Review

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Regenerative Medicine

The promise of autologous and allogeneic cellular therapies in the clinical trials of autism spectrum disorder

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Autism spectrum disorder (ASD) is a consortium of developmental conditions. As scientists have not yet identified the exact underlying cause for these disorders, it is not easy to narrow down a singular therapy to propose a reliable cure. The preponderance of research suggests that stem-cell therapy improves aspects of outcome measure scales in patients with ASD; therefore, future studies should give us more confidence in the results. This overview considers the data that have emerged from the small set of published trials conducted using different approaches in stem-cell therapy for ASD, evaluates their results and proposes additional steps that could be taken if this field of endeavor is to be pursued further.

Tweetable abstract: Summary of the current trends and outcomes of clinical trials conducted using different autologous and allogeneic cellular approaches to investigate cellular therapies' use, safety and efficacy in treating autism spectrum disorder.

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'Autism' was initially used as a diagnostic term in 1943 to describe a particular syndrome seen in children who displayed typical symptoms such as impaired social and emotional connections at an early age [1]. Autism is defined by a substantial impairment in social communication as well as unusual repetitive and/or restricting behaviors or interests [2]. Henceforth, the term has come to be known as autism spectrum disorder (ASD), which is a neurodevelopmental disorder according to the definitions of the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, fifth edition) by the American Psychiatric Association and the ICD-10 (International Classification of Diseases, tenth revision) by WHO [2].

ASD includes several conditions that were previously considered separate entities. These include autism, Asperger's syndrome, childhood disintegrative disorder and an unspecified form of pervasive developmental disorder. Patients with ASD show a wide range of variations in symptoms, condition severity and functional disability [3]. Although scientists have not yet been able to determine the exact cause leading to ASD, it has been theorized that disturbances caused during the embryonic stages may be responsible [4]. ASD may be divided into two main symptom categories:

- Reduced social interaction;
- Repetitive behaviors, curiosities and aggressive actions.

Screening of all newborns is advised by the American Academy of Pediatrics for the presence of early indicators of autism. Autistic children can be diagnosed using evaluation scales such as the Autism Diagnostic Observation

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Schedule and the Autism Diagnostic Interview by qualified professionals together with the child's examination, taking into account their history and clinical appearance [2].

Early signs of ASD can include, but are not limited to [5]:

- Evasion of eye contact;
- Having little interest in other children or caretakers;
- Limited display of language (e.g., having fewer words than peers or difficulty with use of words for communication);
- Getting upset by minor changes in routine.

To date, autism is diagnosed on the basis of the core and linked symptoms such as observed behavioral indicators. ASD can sometimes be detected at 18 months or younger. Other comorbidities linked to ASD include intellectual disability, irregularities in EEG and MRI results with or without epilepsy, dysmorphic features and microcephaly (approximately 10% of the known autistic population) [2]. Comparatively larger head and brain size, especially the frontal lobes with smaller occipital lobes, seem to be characteristic to autistic patients. Recent studies have discussed the genetic correlation between ASD and cancer, with approximately 20% of genes identified as linked to autism also being known cancer genes [2]. Studies where more such relationships are explored and identified may be the key to finding safe and effective remedies for ASD patients [2].

It is difficult to identify a singular cause for the occurrence of ASD. Multiple changes – genetic, mitochondrial, metabolic, environmental and prenatal – have been discussed as having influence on triggering ASD [2]. ASD may be detected in children around the age of 18 months [6].

It is estimated that worldwide about one in 160 children has an ASD. This estimate represents an average figure, and the reported prevalence varies substantially across countries [7]. For 2018, across all 11 Autism and Developmental Disabilities Monitoring Network sites (the US states of Arizona, Arkansas, California, Georgia, Maryland, Minnesota, Missouri, New Jersey, Tennessee, Utah and Wisconsin), ASD prevalence per 1000 children aged 8 years ranged from 16.5 in Missouri to 38.9 in California. The median age of earliest known ASD diagnosis ranged from 36 months in California to 63 months in Minnesota [8]. It is believed that the prevalence of ASD is increasing in Asia. According to Qiu *et al.*, ASD prevalence in east Asia, south Asia and west Asia was 0.51, 0.31 and 0.35%, respectively [9]. For that study, the authors conducted a systematic review by searching English databases (Medline, Embase, Web of Science and Cochrane Library) from inception date to 6 August 2018 [9]. There are no reliable data available regarding the prevalence of ASD in Pakistan. However, according to the estimates of the Pakistan Autism Society, about 350,000 children are affected by ASD in Pakistan [10]. According to Pillay *et al.*, the prevalence of ASD in South Africa is unknown [11]. They conducted a database search of all children with ASD in the formal education system in the Western Cape province of South Africa and found a rate of 0.08%. Their data showed a 76.03% increase in ASD in schools between 2012 and 2016, with an average increase of 15.18% per year. For Europe, the mean prevalence was 80 per 10,000 population. For North America, the mean prevalence was 95 per 10,000 population. For Oceania, the mean prevalence was 112 per 10,000 population [12].

Traditional treatments for autism patients include speech therapies, social interaction training, applied behavioral analysis and use of psychotropic drugs [13]. Alternative therapies include hyperbaric oxygen management [14] music therapy, cognitive behavioral therapy and fast learning therapies [15].

The use of complementary and alternative medicine is common in children with ASD, despite the lack of research and potential side effects. Some regularly used complementary and alternative therapies, such as methyl B12, oxytocin, ginkgo biloba, secretin, hyperbaric oxygen therapy and chelation therapy, have been found to be ineffective [16]. Hence it is imperative that safer alternative therapies such as stem cell therapy be investigated and promulgated further. This review was carried out to get an overview of the current studies that have been registered as completed with clinicaltrials.gov and to get a clear picture of the current scenario regarding autologous and allogeneic settings.

