Stem Cell Therapy In Pediatric Neurological Disorders

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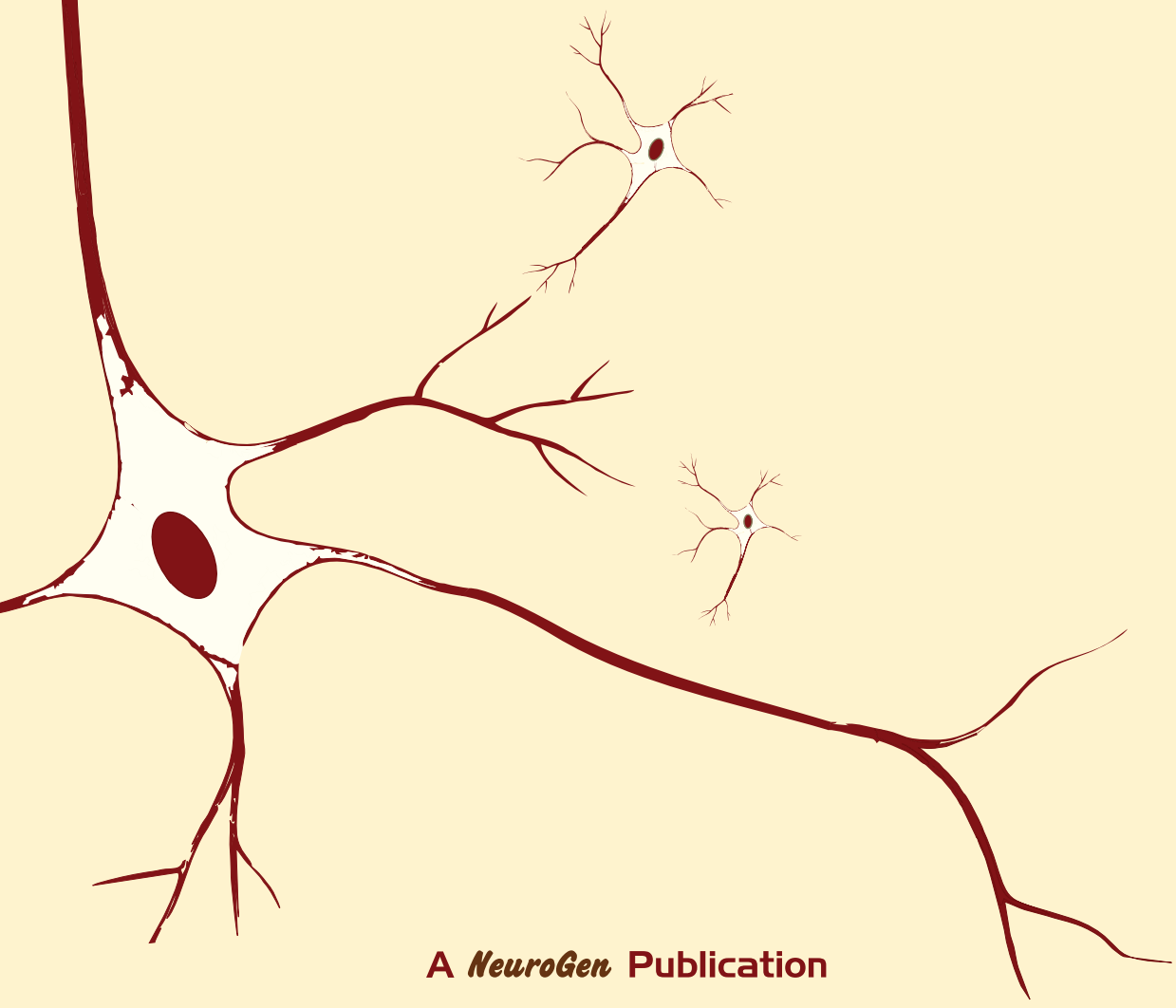

A *NeuroGen* Publication

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Stem Cell Therapy
In Pediatric Neurological Disorders

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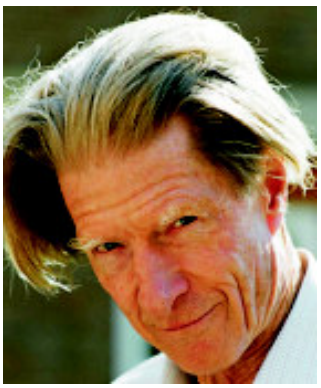
Stem Cell Therapy
"An idea whose time has come"

INTERNATIONAL

2012



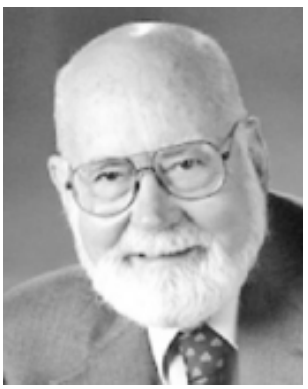
John B. Gurdon

Shinya Yamanaka

2007

Sir Martin Evans

1990



Dr. E. Thomas

Nobel Prize Awarded for Stem Cell Research

Stem Cell Therapy
"An idea whose time has come"

NATIONAL

Prime Minister Narendra Modi visits the Stem cell Institute of Kyoto University Japan and meets with Nobel Prize winner Professor Yamanaka in September 2014.

