,37]. MSCs, a subpopulation of BMMNCs, can release synaptic transmitters that reinforce E/I neural connectivity [23]. Studies have indicated that impaired brain connectivity may underlie behavioral symptoms and deficits in social cognition, language, and executive functions

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in autism [38].

Therefore, the diverse mechanisms of BMMNCs appear to collectively contribute to the amelioration of autism symptoms. Improved brain connectivity leads to better social relationships, emotional responsiveness, speech-language and communication, behavior patterns, sensory processing, and cognitive function. Reduced inflammation and restoration of the balance between inhibitory and excitatory neurotransmission result in decreased hyperactivity and aggression. Enhanced oxygenation and brain metabolism, along with improved neuronal function, can increase glucose metabolism in brain regions that previously exhibited hypometabolism, as observed in brain PET/CT scans.

The intrathecal route of administration was chosen because it is straightforward, safe, and relatively less invasive. Administered cells are mobilized directly to the site of injury via the cerebrospinal fluid (CSF) [31]. Cells in the CSF within the subarachnoid space travel into the perivascular space around blood vessels and subsequently enter the brain parenchyma by diffusion. Additionally, aquaporin-4 channels facilitate the entry of cells into the brain parenchyma [39,40]. Patients with autism have altered blood-brain barrier permeability, which enables transplanted cells to reach dysfunctional brain regions more effectively [41]. This route improves cell migration to the desired area and reduces unwanted entrapment of transplanted cells in other organs, such as the lungs, spleen, and liver [31].

Rehabilitation enhances neural plasticity and facilitates neuroprotection [42]. Exercise and physical activity are associated with the activation and proliferation of various cell types, as well as improved oxygenation and angiogenesis [43]. Rehabilitation aids in the mobilization and homing of transplanted cells to sites of damage and inflammation. The proliferation of these cells expands the available pool for mobilization [44]. Lv et al. [45] demonstrated that in patients with autism, intrathecal and intravenous transplantation of human cord blood cells combined with rehabilitation resulted in better outcomes than rehabilitation alone. Thus, we can postulate that cell therapy in combination with neurorehabilitation produces superior outcomes in patients with ASD.

Our two previously published clinical studies have demonstrated the safety and beneficial effects of BMMNCs combined with standard neurorehabilitation in cohorts of 32 and 254 patients diagnosed with ASD, respectively [17,18]. The first study, published in 2013, revealed statistically significant improvements on the ISAA and Clinical Global Impression-Improvement (CGI-I) scale at a mean follow-up of 12.7 months. On the CGI-II, 96% of patients demonstrated global improvement; few AEs were observed, including seizures in three patients, and were controlled with medication [17]. The second study, published in 2020, included 254 patients with ASD [18]. Following the intervention, symptoms were alleviated, and statistically significant improvements were observed in ISAA and CARS scores; furthermore, brain PET/CT scans showed improved brain metabolism. Other studies support these findings. Villarreal-Martinez et al. [46] and Nguyen Thanh et al. [47] reported that intrathecal administration of autologous BMMNCs in patients with ASD improved clinical outcomes without serious AEs. Kobinia et al. [48] also demonstrated that autologous bone marrow-derived cell administration is a safe therapeutic option, with no reported AEs, in patients with autism. Previous research further indicated that intrathecal injection of autologous bone marrow MSCs appears to be safe and feasible for treating children with ASD [49]. A large meta-analysis and systematic review published by Villarreal-Martinez et al. [46] in 2022 examined the safety and efficacy of various cell therapies in patients with ASD. The results suggested that cell therapy is safe and clinically beneficial, regardless of cell source, dosage, or delivery route [50]. While previous studies on autologous BMMNCs have shown improvement, most had small sample sizes, and some relied only on biochemical tests without clinical correlation [46,47,51]. In contrast, our study is unique in its large sample size and clinical correlation, supported by radiological imaging (PET/CT) evidence.

