

# Stem Cell Therapy in Autism Spectrum Disorders

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## INTRODUCTION

Autism is a very complex neurodevelopmental disorder. Different researchers have tried understanding the basic pathophysiology of autism. It is understood now, that the neural hypoperfusion and immune dysregulation are the two key pathologies associated with Autism. There is reduced blood flow to certain specific areas of the brain (mesial temporal and cerebellum), which in turn could be the cause of reduced functioning in these areas. This coupled with an overall imbalance in the activity of the brain, is possibly responsible for the manifestations associated with autism.

Based on the above understanding, many scientists all over the world, such as, Ichim et al from USA and Siniscalco from Italy (in various scientific reviews and publications) have strongly emphasized the potential of stem cells for the treatment of autism. These proposals are in view of the fact that stem cells have strong angiogenic potential which could facilitate counteractive processes of improving perfusion and balancing inflammation by immune regulation which would exhibit beneficial clinical effects in patients with autism. Other contributing effects of stem cells, which have been proposed are, strong immunosuppressive activities as well as paracrine effects to stimulate neuronal function via growth factors, such as BDNF, VEGF, NGF and PDGF.

Going ahead from this, several groups have studied the effect of stem cells on the course and prognosis of autism in children and adults. The evolving results and information from these studies is revolutionizing the approach to autism and its treatment.

The very first open labelled proof- of- concept study published in 2013 by Sharma et al., not only showed encouraging clinical improvements, wherein children with autism could be mainstreamed, but they also put forth objective evidence based on PET-CT brain scan studies. [2] Subsequently, two more clinical studies from China and Ukraine, were published showing similar improvements [3,4].

With the worldwide growing prevalence of ASD (1 in 45 to 1 in 68), establishing a therapeutic strategy has become the need of the hour. ASD is characterized by deficits in communication, socialization and repetitive behavior. ASD includes a range of clinical presentation from mild (Asperger's Syndrome) to severe (Autism) . The cause of all these symptoms is basically the malfunctioning of the brain. Certain specific areas of the brain are implicated by various neuroimaging studies eg. medial temporal lobe, frontal lobe and thalamus. Depending upon which areas of the brain are affected and the severity of damage, the patient will exhibit the spectrum of symptoms. Hence, conceptually if the abnormal areas of the brain are repaired, it should lead to alleviation of the symptoms in autism. The conventional treatments are mainly behavioral, nutritional and pharmacological, which mainly focus on suppression of neuropsychiatric symptoms. But these treatments fail to address the core pathologies of brain abnormalities in ASD. [5] Stem cell therapy emerged as an effective therapeutic option for ASD due to its ability to repair at a cellular level. Stem cell therapy has already proved its therapeutic potential in various incurable neurological disorders such as cerebral palsy, brain stroke, spinal cord injury, head injury, etc. [6-14].

It is of utmost importance that we understand in depth, the mechanism of action of stem cells in ASD. In this chapter, we describe the various postulated pathways through which the stem cells exert their effect. We have also summarized the worldwide clinical review and enumerated our results involving cases of ASD administered with autologous bone marrow mononuclear cells, intrathecally. We have also discussed different types of stem cells, routes of administration of cells and various factors which can influence the outcome of stem cell therapy.

## **BASICS OF STEM CELLS**

Stem cells are blank, undifferentiated cells defined by their ability to self-renew and differentiate into mature cells. They are highly proliferative and can cross-lineage restriction boundary and give rise to cell types of other lineages. This unique property of stem cells is known as plasticity [15].

Based on their developmental potential they are classified into totipotent cells (which can produce any type of cell of the human body along with extra-embryonic cells), pluripotent cells (which can give rise to the three germ layers viz. endoderm, mesoderm and ectoderm), multipotent

cells (which can give rise to a specialized subset of cells), oligopotent cells (which can give rise to a restricted subset of cells) and unipotent cells (which can give rise to cells of only their type) [16].

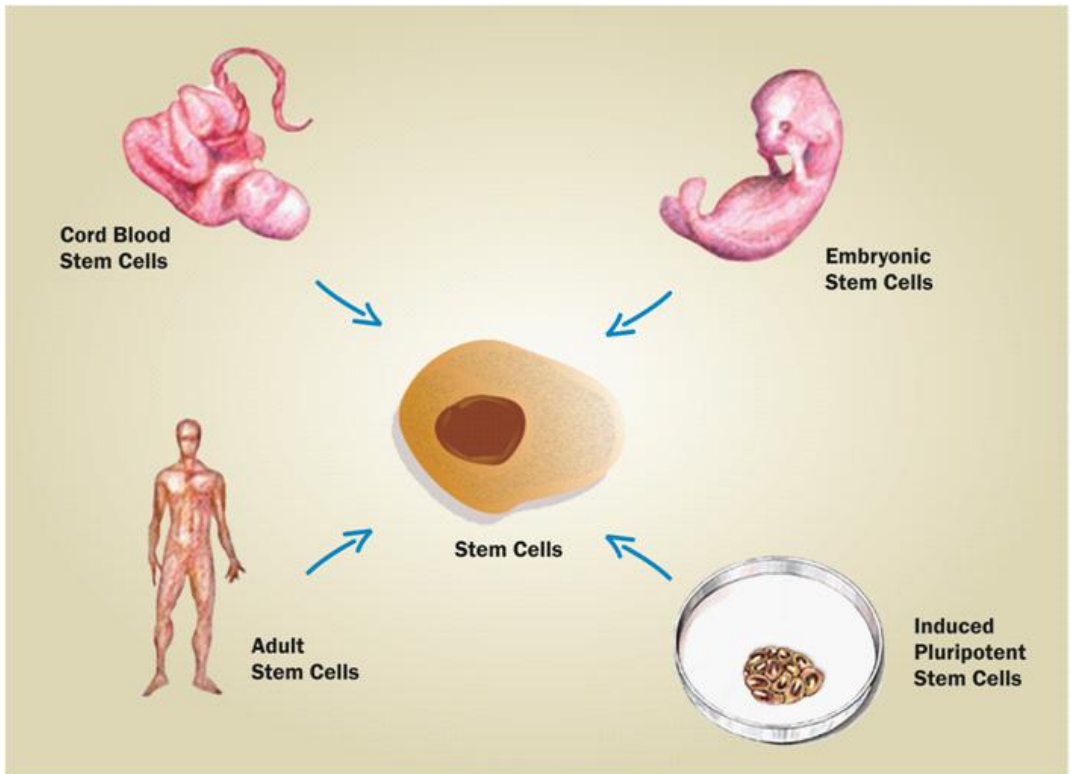
## TYPES OF STEM CELLS

Based on their source they are classified into adult, umbilical cord, embryonic and induced pluripotent stem cells. Here we describe each one of them in detail.