Stem cells & their role in ASD treatment

ASD is a complicated range of illnesses that are based not only on genetic but also on epigenetic factors. It is mainly a developmental disorder that affects the neurological system of the patient. Due to the complex nature of these disorders, there is an increasing rise in the search for efficient therapies [17]. Research is ongoing to investigate the efficacy of mesenchymal stem cells (MSCs) obtained from adipose tissues and umbilical cord tissues, and their

by-product (exosomes), in treating neurodegenerative disorders such as Alzheimer's disease and neurodevelopmental disorders like ASD [18].

Researchers have yet to discover a mechanism to reverse ASD. Fortunately, stem cells with the ability to regenerate are demonstrating positive outcome measures in the therapy of ASD. Several previously conducted preclinical and clinical studies have been performed with improved medicinal effects [19].

Stem cell types in ASD treatment

Fetal stem cells

For therapeutic purposes, stem cells may be extracted from the various stages of human development. Fetal cells have the greatest self-renewal capabilities and grow fast when cultured. These cells are characterized by features that range between those of embryonic and adult stem cells. They self-renew faster in culture, are more pluripotent, have a higher capacity for engraftment and do not cause teratomas *in vivo* [20,21].

Neural stem cells from various fetal brain regions have been studied in order to cure neurological illnesses like ASD. The neural stem cells from fetal tissue contain the highest self-renewal rate, are multipotent and can be differentiated into neurons, oligodendrocytes and astrocytes. These cells release certain growth factors and suppress inflammatory cytokines in ASD treatments [22].

Mesenchymal stem cells

Most adult tissues contain MSCs with cell surface antigens like CD73 and CD79 for identification and the capacity to bind to plastic surfaces. The formation of osteoblasts, adipocytes and chondroblasts can be seen in culture with this differentiation [23]. These MSCs are able to proliferate and differentiate into germ layer (ectodermal and endodermal) cells and perform several immunomodulatory activities which mark them as a suitable candidate for cellular therapies [24,25]. ASD patients can be treated with these MSCs which produce paracrine secretions and are characterized by their immunomodulatory activities [26].

Different neurotrophic factors, including VEGF, BDNF and growth factors for neurons, with nerve-protective effects in cultured MSCs are showing most promising results [26]. MSCs have immunosuppressive properties and do not express molecules like CD80 and CD40. In the treatment of ASD, certain MSC mechanistic hypotheses have been proposed that include secretion properties, anti-inflammatory effects, plasticity, growth factors, survival ports and engraftment into functional neural networks [27].

For *in vivo* neurodegeneration studies in different animal models, MSCs are used because these cells use the neuroprotection to enhance and regulate the regeneration capacity by drafting endogenous stem cells and thus downregulating T, B and NK cells [28]. By looking at these properties, certain clinical trials have been conducted for neurological diseases like ASD [29].

Bone marrow stem cells

Bone marrow mononuclear cells (BMMNCs) are autologous cells which pose very little risk in terms of a negative autoimmune reaction and are widely available. This category of cells is commonly used because the bone marrow stem cell method of transplantation does not disqualify children who lack access to a preserved umbilical cord tissue or blood [30]. This cell population hosts a combination of hematopoietic and MSCs with individual efficacy in reducing core ASD symptoms. Researchers from Vinmec Research Institute of Stem Cell and Gene Technology, Vietnam, inferred from previously available data that as BMMNCs will have both these cell types, they may significantly aid in treatment of ASD core symptoms [31].

Adipose stem cells

These stem cells are obtained from the adipose tissues of the body in an invasive manner and are termed adipose-acquired stem cells. They are similar to MSCs in plasticity and cell surface antigen expression (CD90, CD73, CD44, CD166 and CD105, with no expression of CD45 and CD34). These cells have capacity to differentiate into three germ layers [32,33]. No completed clinical studies have been reported on clinicaltrials.gov for the use of these stem cells in ASD.

Umbilical cord-derived stem cells

Umbilical cord-derived mesenchymal stem cells (UCMSCs) are known as a good source of stem cells [20]. The umbilical cord Wharton's jelly and the amniotic fluid are characterized by high levels of growth factors while

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expressing antigens of MSCs in *in vitro* growth [34]. UCMSCs are derived from the perinatal extraembryonic tissues; because they have advantageous growth and plasticity properties over adult MSCs, they can provide potential benefits in studying autism [20].

Neural stem cells

Neural stem cells are found in fetal and adult human brain and have the ability to differentiate into major cell types of the CNS, neurons and their neural network [35]. These cells can be isolated from the two main regions of the brain (the subventricular zone of lateral ventricles and the subgranular zone of the hippocampus) and can be cultured [36,37]. In order to make these stem cells a perfect candidate for autism treatment, the neural stem cells are cultured. They are multipotent and can divide into nervous system integrations for homeostasis and neuroprotection.

Hematopoietic stem cells

According to research, the two important physiological changes that are connected with the degree of autistic symptoms are immunological dysregulation and neurological hypoperfusion [38]. Previous studies observed that hematopoietic stem cells (HSCs) prompted angiogenesis, facilitating blood perfusion to ischemic brain regions in animal models [39,40]. Studies have demonstrated benefits of HSCs and MSCs in autistic children due to decreased inflammation, facilitation in mobilization, proliferation and differentiation of stem cells in tissues and alleviation of ASD symptoms [41]. Children with ASD may live longer thanks to HSC therapies, which offer a comprehensive enzyme replacement that can help avoid neurological deterioration [41].