In the present study, BMMNCs were administered to 1,011 patients with ASD in combination with standard neurorehabilitation. The efficacy of the intervention was assessed using the ISAA and CARS outcome measures. For analyses of illness severity and degree of improvement, the ISAA was preferred, as the CARS does not include components addressing delayed response, cognitive abilities, or savant skills [13]. Statistically significant improvements were observed on both outcome measures following the intervention. Symptomatic improvements were noted in attention and concentration, command following, eye contact, sitting tolerance, social interaction, hyperactivity, communication, speech, stereotypical behavior, aggressiveness, and self-injurious behavior. All domains of the ISAA demonstrated statistically significant improvement after cell therapy. Previous studies have reported that individuals with autism exhibit abnormal neu-

roplasticity. A 2024 study by Chen et al. [52] summarized the impact of abnormal neuroplasticity and neuroinflammation on information processing, sensory processing, and social cognition. In our study, postintervention improvements in these areas suggest that cell therapy may contribute to symptomatic improvement by enhancing neural plasticity, promoting synaptic reorganization, reducing neuroinflammation, and modifying functional neural connectivity. Our findings are therefore consistent with existing research on neuroplasticity and neuroinflammation in ASD. Preclinical studies conducted in BTBR mouse models treated with cell therapy have also reported significant improvement in social interaction and reduced repetitive stereotypic behaviors, along with increased hippocampal neurogenesis and elevated brain-derived neurotrophic factor levels, indicating improved synaptic plasticity [53]. The observed symptomatic improvement also correlates with improved metabolism in brain PET/CT scans across nine distinct brain regions: amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, and superior and middle temporal poles, as shown in Supplementary Table 3. Thus, the capacity of cell therapy to reduce cerebral hypometabolism in patients with ASD appears to be a key contributor to clinical improvements.

Age-wise analysis of outcome measures (ISAA and CARS) revealed that patients across all age groups demonstrated statistically significant improvement. However, a greater proportion of patients under 10 years old showed improvement in scores compared to those older than 10 years. Significant improvements were also observed regardless of disease severity. The present study further demonstrated that a higher number of cell therapy doses was associated with better therapeutic effects.

One patient in the study group, who had shown improvement following cell therapy, developed severe jaundice after 10 months and subsequently died. This event was determined to be unrelated to cell therapy.

AEs were categorized as either procedure-related or cell therapy-related. Procedure-related AEs involved events associated with bone marrow aspiration and cell administration via lumbar puncture, including spinal headache, nausea, vomiting, pain at the site of aspiration or injection, back pain, fever, skin rashes, etc. In this study, 3.27% of patients experienced procedure-related AEs, all of which were managed with medication during their hospital stay.

In the present study, cell therapy-related AEs were reported in 8.3% of patients. These included increased hyperactivity (4.3%) and increased aggressiveness (3.1%). Seizures were also observed but were classified as AEs only if there was a new-onset or an increase in frequency or duration. Among the study cohort, 116 patients had a history of seizures prior to intervention, and 62 had abnormal EEG findings. Patients with a history of seizures or abnormal EEG were managed with a prophylactic antiepileptic protocol to reduce the risk of seizures following cell therapy [54]. Only nine (0.9%) of the 1,011 patients experienced seizures as an AE following cell therapy. Of these, four had a prior history of seizures, while five (0.5%) had new-onset seizures, all of which were managed with antiepileptic medications. The occurrence of seizures did not impact the overall outcome of the intervention, as these patients still demonstrated improvements.

This study lacks a control group to assess the effects of neurorehabilitation alone. However, since patients were already receiving rehabilitation prior to cell therapy, they may serve as their own controls. The number of female participants was lower than that of males. Comparative brain PET/CT scans could not be performed for all patients, as only 401 patients provided consent for a repeat scan. Future research may benefit from the inclusion of biomarkers of neuroplasticity and neuroinflammation.

This study demonstrates the efficacy of intrathecal transplantation of BMMNC therapy combined with neurorehabilitation in a large cohort of patients with ASD. Both symptomatic and objective improvements, as assessed by the ISAA and CARS scales and brain PET/CT scans, provide evidence for the effectiveness of this treatment approach. These effects result in a reduced degree of impairment and improved quality of life for patients with ASD. The study also suggests that multiple doses of cellular therapy may further increase the effectiveness of the intervention. In summary, intrathecal administration of BMMNCs appears to be a feasible and effective therapeutic option for autism.

ARTICLE INFORMATION

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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Supplementary Materials

Supplementary materials can be found via https://doi.org/10.4285/ctr.25.0009.

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