### Adult Stem Cells

Adult stem cells are multipotent stem cells found in fully developed tissues. The major function of these cells is to facilitate maintenance, repair and restoration of the body tissues by cell replacement and tissue regeneration [17]. These cells are usually present in dormant state within the tissues and become active only when required. However, they maintain their cell pool by self renewal. These cells are found in various regions of the adult individual such as bone marrow, adipose tissue, skin, eyes, brain, liver, pancreas, etc. These cells are safe, do not involve any ethical issues and are easily obtainable in abundance [18]. Hence, extensive research is being performed globally to study the potential of these stem cells. Clinically, bone marrow stem cells are one of the most preferred types of cells due to their safety profile.

Bone marrow stem cells comprise of a mixture of hematopoietic cells, mesenchymal stromal cells (**MSC**), endothelial progenitor cells (**EPC**) and very small embryonic-like cells (**VSEL**) amongst others. Studies have demonstrated the advantage of using a cell mixture over sub-fractionated cells. It imparts the cumulative benefits of all the cell types. These cells can differentiate into various cell types such as muscle cells, neural cells, etc. They promote angiogenesis, immunomodulation, neuroprotection and produce several cytoprotective growth factors and cytokines [19]. Bone marrow derived stem cells have been used for their therapeutic potential in various incurable disorders such as brain stroke, cerebral palsy, head injury, spinal cord injury. Studies involving use of bone marrow stem cells in ASD have been enumerated below.



**Figure 1:** Types of Stem cells.

## Umbilical Cord Blood Stem Cells

Umbilical cord blood contains a heterogeneous population of stem cells. It mainly comprises of hematopoietic stem cells (**HSCs**), multipotent non-hematopoietic stem cells and Mesenchymal stem cells (**MSCs**) [20]. Advantages of using umbilical cord blood stem cells are that they are available abundantly, their use does not involve any ethical or moral controversies, they can be collected via non-invasive procedures and they are highly proliferative [21]. However, the major disadvantage of using these cells is the number of stem cells available per cord unit is low as compared to other sources which could affect its clinical outcome. [22] Use of autologous UCBCs is fairly safe however, few studies have reported incidence of Herpes virus and JC virus infection by allogeneic UCBCs transplantation [23,24]. It has also been recorded that incidence of life threatening opportunistic/viral infections is high in the first 6 months of allogeneic UCBC transplantation [25]. A study demonstrating the use of these cells in autism patients has been discussed in detail below.

## Embryonic Stem Cells

Embryonic stem cells are derived from the inner cell mass (**ICM**) of pre-implantation blastocysts which are 4-7 days old. They are highly proliferative in nature and are capable of differentiating

into any cell type [26]. In spite of these being the most potent type of cells for transplantation, they are involved in moral and ethical issues [27]. The potential of tumor formation is one of the major safety concerns in the use of human embryonic stem cells. These cells are known to possess tumorigenicity and form teratomas which could be fatal [28]. These issues hinder the clinical applications of embryonic stem cells and make them non viable for therapeutic use.

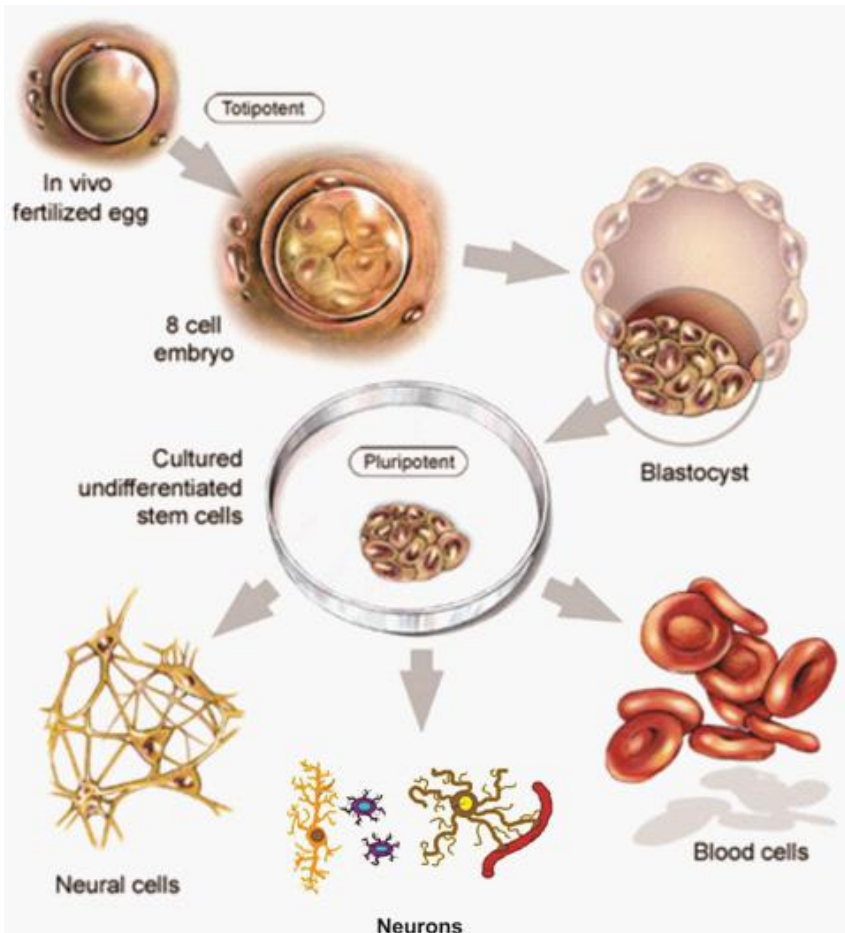
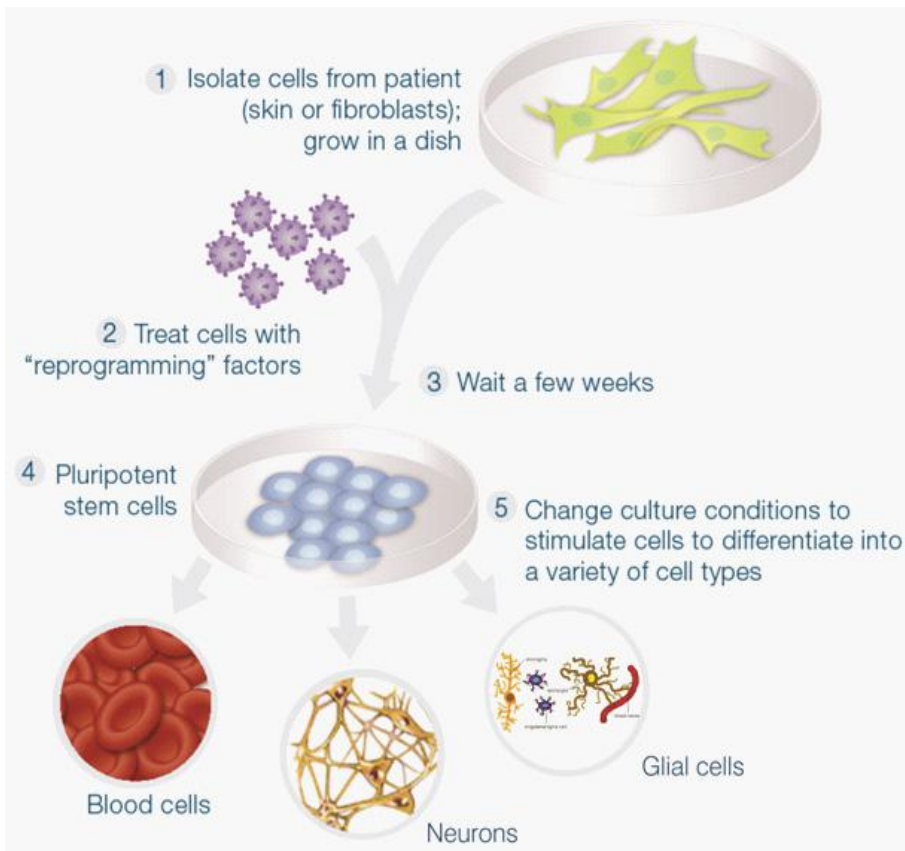