The aberrant control of the blood–brain barrier (BBB) seems to be a challenging aspect of immunological deregulation in ASDs. Although the roles of the BBB are complicated and poorly understood, it is evident that it makes and controls cytokines and acts as an immunological bridge between the CNS and the peripheral immune system [23]. A considerable number of endogenous HSCs have been discovered in the brain which continuously produce macrophages without interfering with the BBB [23]. By eliminating cellular waste products like myelin fragments, these macrophagic cells assist in maintaining the normal equilibrium of brain activity [23].

Transplanted stem cells have the ability to enter the CNS and maintain their differentiation potential [42]. It is obvious that the BBB allows stem cells from the blood to enter the CNS region, where they may perform their functions. Stem cells that have been developed into epithelial cells *in vitro* have been shown to have numerous BBB-linked characteristics, including properly arranged tight junctions, nutrient transporter expression and polarized efflux transporter activity [23]. These properties are very useful in restoring BBB disruption. In this way, in ASDs, transplanted stem cells could restore the BBB characteristics.

HSCs give rise to blood and immune cells and play a crucial role in regulating the immune system and regulating chronic inflammation. They have distinctive cell markers (CD34, CD59, CD90 and CD117) and were discovered in circulating blood, the spleen and the bone marrow. They exhibit self-renewal, recruitment and multipotent differentiation abilities because they can produce lymphoid and myeloid cells, which restock all blood cell types and maintain blood cell homeostasis [23].

The HSC response requires a number of inflammatory signaling molecules, demonstrating that the local microenvironment influences stem cell destiny in a meaningful way. In reality, elevated cytokine and hematopoietic growth factor levels in inflammatory processes cause HSCs to migrate and proliferate, leading to enlargement of the hematopoietic tissue.

HSCs may be drawn to the locations of significant inflammatory conditions by proinflammatory chemicals generated in ASDs, where these cells could exercise their anti-inflammatory effects. Their exact course of action is still unclear, but paracrine capacity has been observed in these cells [23]. Growth factors and cytokines released by stem cells promote tissue-resident stem cell recruitment, retention, mitosis and differentiation while suppressing the immune system's alterations and inhibiting apoptosis [23].

These stem cells reside in the bone marrow, in umbilical cord and in blood. Due to their residency in the umbilical cord and bone marrow, the self-renewal and regeneration capacities are very high. These stem cells can secrete bioactive molecules with the paracrine mechanism. They are used as a fast shuttle to reach the site of inflammation and hence can be used in ASD treatment [23]. Stem cell therapy is providing valuable insight for studying the pathology of autism with an in-depth clinical approach.

Clinical trials in autism studies with cellular therapy

Clinical trials hold importance in safety and efficacy studies for any disease. To date, a number of clinical trials have been carried out to evaluate the safety and effectiveness of stem cells for ASD using multiple types of methods. A search of the US NIH Clinical Trials database (clinicaltrials.gov) using the search term 'autism spectrum disorder AND cell therapy' was conducted in September 2022 and revealed 41 items. From these, only 20 were under the 'completed' category. Out of these, only ten used stem cells to treat ASD (Table 1), and eight have been successfully published so far, along with one case study (Table 2).

Autologous studies

Studies using BMMNCs

In an open-label investigation, researchers used autologous BMMNCs in 32 subjects (aged 3–33 years) diagnosed with autism [25]. Most studies investigating the impact of cellular therapies in ASD participants tend to have a more condensed age range. Given that ASD affects a person for the entirety of their life, a comparison of how transfused cells perform in ASD children versus adults, and what effects/consequences/improvements can be observed, can be vital information. The study was single-arm and no placebo control was employed. Intrathecal mode of delivery for therapy was used to transplant approximately 8.19×10^7 cells to each participant. The cells were isolated from the bone marrow and checked for viability and considered fine with $CD34^+$ marker. These cells were introduced using the intrathecal route of administration, and to enhance their survival, methyl prednisolone was injected intravenously. Follow-up to monitor adverse events was undertaken for a longer period (26 months) compared with some other registered studies to determine the safety [25]. The researchers conducted a proof-of-concept study; however, the participants also received other conventional therapies and interventions after receiving stem cells. Also, due to the lack of a control group, it cannot be conclusively stated that the transplantation of BMMNCs improved ASD symptoms in the participants. In addition, a control group can work as a benchmark to elucidate comparable differences between experimental groups based on safety and efficacy end points. Only vomiting, mild pain and nausea were determined as minor adverse events. According to the study, these events were related to the procedure protocols and not to the cell injection pathways. The participants were evaluated using the Indian Scale for Assessment of Autism (ISAA), Clinical Global Impression (CGI) scale, Functional Independence Measure and Wee-FIM (Functional Independence Measure for Children) scales. Results of the outcome from ISAA indicated enhanced eye contact, better social interaction/social smile, better approach towards learning, better attention to a call out and better time of response. A decrease in unusual noises when the participants spoke was observed. A decrease in the unstable emotional activities and exaggerated emotions with normal excitement to every event was recorded [25]. However, it is interesting to note that after conducting this study, where improvement in various clinical outcomes was observed, a consequent study with a bigger cohort was not published by the researchers.