Pranab Mukherjee's speech at Lok Sabha joint session

President Pranab Mukherjee addressed the joint session of the Lok Sabha at the Central Hall of Parliament in New Delhi on 9th June 2014.

The president shared the Narendra Modi government's agenda for the country. From the economy to the environment, from minorities to terrorism, the President announced a series of programmes on a variety of issues facing the country.

"My government recognises the central role of Science and Technology in raising the quality of life. It will encourage and incentivise private sector investments, both domestic and foreign, in science and technology and in high-end research aimed at nurturing innovation. My government will build world class research centres in the fields of nanotechnology, material sciences, thorium technology, ***brain research, stem cells****, etc. The government will also establish institutes of Technology for Rural Development and a Central University of Himalayan Studies."*

Stem Cell Therapy
"An idea whose time has come"

Scientific Publications on Pediatric Neurodevelopmental Disorders by the Authors

A) AUTISM

1. Alok Sharma, NandiniGokulchandran, Hemangi Sane, Anjana Nagrajan, Amruta Paranjape, Pooja Kulkarni, Akshata Shetty, Priti Mishra, Mrudula Kali, Hema Biju, Prerna Badhe. Autologous bone marrow mononuclear cell therapy for autism - an open label proof of concept study. Stem cell international. 2013 (2013), Article ID 623875, 13 pages.
2. Alok Sharma, NandiniGokulchandran, PrernaBadhe, PoojaKulkarni, Priti Mishra, Akshata Shetty and Hemangi Sane. An Improved Case of Autism as Revealed by PET CT Scan in Patient Transplanted with Autologous Bone Marrow Derived Mononuclear Cells. J Stem Cell Res Ther 2013, 3:2
3. Alok Sharma, Nandini Gokulchandran, Akshata Shetty, Hemangi Sane, Pooja Kulkarni and Prerna Badhe. Autologous Bone Marrow Mononuclear Cells may be Explored as a Novel. Potential Therapeutic Option for Autism. J Clin Case Rep 2013, 3:7
4. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Pooja Kulkarni, Nancy Thomas, Amruta Paranjape, Prerna Badhe. Intrathecal autologous bone marrow mononuclear cell transplantation in a case of adult autism. Autism open access. 2013, 3:2
5. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Pradnya Bhovad, Hema Biju,Akshata Shetty, Mrudula Kali and Prerna Badhe, Cell therapy effects portrayed on positron emission tomography computerized tomography scan of the brain serve as a new dimension for autism: A case report. Journal of Paediatric Neurology, (2014) (In Press).
6. Sharma A, Gokulchandran N, Shetty A, Kulkarni P, Sane H, Badhe P. Neuropsychiatric Disorder Tackled by Innovative Cell Therapy - A Case Report in Autism. The Journal Stem Cell Research and Transplantation. 2014 July (In Press)

(B) CEREBRAL PALSY

1. Alok Sharma, Hemangi Sane, Nandini Gokulchandran, Prerna Badhe, Pooja Kulkarni and Amruta Paranjape. Stem Cell Therapy for Cerebral Palsy - A Novel Option. Cerebral Palsy. Challenges for the future. 2014: 217-242.

2. Alok Sharma, Hemangi Sane, Amruta Paranjape, Nandini Gokulchandran, Pooja Kulkarni and Anjana Nagrajan, Prerna Badhe. Positron Emission Tomography - Computer Tomography scan used as a monitoring tool following cellular therapy in Cerebral Palsy and Mental Retardation - A Case Report. Case Reports in Neurological Medicine. Volume 2013, Article ID 141983, 6 pages
3. Dr. Alok Sharma, Ms. Pooja Kulkarni, Dr. Hemangi Sane, Dr. Nandini Gokulchandran, Dr. Prerna Badhe, Dr. Mamta Lohia, Dr. Priti Mishra. Positron Emission Tomography - Computed Tomography scan captures the effects of cellular therapy in a case of cerebral palsy. Journal of clinical case reports. 2012 J Clin Case Rep 2:195.
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(C) MUSCULAR DYSTROPHY

1. Dr. A. Sharma, Ms. P. Kulkarni, Dr. G. Chopra, Dr. N. Gokulchandran, Dr. M. Lohia, Dr. P. Badhe. Autologous Bone Marrow Derived Mononuclear Cell Transplantation In Duchenne Muscular Dystrophy-A Case Report. Indian journal of Clinical Practice 2012; 23 (3): 169-72
2. Alok Sharma, Hemangi Sane, Amruta Paranjape, Khushboo Bhagwanani, Nandini Gokulchandran, Prerna Badhe. Autologous bone marrow mononuclear cell transplantation in Duchenne muscular dystrophy - a case report. American journal of case reports (Ahead of Print)