Figure 2: Embryonic Stem cell.

## Induced Pluripotent Stem Cells

Induced Pluripotent Stem cells (**iPSs**) are generated from adult somatic cells by genetically reprogramming them. These mature adult cells originally have a limited differentiation potential but, upon reprogramming, they gain plasticity and become pluripotent with ability to give rise to all cell types [29]. iPSC transplantation protocol is patient specific hence bypasses the need of immune suppression. However, the processes to generate iPSCs and their fate are still being investigated due to the threat of uncontrolled differentiation, genetic damage and malignant growth [30].



**Figure 3:** Procedure for induced pluripotent stem cell.

## ROUTES OF ADMINISTRATION OF STEM CELLS

To achieve optimum potential from stem cell therapy, selection of a suitable route of administration is equally important as selection of type of cells. Intrathecal, intravenous and intracerebral transplantations are three routes through which stem cells can be delivered to the brain in an individual with autism.

### Intrathecal route of administration

Intrathecal route of administration is a minimally invasive procedure involving delivery of cells through lumbar puncture. This mode of injection allows efficient delivery of cells as it is the closest environment to the nervous system with the least chance of causing any long term harm to the patient. Tracking studies have confirmed that the intrathecally injected cells travel to the target tissues via CSF [31]. In autism, various findings have suggested altered permeability of blood brain barrier allowing the stem cells to migrate to the affected brain areas [32]. Its safety and feasibility has been established in neurological disorders via many experimental and clinical studies [33-36].

## Intravenous route of administration

Intravenous route of administration is the most minimally invasive and a safe system of delivery of cells. However, a chief limitation of this mode of transplantation is not all the cells which are delivered reach the target site. Majority of the cells get trapped in the pulmonary passage impeding the potential of cell migration to the targeted area [37]. This reduces the efficacy of the stem cell transplantation.

## Intracerebral transplantation (stereotactic method)

This method is intended to deliver the stem cells into the brain at the site of damage or dysfunction through high precision stereotactic method. Though, this would theoretically be the most efficient way of stem cell transplantation, it also becomes the most risky method. Possibility of damaging the normal tissue on the way is one of the major worries.

## Adverse Events

Evidence suggests occurrence of teratomas after use of embryonic stem cells and viral infections after umbilical cord stem cells. Use of allogenic stem cells may also have a risk of immunogenic reactions.

The use of autologous BMMNCs has been found to be relatively safe in autism. There is a study that has reported small incidence of seizures as an adverse event in children with autism who had previous history of seizures or abnormal EEG. In the second part of the same study it was shown that adverse event of seizures can be prevented by prophylactic antiepileptic medications.

Occasionally, there are also minor procedural adverse events associated with cell aspiration and route of administration. For intrathecal route these may include nausea, vomiting, headache, backpain, etc which can be easily treated using medications.