The following published study was a case study on a single individual; it reported transplantation of autologous BMMNCs on a male autistic adult aged 25 years [43]. A dose of 1.45×10^8 BMMNCs (intrathecal) along with methylprednisolone (1 g in 500 ml Ringer Lactate, intravenous) was given. The study took into account the history of the participant, including the age of his mother at the time of birth, delivery mode and other details from different stages of his life. It is interesting to note that the seizures and other ASD core symptoms started manifesting after the participant started going to school aged 3 years. Because researchers have still not been able to determine specific root causes of ASD, studies like these that also monitor brain activity can contribute to further studies analyzing possible underlying triggers for ASD. Improvements in multiple domains of the ASD assessment scales were obtained with no major events; however, the researchers did not specify the parameters they used for adverse and serious adverse events. After the 6-months follow-up, there was improved concentration, stable sleep patterns, proper eye contact, good social interaction and improved memory; different scales (e.g., ISAA, Childhood Autism Rating Scale [CARS]) were used to determine the above-mentioned events. After 6 months, the patient showed improvements in the brain hypometabolism which were verified with PET scan [43]. The first 24–48 h after cell transplantation are crucial to observe for any adverse events; however, this follow-up, if taken, was not specifically mentioned by the authors. As cited by the researchers, the patient received antiepileptics from an early age and special schooling 2 years before his assessment for this study. However, it is not clear what this entailed. Whether the participant received "*personalized neuro-rehabilitative regime which included the same psychological intervention, special education occupational therapy, and physiotherapy*" as was administered in the study is not clearly mentioned. Hence it cannot be conclusively determined that the improvements shown in multiple facets of the outcome measures were significantly due to the cellular intervention.

Table 1. Registered clinical trials of cell therapies in autism spectrum disorder.

Trial reg. no.	Country	Sample distribution	Study design	Subject age (years)	Type of transplantation	Observed parameters	Ref.
NCT01343511	China	CBMNC: n = 14; CBMNC + UCMSC: n = 9; Control: n = 14	Nonrandomized, intravenous and intrathecal	3–12	Allogeneic	6-month follow-up on CARS, CGI, ABC, AEs and serious AEs	[53]
NCT01974973	India	BMMNC: n = 32	Single-arm, open-label, intrathecal	3–33	Autologous	6-month follow-up using ASD-specific scales and brain metabolism values using PET	[54]
NCT02627131	Vietnam	BMMNC: n = 24	Single-arm, open-label, intrathecal	3–16	Autologous	3 and 6 months follow-up on CARS; adverse events during and 6 month follow-up	[55]
NCT03225651	Vietnam	BMMNC and control (therapy + rehabilitation), n = 30	Nonrandomized, parallel, open-label, intrathecal	3–7	Autologous	A year-long follow-up of CARS, ADOS-2, Denver II, ISAA and AEs	[56]
NCT01638819	USA	UCB: n = 15; control: n = 15	Randomized controlled trial, intravenous	2–6	Autologous	Baseline and 6-month follow-up using ROWVT-4, EOWVT-4, VABS 2, ABC and the Stanford–Binet measure	[57]
NCT02176317	USA	UCB: n = 25	Single-arm, open-label, intravenous	2–5	Autologous	A year-long follow-up for AEs and serious AEs, VABS-2, CGI-I, CGI-S, the Stanford–Binet test, EOWVT, ABC, GI indications, PSI, eye tracking and EEG	[58]
NCT02847182	USA	UCB + control = 119 Control + UCB = 61	Randomized controlled trial, intravenous	2–7	Autologous, allogeneic	A baseline to 6 months follow-up for VABS-3, VABS-2, PDD-BI, CGI-S, EOWVT, AEs and serious AEs	[47]
NCT03099239	USA	Cohort 1: n = 3; cohort 2: n = 3; cohort 3: n = 6	Single-arm, open-label, intravenous.	4–9	Allogeneic	A baseline to 12 months follow-up on AEs and serious AEs	[59]
NCT03786744	Russia	UCB: n = 10 Control (standard therapy): n = 10	Non-randomized, parallel	3–15	Allogeneic	A 24-h follow-up of AEs and serious AEs. A baseline to 12-month follow-up for ATEC. A baseline to 6-month follow-up of cytokines and peripheral blood cells	[49]
NCT04710810	Russia	UCB: n = 15 Control (standard therapy): n = 15	Random, parallel, open-label, intravenous	3–11	Allogeneic	A 24-h follow-up of AEs and serious AEs. A 6-month follow-up of CASD, ATEC & WISC	[50]

ABC: Aberrant Behavior Checklist; ADOS-2: Autism Diagnostic Observation Schedule, Second Edition; AE: Adverse event; ASD: Autism spectrum disorder; ATEC: Autism Treatment Evaluation Checklist; BMMNC: Bone marrow mononuclear cells; CARS: Childhood Autism Rating Scale; CASD: Checklist of Autism Spectrum Disorders; CBMNC: Cord blood mononuclear cells; CGI: Clinical Global Impressions; EOWVT-4: Expressive One-Word Vocabulary Test; GI: Gastrointestinal; ISAA: Indian Scale for Assessment of Autism; PDD-BI: Pervasive Developmental Disorder Behavior Inventory; ROWVT-4: Receptive One-Word Vocabulary Test; UCB: Umbilical cord blood; UCMSC: Umbilical cord mesenchymal stem cells; VABS: Vineland Adaptive Behavior and Socialization; WISC: Wechsler Intelligence Scale for Children; PSI: Parenting Stress Index.

Table 2. Published clinical trials of stem cells in autism studies.