(D) MISCELLANEOUS

1. Alok Sharma, Nandini Gokulchandran, Guneet Chopra, Pooja Kulkarni, Mamta Lohia, Prerna Badhe, V .C. Jacob. Administration of autologous bone marrow derived mononuclear cells in children with incurable neurological disorders and injury is safe and improves their quality of life. Cell Transplantation, 2012; 21 Supp 1: S1-S12.
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3. Sharma A, Gokulchandran N, Kulkarni P, Chopra G. Application of autologous bone marrow stem cells in giant axonal neuropathy. Indian J Med Sci 2010; 64 : 41-4
4. Alok Sharma, Hemangi Sane, Amruta Paranjape, Nandini Gokulchandran, Mansi Takle, Prerna Badhe, Seizures as an adverse event of cellular therapy in pediatric neurological disorders and its prevention. Journal of Neurological Disorders. 2014 2(164), 2.

Chapter on "Stem Cell Therapy for Cerebral Palsy" written from NeuroGen, published in an international book- " Cerebral Palsy- Challenges for the Future" (Publisher- Intech)

Chapter 7

Stem Cell Therapy for Cerebral Palsy – A Novel Option

Alok Sharma, Hemangi Sane,
Nandini Gokulchandran, Prerna Badhe,
Pooja Kulkarni and Amruta Paranjape

Additional information is available at the end of the chapter

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1. Introduction

Discovery of stem cells by James Till and Ernest McCulloch in 1961, stands as one of the most remarkable medical-research achievements of the 20th century. This discovery provided a foundation for further breakthroughs in the field of stem cells. Sir Martin J. Evans along with Mario R. Capecchi, and Oliver Smithies were jointly awarded a Nobel Prize in 2007 for their contribution in introducing specific gene modifications in mice by the use of embryonic stem cells. Later in 2012, John B. Gurdon and Shinya Yamanaka were also jointly awarded a Nobel Prize for discovering that mature cells can be reprogrammed to become pluripotent cells. [1]

Ramon y Cajal in 1926 stated "Once the development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, ended, and immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree." [2]. It was a long-standing belief that cells of the central nervous system once damaged cannot be regenerated. The medical science of stem cells has finally made restoration of CNS possible which has changed the old concept of medicine. Not too long ago, this therapy was hamstrung by various controversies, ethical and moral issues. But, tremendous progress of research in this field has finally led to its translation from laboratory to innovative cellular therapies.

A variety of cells including embryonic stem cells, adult stem cells, umbilical cord blood cells and induced pluripotent stem cells have been explored as a therapeutic alternative for treating a broad spectrum of neurologic disorders including stroke, Alzheimer's, Parkinson's, spinal cord injury, cerebral palsy etc. amongst others. It is essential to select suitable cells depending on the nature and status of neurological dysfunctions to achieve optimal therapeutic efficacy. Along with the selection of cells, the route of administration also plays an important role to

Scientific Publications on Other Incurable Neurological Disorders by the Authors

1. Alok Sharma, Hemangi Sane, PrernaBadhe, Nandini Gokulchandran, Pooja Kulkarni, Mamta Lohiya, Hema Biju, V.C.Jacob. A Clinical Study Shows Safety and Efficacy of Autologous Bone Marrow Mononuclear Cell Therapy to Improve Quality of Life in Muscular Dystrophy Patients. Cell Transplantation. 2013; Vol. 22, Supplement 1, pp. S139-S146.

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4. A. Sharma, P. Badhe, N. Gokulchandran, P. Kulkarni, V.C Jacob, M. Lohia, J. George Joseph, H. Biju, G. Chopra. Administration of Autologous bone marrow stem cells intrathecally in Multiple Sclerosis patients is safe and improves their quality of life. Indian Journal of clinical Practice. 2011:21(11):622-625

5. Alok Sharma, Prerna Badhe, Pooja Kulkarni, Nandini Gokulchandran, Guneet Chopra, Mamta Lohia, V.C.Jacob. Autologous Bone marrow Derived mononuclear cells for the treatment of Spinal Cord Injury. The Journal of Orthopaedics. 2011; 1(1): 33-36

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7. Dr. Suvarna Badhe, Ms. Pooja Kulkarni, Dr. Guneet Chopra, Dr. Nandini Gokulchandran, Dr. Alok Sharma Dystrophin Deletion mutation pattern and Cardiac involvement in 46 cases of Dystrophinopathies. Asian journal of clinical cardiology. Asian Journal of Clinical Cardiology, Vol. 15, No. 6, October 2012: 211-214

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mononuclear cell therapy for vascular dementia – Case report. Journal of stem cell research and therapy. J Stem Cell Res Ther 2:129.