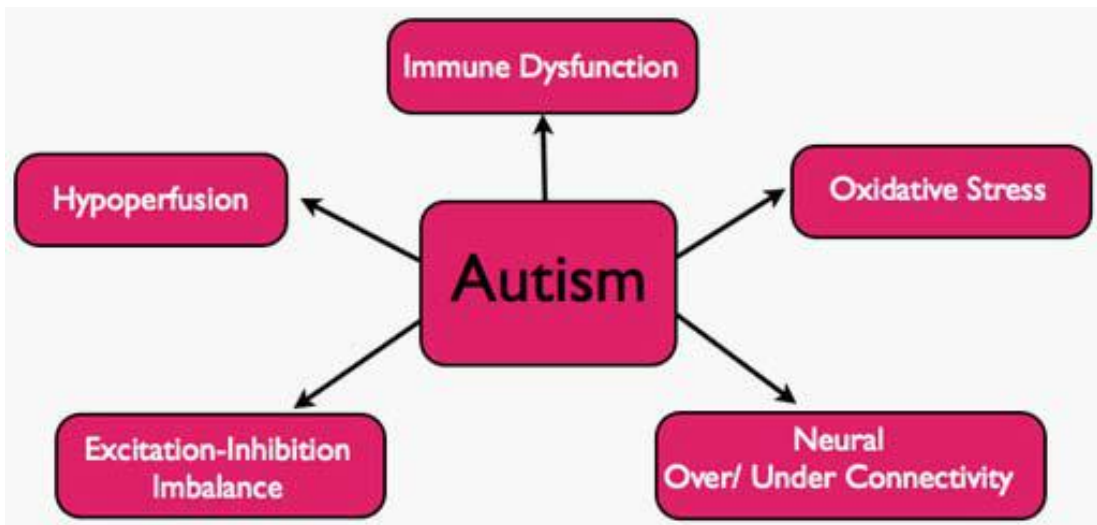
Adverse events related to procedures, such as in stereotactic surgery also needs consideration.

Hence, while using stem cells in autism, two variables need to be factored in a) the type of stem cells used and b) the route of stem cell transplantation. The safety and efficacy of the stem cell transplantation process is dependent on these factors.

## Pathophysiology of Autism Spectrum Disorders

To understand how stem cells work in autism, we must first understand the underlying pathophysiology of autism. There is published data which attributes its pathogenesis to damaged neural circuitry, disruption in excitation-inhibition balance, oxidative stress, inflammation, immune dysfunction and hypoperfusion [38,39].





**Figure 4:** Pathophysiology of Autism Spectrum Disorders.

Neural connectivity plays a vital role in normal brain functioning. There are theories which state early brain overgrowth and neural overconnectivity as key pathological factors leading to autism [40] But, conversely, other theories have also postulated reduced intracortical connectivity i.e. underconnectivity to be responsible for autism which may lead to lesser information processing [41]. This abnormal neural connectivity is responsible for the defective functioning of the brain in autism.

Cellular imbalance of excitation and inhibition ratio within the neuronal systems could give rise to cognitive and social deficit in autism [42]. Abnormal synaptogenesis can affect the balance of excitation/inhibition ratio during development [43].

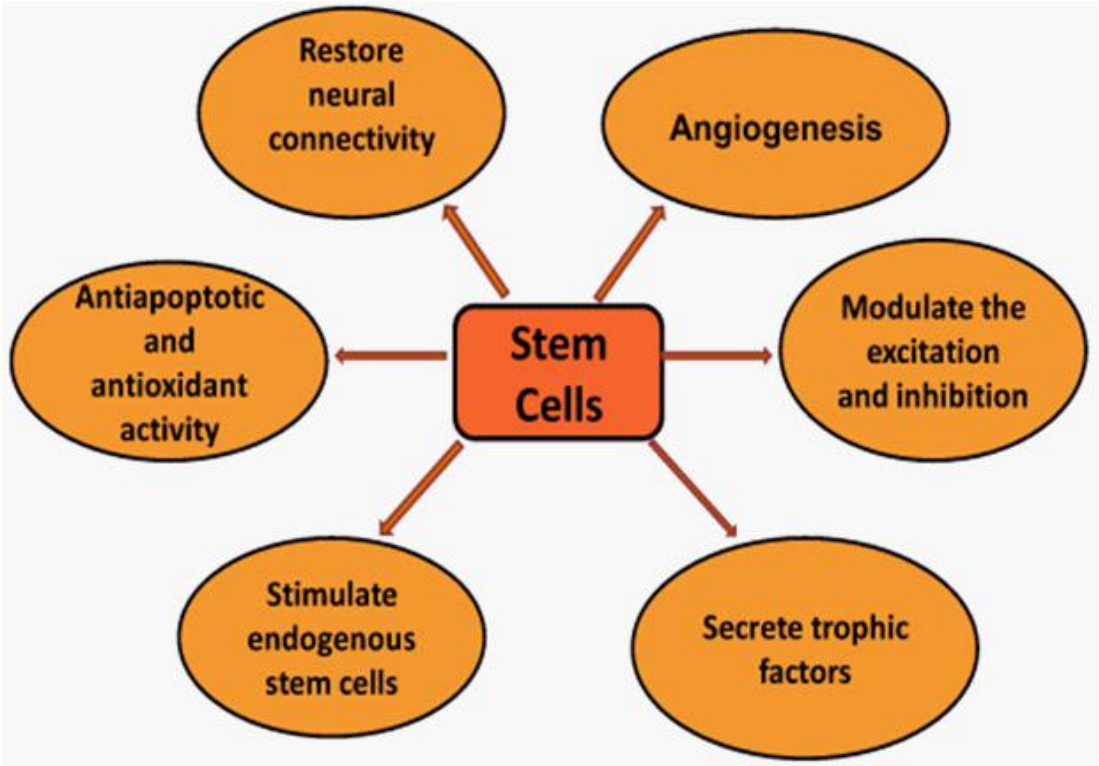
Myelination defects have also been implicated in recent studies. White matter is composed of neuronal matter coated with myelin sheath. Defect in myelination may affect learning, cognition and memory [44].

Neuroinflammation and oxidative stress are postulated to play a major role in pathology of autism. Microglial activation has been observed in autism, which may increase inflammation through release of proinflammatory cytokines and free radicals [45]. Proinflammatory cytokines such as TNF-alpha, IL-6, IFN-gamma, IL-8 are found to be elevated in autism. Increased levels of free radicals may result in oxidative stress. Number of studies have reported oxidative stress to be associated with autism [46].

Hypoperfusion has been linked to autism through various neuroimaging studies. Hypoperfusion could lead to hypoxia along with abnormal metabolite and neurotransmitter accumulation causing toxicity. A PET study in 21 children showed significant hypoperfusion in temporal lobes in 76% patients [47]. Similar result was also observed in a SPECT study reported by Gendry Meresse et



al., in 2005 involving 45 autism patients [48]. Kaya et al, in 2002 also reported hypoperfusion in frontal, frontotemporal and temporo-occipital along with temporal regions [49].