Autologous						Allogeneic					
Cell type	n / Age	Dosage / Route	AEs	Improvement	Ref.	Cell type	n / Age	Dosage / Route	AEs	Improvement	Ref.
BMMNC						**Hct-MSC**					
Study 1	32 / 3–33	8.19×10^7 cells / Intrathecal	Seizures, vomiting, mild pain, nausea	Social interaction and speech. Decrease in unstable emotions	[25]	Study 1	12 / 2–11	2.0×10^6 (TNC/kg). One subject received 1.79×10^6 TNC/kg due to a miscalculation / Intravenous	Two subjects faced AEs during or immediately after dose infusion. One subject had hypotension after the third dose. One subject had an adverse reaction (due to no diphenhydramine on insistence of parents)	Four participants experienced improvement in at least three categories of assessments. The other participants had improvement in one or two categories	[48]
Study 2	1 / 25	1.45×10^8 MNCs / Intrathecal	No major AEs	Cognition, social behavior, speech, reduced hyperactivity	[43]						
UCB						**UCB**					
Study 1	30 / 2–7	Min. 10 million (TNC/kg) / Intravenous	None	No statistically significant changes in the outcomes measured	[44]	Study 1	25 / 6–7	(250 ± 20 million cells) × 4 / Intravenous	Well tolerated. No significant AEs	Enhanced cognitive functioning and alleviation of autistic symptoms in the test group 6 months after the first dose. Test group significantly better than the control group	[60]
Study 2	180 / 2–7	$\geq 2.5 \times 10^7$ cells/kg / Intravenous	535 AEs were reported during the 6-month period (485 mild, 45 moderate, 5 severe). Childhood infections or surgical procedures or infusion reactions were frequently observed	Participants without ID showed significant improvement in communication skills (VABS-3 communication domain) and exploratory measures including attention to toys and sustained attention (eye tracking) and increased α and β EEG power	[18]	**UCMSC/CBMNC**					
						Study 1	37 / 3–12	2×10^6/kg CBMNCs and 1×10^6/kg UCMSCs / Intravenous/ intrathecal	Three participants receiving CBMNCs and two receiving both cell types had low fever. No serious AEs reported	The CGI scales showed statistically significant improvements in combined group compared with the control group	[52]
						FSC					
						Study 1	45 / 3–15	$>30 \times 10^6$/ml per transplantation / Intravenous	No AEs or serious AEs were reported. No transmittable diseases were noted for 1 year after treatment	Overall scores of ATEC, including social, cognition and behavioral subscales, showed improvement in participants who received treatment	[22]
Study 3	25 / 2–6	$1–5 \times 10^7$ cells/kg / Intravenous	92 AEs reported in 23 participants (allergic and skin reaction, vomiting and diarrhea)	VABS-II socialization, communication and adaptive behavior scores and the PDDBI, CGI-S, CGI-I and EOWVT and objective eye-gaze tracking showed improvement	[45]						

AE: Adverse event; ATEC: Autism Treatment Evaluation Checklist; BMMNC: Bone marrow mononuclear cells; CBMNC: Cord blood mononuclear cells; CGI: Clinical Global Impressions; EOWVT: Expressive One-Word Vocabulary Test; FSC: Fetal stem cells; Hct-MSC: Human umbilical cord tissue-derived mesenchymal stromal cells; ID: Intellectual disability; PDDBI: Pervasive Developmental Disorder Behavior Inventory; TNC: Total nucleated cells; UCB: Umbilical cord blood; UCMSC: Umbilical cord mesenchymal stem cells; VABS: Vineland adaptive behavior and socialization.

Studies using umbilical cord blood

In 2018 a phase I/II clinical trial was conducted using autologous umbilical cord blood (UCB) in patients with ASD by Sutter Pediatric Neurology (Sacramento, CA, USA) [44]. A total of 30 patients were enrolled for the randomized, triple-blinded (participant, care provider and investigator), placebo-controlled and crossover study. An age range of 2–7 years was maintained. The subjects were infused with autologous UCB and placebo (saline). The dosage used was a minimum of 10 million total nucleated cells/kg in one infusion of 60 ml of study product. Twenty-nine patients completed the post-treatment follow-up. In our opinion, this was a very well-planned and executed study. The researchers allowed for a good 6-month period before administering the crossover dose. The timelines for crossover and follow-up seem very appropriate for a study of this scale. The banked UCB was tested to match with their respective recipients and the viability of cells was measured to be more than 85% before administration. Two groups were formed in the study. Treatment for one group included the administration of UCB first and then saline. This order was reversed for the second group. The results were examined at baseline, 12 and 24 weeks after the first treatment, and then again 12 and 24 weeks after the second treatment. The authors reported a very detailed standard operating procedure starting from the point where the UCB was obtained to the processing in the lab before administration. A very thorough description was provided of how the masking was done for each party involved, from the use of garlic to mask the scent of dimethyl sulfoxide to covering all the injection equipment and placement of dimethyl sulfoxide in the room so that the participants remained unaware of what treatment they were receiving [44].

The outcome measures used included CGI, expressive and receptive one-word picture vocabulary tests, Stanford–Binet Fluid Reasoning and Knowledge and Vineland Adaptive Behavior and Socialization. According to the study, none of the participants experienced serious adverse events, nor did they have to be hospitalized as a consequence of cellular therapy during the period of the study [44]. The researchers noted positive results, especially in the outcome measures for social activities; however, when statistically analyzed, no significant changes were obtained in the outcomes. Nevertheless, it may be inferred from the study results that autologous UCB infusions may be safely used in ASD subjects [44]. As scientists, we recognize the impact of biodiversity among individuals; that is, how every individual reacts differently to various treatment types and dosages. Hence more such crossover studies, with ample time between each treatment, need to be carried out to efficiently determine the impact and efficacy of cellular therapy.

A safety study with 25 autistic children between the ages of 2 and 6 years for phase I clinical trials was conducted by Duke University. The participants selected had their own autologous UCB unit, and no masking was done. The researchers discussed the therapeutic utility of UCB by-products for the treatment of children with ASD. In this study, the intravenous mode of delivery for treatment was chosen [45].

A dose of 1–5 $\times$ 10^7 cells/kg was given to the participants. According to the authors, "*Intravenous fluids were administered at 1.5-times maintenance for 30 min to 2 h after the UCB infusion*" [45]. However, it was not specified what these fluids contained. The researchers had previously conducted a study investigating privately and publicly banked UCB units for quality in a small trial which had employed the same methodology as in this trial. However, they noticed that total nucleated cell count (TNCC), CD34 and colony-forming unit (CFU) figures in the previous trial appeared better [46]. It was also observed that bacterial contamination was present in one UCB unit, even though the sample was reported as sterile by the UCB banking facility.