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14. Alok Sharma, Hemangi Sane, Nandini Gokulchandran, Dipti Khopkar, Amruta Paranjape, Jyothi Sundaram, Sushant Gandhi and Prerna Badhe Autologous Bone Marrow Mononuclear Cells Intrathecal Transplantation in Chronic Stroke. Stroke Research and Treatment, Volume 2014, pages 1-9

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16. Alok Sharma, Hemangi Sane, DiptiKhopkar, NandiniGokulchandran, V. C. Jacob, Joji Joseph, PrernaBadhe. Functional recovery in chronic stage of spinal cord injury by Neurorestorative Approach. Case Reports in Surgery 2014 (In Press)

Preface

There are some medical disorders that have remained very difficult to treat over the last many years. Pediatric neurological disorders like autism, cerebral palsy and muscular dystrophy have been some of them. All the advances in imaging, pharmacology and surgical techniques failed to make a significant dent in the quality of lives of these children. However in the recent few years there has been a development in the field of regenerative medicine whereby stem cell therapy has become available to us as a new treatment method . The biggest impact of stem cell therapy has been on the otherwise incurable pediatric neurological disorders. These are new and exciting times for pediatric neurology. With increasing research in this field and greater publications coming out showing the safety and efficacy of stem cell therapy, a quantum shift is happening in our approach to these disorders . We have shifted from hopelessness to hope and from lack of options to the availability of multiple options in all aspects of their care. We can safely say now that *"Stem cell therapy is an idea whose time has come"*

Despite this there is a lack of awareness about stem cell therapy and the clinical conditions it could possibly benefit. We have therefore written this book for pediatricians since they are the primary caregivers to all the children with pediatric neurological disorders. Nowadays with availability of information on the internet, parents of these children obtain a lot of knowledge about newer treatment options and then ask their doctors about it. It's important that pediatricians understand all aspects of this new therapy so that they can counsel and advise the parents appropriately. In this book we share with our pediatric colleagues general information about what stem cell therapy is, the indications where it can be used, a relevant review of literature as well as our own clinical results. We hope that with this book we are able to bring some clarity about different aspects of stem cell therapy in pediatric neurological disorders. From our own clinical experience we can now say with reasonable confidence to the parents of these children that with the availability of stem cell therapy for your children now : *"Aache din aane wale hain".*

– Dr. Alok Sharma

Contents

1. Introduction

Neurodevelopmental and other neurological disorders represent one of the leading causes of disability in children throughout the world. These disorders are currently estimated to affect as many as a billion people worldwide and the number is expected to increase considerably in years to come. Very few of these conditions have a cure and they may worsen over time. These children demonstrate a range of symptoms and functional limitations affecting their daily activities. Neurological conditions also pose an economic burden to the society. Hence, finding a treatment/ cure for these disorders is a goal of increasing urgency.

The devastating nature of neurological disorders is attributed to the longstanding belief that the cells of the brain and central nervous system (CNS) are incapable of regeneration.

In 1928, Ramon Y Cajal stated in his work Degeneration & Regeneration of the nervous system that "In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree."

However, recent progress in regenerative medicine has provided hope that injured CNS can be repaired using stem cells.

Stem cells are unspecialized cells with a capacity of self renewal and differentiation into specialized cells. The main aim of stem cell therapy is axonal regeneration, replacement of damaged neural cells and recovery of lost neural functions. These cells either carry out regeneration of new cells or stimulate the endogenous stem cells to reverse the CNS damage. A number of stem and progenitor cell types have been proposed as therapy for neurological diseases. These include bone marrow stem cells, neural stem cells, embryonic stem cells and umbilical cord blood stem cells. Extensive research has been carried out in this field. Researchers have anticipated that cell therapy may replace the conventional management strategies presently available for disorders such cerebral palsy, autism, brain injury, spinal cord injury, etc in children.

Stem cell therapy has already been translated from bench to bedside. To make it more efficacious some unsolved queries need to be answered such as the ideal source of cells, potent type of cells, optimal route of administration, effective therapeutic

time window and identification of the respondent group of patients. In this book we have made an attempt to address these queries. We have described in detail the mechanism of stem cell therapy in various pediatric neurological disorders.

2. What are Stem Cells?

Stem cells are defined as undifferentiated cells which are capable of proliferation, self maintenance, production of a large number of differentiated, functional progeny, regenerating tissue after injury and flexibility in the use of these options. These cells exhibit a unique property of "plasticity" where in cells isolated from one tissue convert to cells of different tissues by crossing lineage barriers and adopting the expression profile and phenotype of cells that are unique to other tissues.

Types of Stem Cells

Stem cells are categorized based on their potential to differentiate into other types of cells.

1. Totipotent cells: These cells have the ability to differentiate into all possible cell types of the human body including extraembryonic and placental cells.
2. Pluripotent cells: These cells have ability to differentiate into any of the three germ layers viz. endoderm, mesoderm and ectoderm.
3. Multipotent cells: These cells have the ability to differentiate into specialized cells.