**Figure 5:** Mechanism of Action of Stem Cell Therapy in Autism Spectrum Disorders.

### Mechanism of Action of Stem Cells

Various human studies have suggested potential role of stem cell therapy in addressing the above mentioned mechanisms associated with autism. Stem cells, when injected, migrate to the target tissue and differentiate into mature cells. Along with regenerating and restoring the neurons and glial cells, they have neuroprotective effect.

#### Reduce inflammation

These cells secrete trophic factors which may reduce inflammation, stimulate endogenous stem cells and promote repair. Stem cells have shown immune modulating and neuroprotective effects. They inhibit microglial activation and reduce the production of proinflammatory cytokines TNF-alpha and IFN-gamma which are elevated in autism by producing immune inhibitory factors such as IL-10 and TGF-beta which helps in decreasing inflammation [50,51].

#### Restore neural connectivity

They promote neuroprotection and participate in functional recovery by directly integrating

into the neural circuitry. They possess property of synaptogenesis which includes new synapse formation and balancing synaptic neurotransmission. Stem cells also modulate the excitation and inhibition of neurons by controlling secretion of neurotransmitters. Thus they re-establish neural connectivity in the brain of autism. This improves the information processing and results in better brain functioning [52].

## Angiogenesis

Stem cells reverse the hypoxia caused due to hypoperfusion in autism through angiogenesis. These cells secrete cytokines, which through paracrine activity stimulate endogenous cells, promote angiogenesis and trigger differentiation of tissue specific cells, in this case, endothelial cells. The formation of new blood vessels increases the blood flow consequently, improving the oxygen supply in the brain and reverse hypoxia [53].

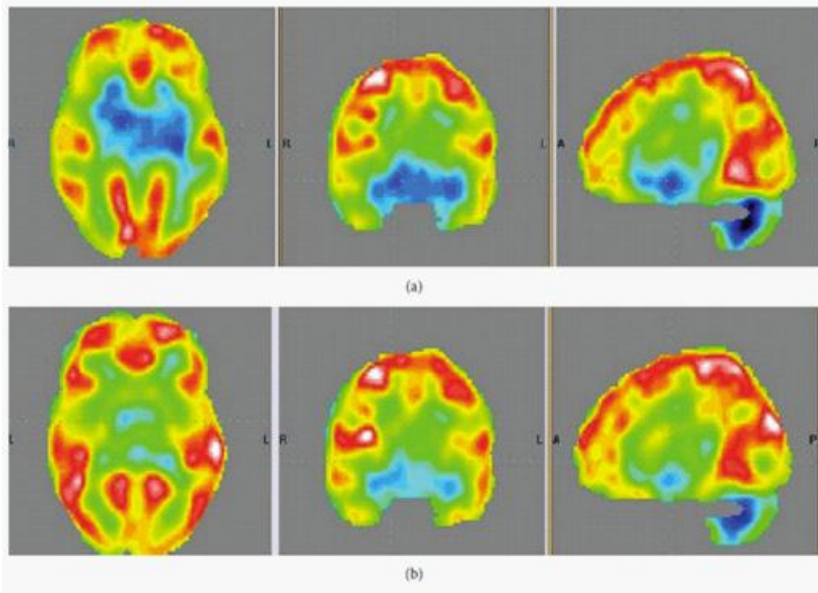
## Antioxidant activity

Stem cells have demonstrated antioxidant protection which reduces oxidative stress noted in autism. Stem cells exert antioxidant effect by reducing the superoxide production [54].

Worldwide published literature on clinical studies of effect of stem cell therapy in autism

There are numerous ongoing clinical trials being conducted worldwide to study the benefits of stem cells in autism. Though, only three case series and six case reports have been published so far, all the studies as well as individual case reports indicate significant clinical improvements based on standardized assessment scales, which point towards a shift towards functional improvement in an individual with autism. The case reports, in addition, also reveal the possibility of objective understanding of the changes that stem cells bring about in the brains of these individuals, viz a viz, use of PET CT Scan brain.

The world's first publication demonstrating the effect of autologous bone marrow mononuclear cells administered intrathecally, was published by Sharma et al, in 2013. They administered 32 patients and combined cell therapy with neurorehabilitation. All patients were followed up for 26 months (mean 12.7 months). Indian Scale for Assessment of Autism (**ISAA**), Clinical Global Impression (**CGI**), and Functional Independence Measure (**FIM/Wee-FIM**) scales were used as outcome measures. Out of 32 patients, a total of 29 (91%) patients improved on total ISAA scores and 20 patients (62%) showed decreased severity on CGI-I. The change in the scores was statistically significant. On CGI-II 96% of patients showed global improvement. Symptomatically, patients showed improved eye contact (70%), social interaction (55%), emotional responsiveness (56%), speech and communication (78%), sensory aspects (44%), attention and concentration (71%) and reduced hyperactivity (71%) and aggressiveness (48%). Along with improvements in symptoms and outcome measures, objective changes were recorded on Positron Emission Tomography-Computed Tomography (**PET-CT**) scan brain. The results of this open labelled proof of concept established the safety and efficacy of stem cell therapy in autism [2].



**Figure 6:** On comparing the PET CT scans, it shows increased FDG uptake (indicating improved metabolism and function) in the following areas: superior temporal gyrus, amygdala, fusiform gyrus (social brain) bilateral frontal, temporal, parietal and occipital lobes, bilateral cerebellar lobes, bilateral basal ganglia, hippocampus and parahippocampus.