The outcome measures were determined using behavioral and comprehensive tests (Vineland Adaptive Behavior and Socialization-II, CGI, Pervasive Developmental Disorder Behavior Inventory, Expressive One-Word Vocabulary Test-IV [EOWPVT-4], Behavior Assessment for Children – social skills subscale, Aberrant Behavior Checklist [ABC], Sensory Experiences Questionnaire, Repetitive Behavior Scale, Intelligence Scales [Mullen Scales of Early Learning or Stanford–Binet], Language Environment Analysis, Preschool Age Psychiatric Assessment, Gastrointestinal Symptom Inventory by the Autism Treatment Network and Parenting Stress Index) along with eye gaze tracking of social stimuli, EEG and brain MRI. These tests are multidimensional and have been used previously by researchers to effectively assess different core ASD symptoms. These tests were conducted at baseline and after 6 and 12 months, and questionnaires were taken from caregivers after 3 and 9 months of cell therapy. The researchers used predetermined categories for recording and analyzing adverse events (Common Terminology Criteria for Adverse Events v. 4.0). More than 90 adverse events were recorded; however, the ratio of events considered to be related to the administration of cellular therapy was low and involved mild and moderately severe incidents. After the evaluation of the adverse and serious adverse events for a whole year, the researchers found this treatment

to be safe for usage for ASD patients. The results indicated a general improvement in the symptoms specific to autistic children. These also included speech and verbal/nonverbal socialization skills. The researchers observed that participants with an initial lower score for nonverbal IQ had greater improvement in behavioral domains [45].

A phase II study was completed in 2019 by researchers at Duke University, using single intravenous autologous or allogeneic, unrelated UCB on 180 participants [18]. A minimum banked total nucleated cell dose of $\geq 2.5 \times 10^7$ cells/kg or $\geq 4/6$ HLA-matched allogeneic, unrelated UCB was used. This study was conducted after the researchers had found autologous UCB to be safe in the first phase of clinical trials using a smaller cohort. A better approach to check for the efficacy of UCB transfusion in ASD participants would have been to continue using only autologous UCB first. The crossover study was randomized with quadruple masking and consisted of two arms [47]. A quadruple (participants, care providers, investigators and outcomes assessors/research coordinators) masking study is one in which only the study's primary investigator will have information about the arms and their interventions. The participants in the first arm were infused with UCB first, followed by the placebo infusion. The participants in the second arm were injected with the placebo followed by UCB infusion. The participants were aged 2–7 years [18]. The researchers noted a nonsignificant association between UCB and improvements in the primary outcome (social communication) or the secondary outcomes, autism symptoms and vocabulary. It was also observed that there was no significant difference associated with the type of UCB infused. A side analysis on autistic children using stem cells from UCB of allogeneic origin was conducted. Significant recovery was observed for scores of CGI-I. However, the odds ratio for improvement was insignificant [18]. Significant improvement was observed in the communication activities involving toys and employing focus. The researchers observed insufficiency of the single dose of UCB toward alleviating symptoms of autism or improving social skills. Hence, the authors recommend further research on the efficacy of UCB for ASD [18].

Allogeneic studies

Studies using human umbilical cord tissue-derived mesenchymal stromal cells

A phase I/II study using human umbilical cord tissue-derived mesenchymal stromal cells (hCT-MSCs) was conducted at the Duke University Medical Center. The study design incorporated three cohorts whereby the participants received treatment in either one, two or three doses. The first trial was a phase I prospective, open-label and interventional investigation in which participants aged 2–11 years were given hCT-MSCs intravenously [48].

The cells for treatment were sourced from digested umbilical cord tissue that was cultured, cryopreserved and banked. The results were reported accumulatively after the phase II study was conducted. Patient outcome measures were taken in person right after the treatment administration and then after 6 and 12 months. At baseline and subsequent follow-ups, the complete blood picture and chemistries along with C-reactive protein, erythrocyte sedimentation rate, HLA typing, type and screen with Coombs test, humoral immune profile, immune reconstitution panel and anti-HLA antibodies were tested. Of the assessment outcomes recorded, only two participants did not show improvement for any measure. Four participants experienced improvements in at least three categories of assessments. The other participants had improvement in one or two categories [48].

Only some subjects experienced discomfort during the intravenous insertion and infusion, but because the participants were ASD patients, the need to stay in one room for the duration of intravenous treatment was a cause for annoyance. The authors cite one patient from the second cohort to have experienced mild hypotension and another patient from cohort three also to have experienced hypotension. However, as shown in Table 2 (Hct-MSC, Study 1), only one patient actually had hypotension. Accumulatively, 11 participants experienced multiple mild adverse events. However, these were not profound, and no direct link between these events and the treatment has yet been found. For seven other participants, aggression, agitation and other psychiatric adverse events, along with behavioral issues, were observed. The researchers observed increased adverse incidents with the higher dosages. However, this was not statistically significant. The authors concluded that hCT-MSCs may be used safely and feasibly in young children with ASD. The phase II part of this study included autologous stem cells [48].

Studies using UCB

A phase I and II, non-randomized, open-label, parallel assignment was conducted by Medical Center Dynasty Samara (Russian Federation) under the NIH registration number NCT03786744 [49]. A total of 20 ASD patients aged 3–15 years were selected. The arms of the study included a combination of cord blood mononuclear cell (CBMNC) injections and other conventional therapies and a control group treated with conventional therapies alone. CBMNC injections from different donors were used. Each dose consisted of 20–50 $\times 10^6$ MNC/kg.