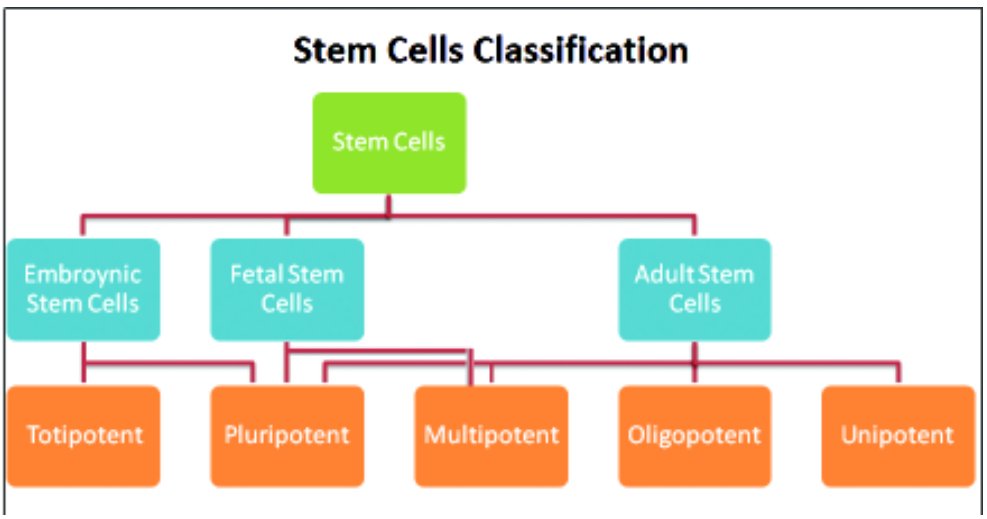

Figure 1: Classification of Stem cells

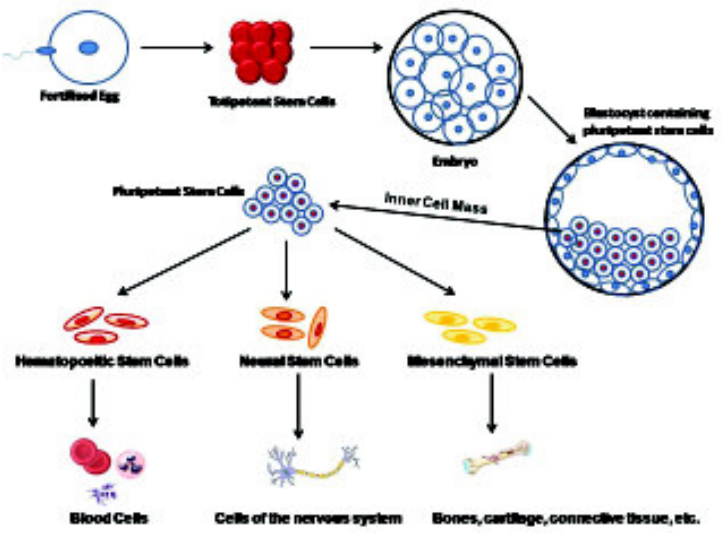

Figure 2: Differentiation of stem cells

4. Oligopotent cells: These cells have the ability to differentiate into a few cells.
5. Unipotent cells: These cells have the ability to produce cells only of their own type, but are capable of self-renewal to be classified as a stem cell.

Stem cells are broadly classified based on their origin, as follows:

1. **Embryonic stem cells (ESCs):** These cells are pluripotent cells derived from a 4-7 day old blastocyst stage embryo. The cells are harvested from the inner cell mass (ICM) of the blastocyst. They can be indefinitely maintained and expanded as pure populations of undifferentiated cells, in culture. Inspite of their clinical potential in tissue repair, these cells have triggered various ethical and moral issues as they involve destruction of human embryos. They also have tumorigenic side effects as ESCs and tumor cells share cellular and molecular phenotypes such as rapid proliferation rate, lack of contact inhibition, a susceptibility to genomic instability, high activity of telomerase, high expression of oncogenes and epigenetic status amongst others. They form teratomas which have the potential to degenerate into malignant teratocarcinomas. The likelihood of development of tumors in children cannot be overlooked as they have many years of life ahead of them for the tumor formation to occur. According to the ICMR, use of these cells fall into the restricted category.
2. **Fetal Stem Cells:** These cells are isolated either from the aborted fetus or from the extra embryonic structures of the fetal origin such as the amniotic fluid and placenta. Fetal blood is a rich source of haemopoietic stem cells (HSC). Non-haemopoietic mesenchymal stem cells (MSC) are also found in the First trimester fetal blood. These cells have better homing capacity, greater multipotentiality and differentiation potential and lower immunogenicity as compared to the adult

stem cells. Although these cells have a greater therapeutic potential they are also susceptible to infections. Studies have demonstrated that these cells are prone to KS-associated herpesvirus (KSHV) infections. As the safety is not yet substantiated, fetal cells are not often used for transplantation. According to the ICMR, use of these cells fall into the restricted category.

3. **Umbilical cord stem cells:** Umbilical cord contains a heterogeneous mixture of stem / progenitor cells at different lineage commitment stages. Cells are isolated either from the cord blood or the Wharton jelly. They consist of embryonic stem cell-like and other pluripotential stem cells, which can give rise to hematopoietic, epithelial, endothelial, and neural tissues. Various banks have evolved to collect and preserve the umbilical cord blood. But the utility of these centers is still questionable. The protocols and guidelines for collection and retrieval of cells are still being standardized. Other disadvantages of use of UCBCs are that the time for platelet engraftment is prolonged in the transplantation of cord blood due to insufficient cell dose. According to the ICMR, use of these cells fall into the permitted category.