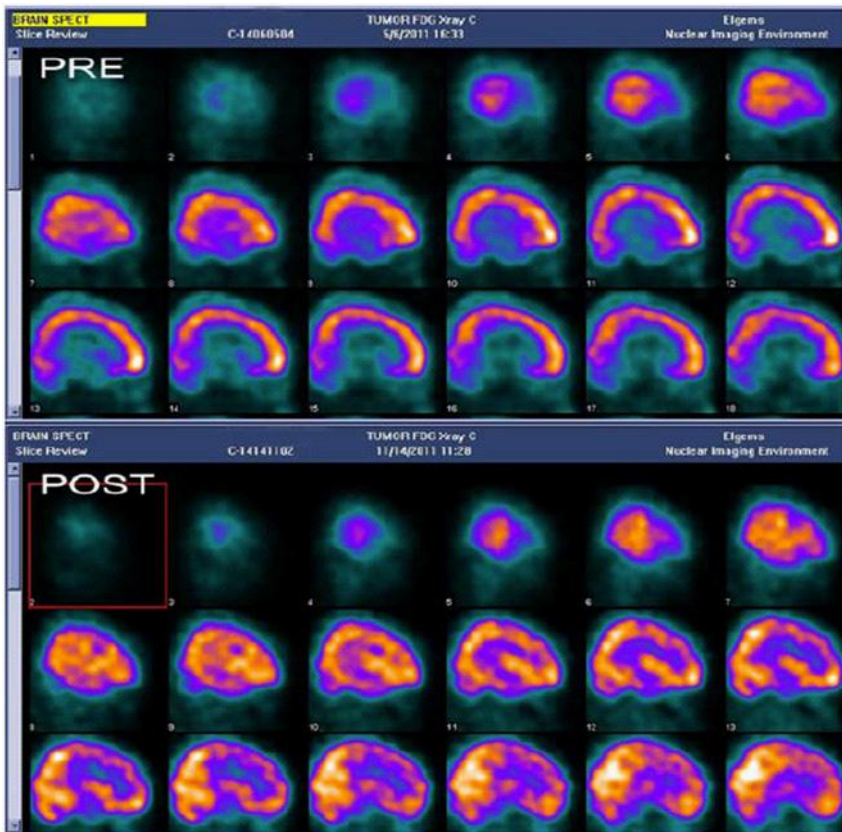
Yong-Tao L V, China simultaneously, published a study in 2013, which demonstrated the safety and efficacy of human umbilical cord mesenchymal stem cells (**hUC-MSCs**) and human cord blood mononuclear cells (**hCB-MNCs**) transplantation in autism. This study comprised of 37 patients divided into three groups: CBMNC group (14 subjects, received CBMNC transplantation and rehabilitation therapy), combination group (9 subjects, received both CBMNC and UCMSC transplantation and rehabilitation therapy), and control group (14 subjects, received only rehabilitation therapy). Transplantations included four stem cell infusions through intravenous and intrathecal injections once a week. They used the CARS, CGI scale and Aberrant Behavior Checklist to assess the therapeutic efficacy. Statistically significant differences were shown on CARS, ABC scores and CGI evaluation in the two treatment groups compared to the control at 24 weeks post treatment. They concluded that transplantation of CBMNCs demonstrated efficacy compared to the control group; however, the combination of CBMNCs and UCMSCs showed larger therapeutic effects than the CBMNC transplantation alone [55].

Following the above two studies, Bradstreet et al, Ukraine in 2014, published their study using fetal stem cells in autism. The study was carried out on 45 children with autism. On follow up after 6 months and 12 months, there was a significant change in Autism Treatment Evaluation Checklist test and Aberrant Behavior Checklist (**ABC**) scores. Improvement was also seen in behavior, eye contact, appetite, etc [64].

Apart from the above studies, case reports of individual patients, with varied clinical profiles, also add to our understanding of how stem cells can be effective in autism.

Sharma et al, India have also published case reports showing evidence of stem cell therapy resulting in functional recovery [56-62]. These cases have been described below.

A 14 yr old boy with severe autism was administered with autologous BMMNCs, intrathecally and followed up after 6 months and 1 year. He showed significant functional improvements in his behavior, social interaction and emotions. Aggression and hyperactivity had reduced. Improvement in impulse control, reading skills, tracing, recognition of all shapes, learning new tasks and command following was also noted. His score on CARS reduced from 42.5 (Severely autistic) to 23.5 (No-Autism). On repeating brain PET scan after 6 months, there was marked increased uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex [57].



**Figure 7:** The Pre and the Post cell therapy PET CT scans. Comparative study of pre and post cell therapy PET CT scan shows increased FDG uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex. (Increase in orange areas in the post cell therapy scan).

A case of an adult with autism (33 year old), administered with autologous BMMNCs twice at an interval of 6 months was also reported. Over a period of 9 months, his ISAA scores reduced from 94 (Mild autism with 60% disability) to 64 (no autism). The CGI showed improvement by change in severity of illness from 3 (mildly ill) to 1 (borderline mentally ill). Global improvement on CGI was scored 2 (much improved) with an efficacy index of 5 (moderate therapeutic effect). PET CT scan repeated at 6 months, showed a balancing effect in the metabolism of frontal, temporal, mesial temporal, amygdale, hippocampus, para hippocampus, parietal, para hippocampus, basal ganglia, cerebellum amongst others. Functionally, improvements were observed in his attention span, tongue movements, eye contact, eye-hand co-ordination, behavior pattern, language and communication, sensory aspects, problem solving. There was significant decrease in aggressive behavior and hyperactivity [58].

In another case of autism with comorbid mental retardation treated with intrathecal administration of autologous BMMNCs, there was a significant clinical improvement recorded in social relationship, communication and behavior. On the ISAA, there was improvement from 111 (Moderate autism) to 73 (Mild Autism). A comparison of the PET CT scans of the brain before and after the intervention showed improved metabolism in Brocas region, external frontal, medial temporal pole, precentral, sensory motor, prefrontal and insula in the right hemisphere and medial prefrontal and thalamus in the left hemisphere. Intelligent Quotient (**IQ**) on The Malin's Intelligence Scale for Indian Children increased from 44 to 49.3. Functional improvements were recorded in social relationship and reciprocity, emotional responsiveness, speech-language and communication, behavioral patterns, sensory aspects and cognitive component [59].

In a case of an 11 year old boy with autism who underwent intrathecal autologous BMMNC transplantation, improvements were recorded in his speech, awareness of the surroundings, eye contact, attention and concentration, logical thinking, command following, emotional responses and writing speed. His stereotypical and self stimulatory behavior along with aggressive behavior and hyperactivity had reduced. He could now eat on his own which he could not do before. His scores on CARS improved from 31 to 25, on ISAA from 130 to 98, on CGI-I from 6 to 5 and on FIM from 104 to 110 [60].