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The protocol included three injections administered at monthly intervals. In the group receiving treatment, ten patients were given infusions with UCB. The control group with equivalent number of patients was treated with conventional therapy. The patients were evaluated primarily using CARS and the Autism Treatment Evaluation Checklist and by noting the adverse and serious adverse events. No participants faced serious adverse reactions, but six participants in the treatment arm and eight participants from the control group encountered adverse events. These events varied from hypersensitivity to infectious and noninfectious events of nervous, respiratory, thoracic and mediastinal origin [49]. Note that the data for this study were only taken from the available material on clinicaltrials.gov; a published journal article for this study was not available for review. Hence the reporting of any adverse events or improvements reported in the raw data presented in the clicialtrials.gov database needs to be statistically analyzed for significance.

Investigators from St Petersburg Bekhterev Research Psychoneurological Institute in the Russian Federation undertook a phase I trial to examine the safety of UCB cell administration in autistic patients aged 3–11 years, along with variations in social interaction, communication and cognition after intravenous transfusion of four doses of blood group-matched UCB stem cells [50]. The treatment was a randomized, open-label and parallel intervention carried out on 30 patients. The patients were first intravenously given 0.025 mg/kg clemastine, then they received four doses of UCBs at 2-week intervals. Usually, most related and serious reactions to treatment can be observed in the first 2-day window, so the 14-day gap seems to be an appropriate time. A single dose consisted of $250 \pm 10 \times 10^6$ viable cells. The control group received conventional therapy (applied behavioral analysis and speech therapy). The results of the study have not yet been posted on clinicaltrials.gov (as of 2021). The data taken for this study were also only taken from the available material on clinicaltrials.gov and a published journal article was not available for review; hence the reporting of any adverse events or improvements reported in the raw data presented in the clinicaltrials.gov database needs to be statistically analyzed for significance.

Studies using UCMSCs

Researchers from Shandong Jiaotong Hospital Jinan, Shandong (China) conducted a phase I/II clinical trial to measure the safety, feasibility and efficacy of UCMSCs and CBMNCs in autistic children. It was an open-label and parallel intervention in which 37 autistic subjects were enrolled. The cohort was divided into CBMNC and combined groups. The injected cells were isolated from the healthy umbilical cord and gifted UCB. CBMNCs and UCMSCs were administered intrathecally (2 ml) and intravenously (20 ml) in one treatment. The patients were assessed using CARS, CGI scale and ABC outcome measures at baseline and then at months 1, 2, 4 and 6 post-treatment. Especially given that two different cell types in different quantities were administered, at least a 12-month follow-up period would have been better [51,52].

Patients received both types of cells. At the time of cell therapy, no adverse event was measured. There were only five children who got low fever. The significant changes observed included improved social and behavioral withdrawals, enhanced eye contact, less emotional and aggressive response, adaptability, and less hyperactivation and unstable speech patterns. At 24-week follow-up, the results were compared with the control group, and considerably higher improvements were seen in the combination group. For the primary outcome measure (CARS), the cohort receiving both cell types observed significantly better results (37.9%) compared with the one receiving only CBMNCs (20.0%) and the control (13.7%). Similarly, for the CGI outcome measures, significant improvement was seen in participants belonging to the cohort receiving combined cell types compared with the CBMNC and control cohort for Clinical Global Impressions-Severity of illness (CGI-SI), Clinical Global Impressions-Global Improvement (CGI-GI) and Clinical Global Impressions-Efficacy index (CGI-EI). For the secondary outcome measure, there was a significant reduction in aberrant behavior among participants belonging to the cohort receiving combined cell types (59.9%) compared with the CBMNC (38%) and control (17.4%) cohorts. However, it is worth noting that the division of participants in this study was not equal for all the cohorts (n = 14, 9 and 14) and that the design of this study used not only different cell types but also two different routes of administration [51,52].

Studies using fetal stem cells

An open-label pilot study with 45 autistic children was conducted by Bradstreet *et al.* [22]. 39 male and six female children aged 3–15 years were recruited to investigate the safety and efficacy of stem cells in autism. An important point to be noted about this study was the presence of ethnic diversity in the participants (Great Britain, Italy, Canada, Kuwait, UAE, Poland, Serbia, USA and Georgia), allowing researchers to observe how fetal stem cell transplantation may be helpful for people from different origins, which may further be used in research regarding

personalized medicine. According to the study design, the participants were to keep using the conventional therapies they had been using; their core symptoms were assessed using Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule (ADOS), CARS and ABC prior to cell transplantation.

This study used fetal stem cells that were harvested from aborted human fetuses (5–9 weeks), which can were lawfully obtained in Ukraine. The donor samples were tested for any infection (bacterial, fungal, viral etc). On day 1, participants received 1.6 ml of HSC suspension with a nucleated cell count $>30 \times 10^6$/ml per transplantation from liver using intravenous administration. On day 2, progenitor cells from the fetal brain amounting to 2.12 ± 0.49 ml with a nucleated neuroprogenitor cell count $>8.70 \times 10^6$/ml per transplantation was given via the subcutaneous abdominal adipose tissues. The researchers noticed significant positive changes in participants. Improvements were observed in the calmness, eye contact and appetite of a considerable number of participants (78%). The pre-treatment values for these children were abnormal. No adverse events were observed in the participants treated with fetal stem cells. In the post-treatment follow-up no transmittable diseases were noted for up to 1 year. The post cellular therapy predicted a significant increase in $CD3^+$ T lymphocytes and $CD4^+$ T helpers to maintain the stable immunity. A decrease in $CD19^+$ B lymphocytes was recorded after the therapy [22].

Limitations

Clinical research on ASD using stem cells has revealed heterogeneity. It is currently difficult to develop a well-defined general agreement of practice to ensure maximum therapeutic effect while reducing side effects due to numerous discrepancies in clinical studies regarding the lack of control groups, subjects enrolled, unclear mechanisms, route of administration with different time points of infusion, cellular source and type, doses and outcome measures used. One of the most intriguing and important aspects of stem-cell therapy in ASD is its long-term safety and efficacy.