4. **Induced pluripotent stem cells (iPSC) :** To circumvent the ethical issues involved in the use of embryonic stem cells, pluripotent cells were generated directly from the patients' own cells. Induced pluripotent stem cells are non-pluripotent adult cells (somatic cells) which have been genetically reprogrammed to form pluripotent cells. But, to initiate the clinical trials involving iPSCs, the reprogramming efficiency and safety has yet to be established.According to the ICMR, use of these cells fall into the restricted category.

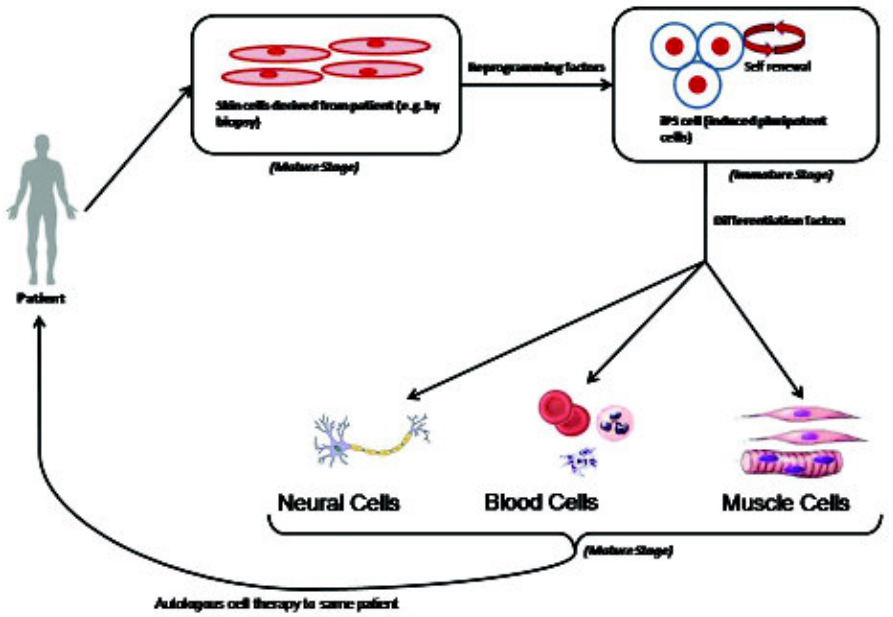

Figure 3: Induced pluripotent stem cells (iPSC)

5. **Adult stem cells:** These cells are multipotent stem cells, isolated from adult tissues. They include hematopoietic stem cells, bone marrow derived stem cells, adipose tissue-derived stem cells, neural stem cells amongst others. Adult stem cells are found in almost all the tissues of the body and help to maintain and repair organs and tissues throughout a person's life. These cells are majorly derived from the bone marrow, brain, skeletal muscle, liver, pancreas, fat, skin and skeletal muscle. According to the ICMR, use of these cells fall into the permitted category.

Major sources of adult stem cells

Bone marrow: Anterior or posterior superior iliac crest is the preferred site for the bone marrow aspiration. If bone marrow cannot be obtained from the iliac crest due to positioning difficulties or obesity, sternum may be used in adults. However, aspiration from sternum poses a great risk of complications.

Bone marrow is a proficient source of autologous cells with distinct regenerative properties, which can be quickly harvested and are thus applicable for both chronic and acute diseases. It is the only known organ in which two or more separate and distinct stem cells and dependent tissue systems not only coexist but functionally cooperate. The mononuclear cell fraction derived from the bone marrow is a heterogeneous population containing differentially matured B-cells, T-cells and monocytes, as well as rare progenitor cells such as hematopoietic stem cells (HSC), mesenchymal stromal cells (MSC), endothelial progenitor cells (EPC) and very small embryonic-like cells (VSEL). The hematopoietic cells are the blood cells which give rise to the myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells). Bone marrow mesenchymal stem cells (BMMSCs) give rise to mesodermal lineage cells such as osteoblasts, chondrocytes, adipocytes, and muscle cells along with neuroectodermal cells. It has been observed that use of cell mixture is more efficacious than individual subfractionated cells of the bone marrow. They promote angiogenesis, mediate vascular repair, and express several cytoprotective growth factors and cytokines. These cells are also safe and due to its easy availability they are most preferred for cellular therapy. These cells are used for the treatment of various neurological disorders such as autism, cerebral palsy, stroke, Parkinson's, Spinal cord injury, etc along with diabetes, orthopedic conditions, cancers and wound healing.