In another similar reported case of a 7 year old boy with autism , clinically significant improvements were observed in behavior, social interaction, speech, communication, cognition and command following. The ISAA score improved from 131 to 112, CARS from 40.5 (severely autistic) to 32 (mild to moderate autism), and WeeFIM score from 31 to 36. Severity of illness on CGI (**CGI I**) changed from 4 (moderately ill) to 3 (mildly ill). Global improvement on CGI (**CGI II**) was measured at a score of 2 (much improved), along with an efficacy index (**CGI III**) of 5 showing moderate therapeutic effect [61].

In a 9-year-old boy with autism, 2 doses of autologous BMMNCs were administered intrathecally. On follow up, significant clinical improvements were noted in social relationship, communication

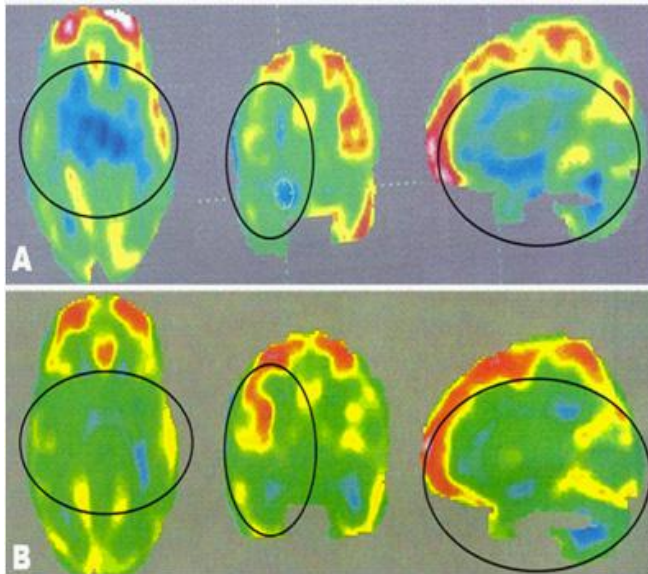


and behavior. His ISAA score improved from 132 (moderate autism) to 103 (mild autism) and CARS improved from 31 (moderately autistic) to 26 (non-autistic). On comparison of the PET-CT scan, changes in metabolism were recorded in parahippocampal and hippocampal regions, the cingulate and the paracingulate gyrus, the mesial temporal structures and the temporal lobe which further correlated with the clinical improvements [62].

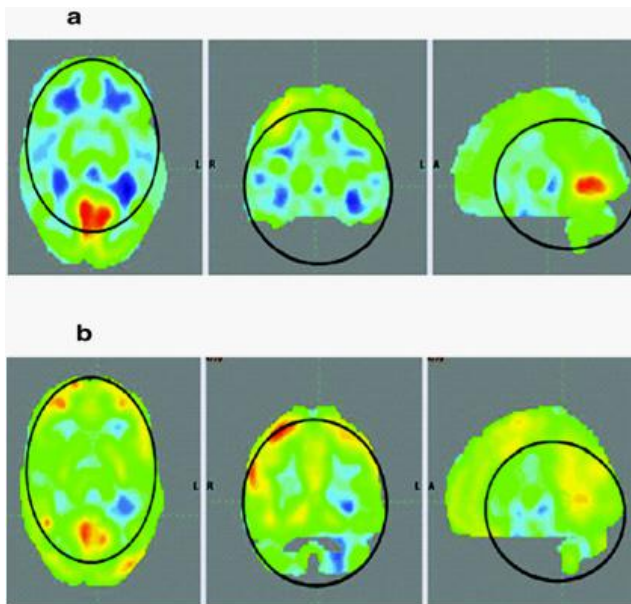
Objective evidence and tools used to establish the role of stem cell therapy in autism.

Magnetic resonance imaging of the brain is usually not very useful in autism as there are no obvious structural defects. Hence, this tool cannot be used to prognosticate and monitor the effects of stem cells. Since, as evidenced by the various clinical studies and case reports, stem cells lead to functional changes in the brain. Hence, functional neuroimaging studies like PET-CT scan and Functional MRI (**fMRI**) scans would be better able to give an insight into the brain connectivity, functional and metabolic activity. These could, therefore, be good tools to study the effect of intervention in autism.

PET-CT utilizes 18-FDG, to study the metabolic activity of the brain [63]. Uptake of the FDG is denoted as standard uptake value (**SUV**). The SUV of the patient undergoing PET-CT scan is then compared that of control population and standard deviation (**SD**) is computed. Function of the brain cells is directly proportional to the glucose uptake and metabolism. Thus, hypofunctioning areas will depict reduced FDG uptake and hypometabolism (represented by shades of blue and black); while hyperfunctioning areas will depict increased FDG uptake and hypermetabolism (represented by shades of yellow and red). This imaging technique can be used as a monitoring tool for stem cell therapy. An increased FDG uptake in hypofunctioning areas or decreased FDG uptake in hyperfunctioning areas may be implicated as improvement in brain function depending upon the correlation with clinical improvement.