Another issue is the reproducibility and validity of cell treatments. There is currently no published evidence that repeated cell transplantation is effective in ASD, and clinical trials should be conducted. There is also increasing concern over the lack of research on socioeconomic and cultural factors related to diagnosis and treatment of ASD. According to Dawson *et al.* [45], economic status may influence the availability of cell therapy. It is assumed that some ethnic groups may not be able to afford the cost of biobanking.

To get a general overview of current progress in cellular therapies for ASD, only completed studies registered with clinicaltrials.gov were considered. It was observed that some of the studies performed had no or very limited published data available in the English language. It is possible that due to this limitation, some of the studies that may have been registered with other repositories were not included in this overview. Hence further studies investigating other sources of data would be beneficial in understanding the scope of cellular therapy for ASD patients.

Conclusion

Stem-cell therapy is an emerging treatment option for patients diagnosed with ASD. This review explored the studies conducted in different countries using bone marrow, UCB, hCT-MSCs, UCMSCs and fetal stem cells. Apart from some common minor adverse events such as vomiting, nausea and mild pain, the results of the trials propose this therapy to be safe for different age groups. It is important to note that the mode of transmission and dose of stem cells is imperative in result outcomes. However, it was observed that individuals react differently to the same treatment. Further research is needed to determine the efficacy of each stem cell type.

Future perspective

Even though multiple studies have been carried out using similar dosages and cell types along with methods of transplantation, there is a dearth of studies performed using a larger sample size. It would also be pertinent to note that not many phase II studies have been carried out for the different cell types. The authors would like to recommend researchers to carry out studies that also focus on observing how long the efficacy of stem cell transplantation remains in the body.

Experimental studies with a standard set of strategies are urgently required to investigate various avenues concerning better expectations and more prominent examples. More human studies showing comparable differences with control group standardization are expected to be directed in the future to demonstrate adequate viability and safety. More research on the pediatric population is required because little is known about long-term outcomes, and

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the follow-up period after current clinical trials is generally brief, implying that the long-term safety and efficacy of stem cell therapy must still be adequately investigated.

There is a dire need for a methodological approach using cellular therapies to compare community- and clinic-based samples, because this has the advantage of reducing the circularity of only focusing on individuals already diagnosed with ASD using current diagnostic tools that may be gender biased.

Additionally, it may be expected that in the near future, researchers will conduct experiments to find ways to eliminate/reduce the side effects experienced by patients receiving cellular therapy and to find avenues to improve their efficacy in ASD patients. Given that cellular therapies are a relatively new method of treatment, there are concerns among the public about their sources and possible side effects. However, as more studies with positive impacts on the lifestyle of ASD patients are conducted, it is possible that more people will invest in this treatment to make it more reachable, feasible and safe for a greater number of people.

Executive summary

Background

- Autism spectrum disorder (ASD) includes several conditions that were previously considered separately. These include autism, Asperger's syndrome, childhood disintegrative disorder and an unspecified form of pervasive developmental disorder.
- Cellular therapy is emerging as an alternative form of therapy to aid ASD patients.
- This overview focused on the current scenario of both allogeneic and autologous cellular therapies.

Stem cell types in ASD treatment

- Stem cells may be sourced from different regions of the human body in different stages of life. The ability of the cells to proliferate changes in each cell type.
- The cell types used include fetal stem cells, mesenchymal stem cells, bone marrow stem cells, adipose stem cells, umbilical cord-derived stem cells, neural stem cells and hematopoietic stem cells.

Clinical trials in autism studies

- A search of the US NIH Clinical Trials database (clinicaltrials.gov) using the search terms 'autism spectrum disorder AND cell therapy' was conducted in September 2022 and revealed 41 items.
- From these, only 20 were under the 'completed' category. Out of these, only ten used stem cells to treat ASD and eight have been successfully published so far, along with one case study.

Autologous studies

- Two studies using bone marrow stem cells were reported.
- Three studies using umbilical cord blood were reported.

Allogeneic studies

- One study using human umbilical cord tissue-derived mesenchymal stromal cells was reported.
- One study each was reported for umbilical cord blood and fetal stem cells.
- A single study using a combination of umbilical cord mesenchymal stem cells and cord blood mononuclear cells was reported.

Limitations

- Long-term safety and efficacy, reproducibility and validity of cell treatments limit the cellular therapy scenario.
- Lack of data and unavailability of studies in English and the singular source of data were limitations of this study.

Conclusion

- Apart from some common minor adverse events such as vomiting, nausea and mild pain, the results of the trials propose this therapy to be safe for different age groups.
- It is important to note that the mode of transmission and dose of stem cells is important in result outcomes.
- Individuals react differently to the same treatment. Further research is needed to determine the efficacy of each stem cell type.

Future perspective

- More controlled studies with a larger sample size need to be conducted.
- More phase II studies using different cell types are needed.
- Studies are also needed that focus on observing how long the efficacy of stem cell transplantation remains in the patient body.
- As more studies with positive impact on lifestyle of ASD patients are conducted, it is possible that more people will invest in this treatment to make it more reachable, feasible and safe for a greater number of people.

Author contributions

S Shamim wrote the original draft and prepared the tables. N Khan revised the draft and supervised the team. D Greene reviewed and revised the draft. U Habiba and A Umer assisted in the collection and writing of the original draft.

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•• **The researchers noticed a significant improvement in the treatment group 6 months after transplantation, including improvement in cognitive functioning, an aspect of autism which is still a cause for concern for researchers. Long-term effects of this therapy are still being investigated.**