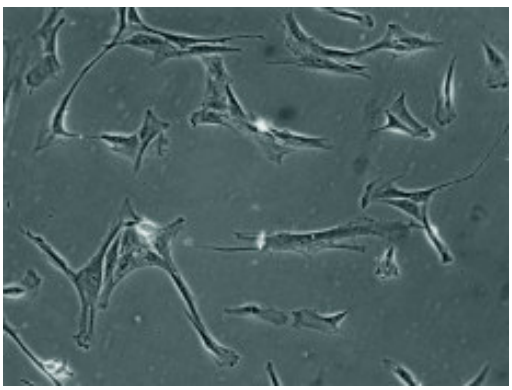

Figure 4: Bone marrow stem cells transforming into neuron like cells.

Adipose tissue: Adipose tissue derived

stem cells (ASCs) are multipotent cells, found abundantly in fat tissue. They can differentiate into several lineages, including adipose cells, chondrocytes, osteoblasts, neuronal cells, endothelial cells, and cardiomyocytes. These cells are obtained either through liposuction or lipectomy. Mesenchymal stem cells make up the majority of the adipose derived stem cells. Due to their plasticity, they are a preferred alternative to the BMSCs. One of the major disadvantages of adipose derived stem cell is that they are not a completely homogeneous cell population in addition to complicated isolating process. Therefore, an expert is required for cell isolation.

Dental pulp: A population of stem cells has been isolated from the human dental pulp known as dental pulp stem cells (DPSCs). They have an ability to regenerate a dentin-pulp-like tissue. DPSCs are a heterogeneous population of cells as they are composed of both mesenchymal and ectodermic cells. These cells are readily obtained (from milk teeth, routine dental procedures such as removal of impacted third molars) and have been shown to possess properties similar to neural stem cells and mesenchymal stem cells. Under appropriate conditions, these cells also undergo neuronal differentiation. One of the disadvantages of DPSCs is that it takes longer to culture mesenchymal stem cells from teeth active tissue. Also, it is difficult to harvest a large quantity of stem cells from teeth.

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3. How is stem cell therapy done?

The process of stem cell therapy is carried out in 3 steps (1) Procurement of stem cells (2) separation, harvesting, enriching &/or expansion and differentiation in the laboratory and (3) transplantation or delivery of the cells. In this chapter, we have discussed each aspect with respect to autologous bone marrow stem cell therapy, in detail. (Others are mentioned in short).

1. Procurement of Stem cells

a) Bone marrow aspiration

The choice of site may be dependent on various factors such as age, weight, marrow distribution, physical status of the patient, physicians experience, etc. However, the most common site is the pelvis. The aspiration is easily done from either posterior

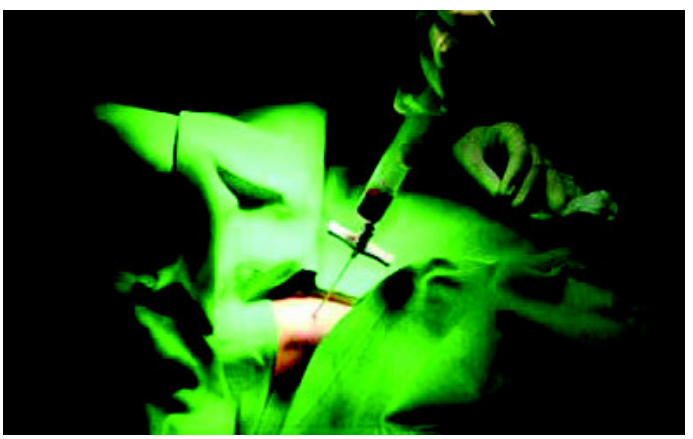

Figure 1: Bone marrow aspiration

or anterior iliac crests. The posterior superior iliac spine is easily accessible and identifiable, however to access this, the patient has to be in the lateral or prone position which can be troublesome and cumbersome. The anterior superior iliac spine can be accessed with the patient lying comfortably in the supine position. In obese patients, the landmarks may be obliterated due to fat distribution.

The site of the aspiration is palpated. For the posterior superior iliac spine, in thin individuals, it is usually palpated as the bony prominence superior and three finger breadth laterals to the intergluteal cleft. The anterior superior iliac spine can be palpated as an anterior prominence on the iliac crest. The overlying skin is prepared in a manner similar to preparation of any site for surgery. The area is anaesthetized by intradermal administration of a local anesthetic such as lignocaine using a 25G or 26G needle. A 1 cm area is anesthetized.

A standard bone marrow aspiration needle is inserted through the skin till the bone is felt. Before using the needle, it is flushed with heparin. Some surgeons make a small incision with a surgical blade and expose the bone before putting in the needle, however in our experience this is rarely required. The needle which is firmly fixed to the obturator is firmly inserted inside, clockwise and anticlockwise, in a screwing motion with exertion of downward pressure, until the periosteum is reached. With similar motion, the needle is inserted till it penetrates the cortex. At this point initially a sudden giving way of the resistance is felt as the needle enters the soft trabecular bone and then the needle feels firmly fixed in the bone. The angle of insertion of the needle is important as it has to be in alignment with the curve of the bone. If this is not done properly the needle will make a through and through penetration across both the cortical surfaces with the tip now being outside the marrow. A study of the anatomy of the pelvis with a model and personal experience over time makes this a very simple procedure.