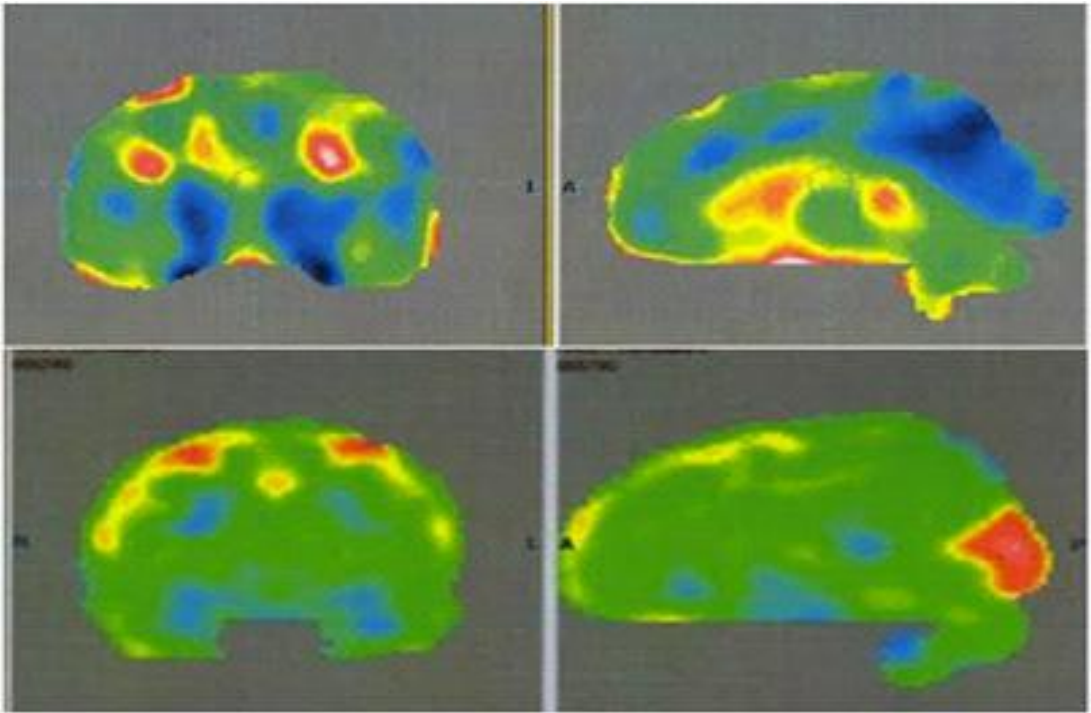


**Figure 8:** A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image which have changed to green areas depicting normal metabolism.



**Figure 9:** a) PET-CT scan before stem cell therapy showing low functioning highlighted in blue and black colour in the regions of brain. b) PET-CT scan after stem cell therapy showing improved functioning in the regions of the brain; indicated by reduction in the blue & black areas which are turning into green (Normal).





**Figure 10:** a) PET-CT scan before stem cell therapy showing low functioning highlighted in blue and black colour in the regions of brain. b) PET-CT scan after stem cell therapy showing improved functioning in the regions of the brain; indicated by reduction in the blue & black areas which are turning into green (Normal).

Functional MRI (**fMRI**) studies reveal brain activity through the measurement of changes in blood flow, blood volume, and blood oxygenation [64]. Functional magnetic resonance imaging (fMRI) of subjects with ASD demonstrates reduced activation in the fusiform gyrus, the portion of the brain associated with facial recognition, and increased activation of adjacent portions of the brain associated with recognition of objects. The enlarged right superior temporal gyri observed in a cohort of males with high-functioning autism, provides a possible anatomic basis for the visual and social deficits of those individuals. fMRI also suggests that in some individuals with Asperger syndrome and high-functioning autism, dysfunctional connections among limbic and paralimbic regions, the cerebellum, and the extrastriate visual cortices occur during the process of identification of the emotion expressed by faces and the sex of the face [65]. But, fMRI can be performed only in high functioning autism patients. Though fMRI provides valuable information, the use of this as a monitoring tool in children with autism is practically not feasible.

### Importance of NeuroRehabilitation in combination with stem cell therapy

Neurorehabilitation therapies in autism, involves behavioral therapy, sensory integration, occupational therapy, speech therapy, psychological intervention, physiotherapy, etc. These

therapies when given in combination, enhances the outcome of stem cell therapy. Evidence suggests that exercise induces mobility in the injected stem cells, thereby helping in migration of the cells and helps upregulate neural plasticity, [66] Exercise also improves oxygenation and blood supply to the brain. Hence, the synergistic effect of stem cell therapy and neurorehabilitation brings about maximum functional recovery.

## Ongoing Trials

Currently, there are 6 ongoing clinical trials on stem cell therapy in ASD, as registered in [clinicaltrials.gov](http://clinicaltrials.gov). [67].

2 clinical trials from India and 1 from Mexico are studying the safety and feasibility of autologous bone marrow derived stem cells in autism spectrum disorders. 2 registered trials are studying the effect of umbilical cord blood stem cells. The trial from USA is studying the effect of autologous cord blood cells while the one from Panama is studying the safety and efficacy of allogenic cord blood cells in ASD. Another trial registered from Mexico is studying the safety and efficacy of adipose derived stem cell therapy in ASD.

## Future Direction

Stem cell therapy has shown immense potential as a treatment for ASD. Studies described above have established its safety and efficacy. However, it requires larger, randomized clinical studies to validate its use as a standard therapy.

It is difficult to conduct pre-clinical studies showing effect of stem cell therapy in animal models of ASD as it is not possible to replicate the behavioral and emotional aspects of humans in animals. Therefore, better animal models are required to be studied in the future.

There are no standard biomarkers available for autism. This aspect can now be studied in depth with iPSC technology. The discovery of biomarkers will be immensely valuable in monitoring the outcome of novel therapeutic modalities.

The available human studies have a small sample size. Hence, currently it is difficult to generalize the findings of stem cell therapy. Long term, large, multi centre trials in future will help corroborate its benefits in ASD.

The pathophysiology of ASD and the mechanism of action of stem cells still needs to be studied in detail. We need studies to track and monitor the effects of stem cells at a cellular level confirming the postulated theories.

To gain optimum benefit of this intervention, it is important to standardize the protocol with respect to types of cells, source of stem cells, number of cells to be injected, frequency of injection, route of administration, etc. Currently available studies are diverse in nature. Hence, more studies comparing these aspects should be conducted to establish the most effective protocol.

Along with the protocol, other details which need to be investigated are outcome measures and monitoring tools to assess the effect of stem cells in ASD. PET CT scans and fMRIs should be explored in depth as they can effectively extract maximal information regarding the effect of intervention on the brain activity.

## CONCLUSION

Stem cell therapy has opened new avenues for the treatment of autism. The worldwide literature has supported the beneficial effects of stem cells to repair the abnormal brain function and produce symptomatic improvements in autism. The clinical studies have provided an excellent foundation upon which future research can be advanced. There are different protocols of stem cell therapy that are being used by researchers which have varied cell type, cell processing and route of administration. An attempt should be made in recent future to find the most effective protocol. The safety of autologous bone marrow stem cells is well known and hence can be used therapeutically. The encouraging clinical results substantiated with objective evidence on PET-CT scan warrant further larger multi-centre trials to establish cell therapy as a standard treatment for autism.

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