The stylet is now removed and a 10 ml or 20 ml syringe, with some heparin in it, is attached and the aspiration is carried out. A total of 100-120 ml is aspirated in adults and 80-100 ml in children is collected. The bone marrow is then transported to the laboratory in a special transporter under sterile conditions for further cell separation.

b) Umbilical Cord Stem Cells

These are procured from the preserved umbilical cord at the time of birth. Nowadays there are many cord banks who collect and store cord blood stem cells. A kit is provided by the banks to the parents or doctors for collection.

2. Cell Separation

Separation of stem cells from its source is one of the most important steps in the process of stem cell therapy. An effective method of cell separation is crucial to gain optimum benefit from the cells. As discussed in the previous chapter, stem cells are primarily obtained from haematopoietic sources such as the bone marrow, peripheral blood and umbilical cord, due to easy accessibility and absence of ethical issues. The protocol for processing these cells is varied.

Harvesting stem cells from peripheral blood.

The most common method of collecting hematopoietic stem cells (HSCs) is by mobilization from the peripheral blood. Since, negligible HSCs are detectable in the

peripheral blood during the steady state, either a hematopoietic growth factor such as granulocyte colony-stimulating factor (GCSF) or chemotherapy (usually cyclophosphamide) with or without granulocyte colony-stimulating factor is necessary to mobilize them.

Most mononuclear cells are collected by peripheral blood apheresis/ leukaphereses. In general, a minimum number of 2x106 CD34 cells per kilogram of recipient weight will ensure engraftment. Hematopoietic stem cells may be positively selected or enriched, ex-vivo using antibodies to CD34 or CD133 or purified by negative selection by using antibodies to remove lymphocytes. In practice, the most common method of purging lymphocytes is via CD34-positive selection.

Harvesting stem cells from bone marrow.

a) Open Method

Bone marrow blood (100-150 mL) aspirated from the iliac bone(generally either anterior or posterior superior iliac spine) and is diluted in Hanks' balanced salt solution (HBSS) at a ratio of 1:1. After centrifugation of samples at 1000 x g for 30 min through a density gradient (Ficoll-Paque Plus, 1.077 g/L; Amersham Biosciences,Piscataway, NJ), the mononuclear cell layer is recovered from the gradient interface and washed with HBSS. The cells are centrifuged at 900 xg for 15 min and resuspended in 1.8 mL of phosphate buffered saline (PBS) at a density of 1.1 x 10^6cells/L.

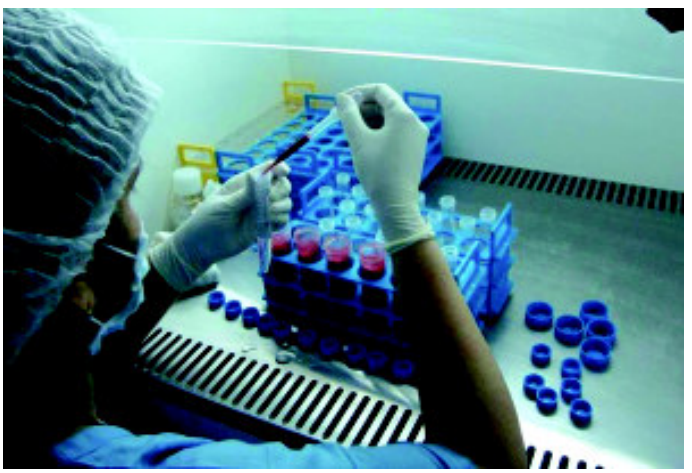

Figure 2: Stem cell Separation

b) Closed Method

Commercial platforms for harvesting bone marrow concentrates are being engineered to facilitate harvesting in a closed system. Various companies such as Harvest Technologies, Miltenyl Biotec, StemCell Technologies, etc have developed fully automated systems to simplify and accelerate the cell separation.

Harvesting stem cells from Umbilical Cord blood

Currently, there are two types of processing methods used to separate cells from the cord blood, manual and automated. Some companies choose to use manual processing systems while others have moved to automated processing systems.

Manual processing involves allowing the blood to sit for a period of time and then manually extracting cells from the middle of what has "settled" out from the cord blood. There are two potential problems however with manual processing. Manual methods recover only 40%-80% of cells necessary for transplant purposes and can potentially subject the cord blood to potential airborne contaminants.

Automated processing has avoided these potential problems by working in a completely closed system eliminating excess air contamination and, most importantly, allowing for up to 99% recovery of necessary cells for transplantation. In addition, the possibility of human error is reduced. Unfortunately, these advancements make automated processing costly, and those costs are passed on to customers.

3. Transplantation of stem cells

Stem cell transplantation is carried out via various delivery routes such as intravenous, intrathecal, intraarterial, intramuscular, direct injection, etc. Every mode of administration has its pros and cons. Comparative studies need to be carried out to optimize the selection of route of administration to ensure a positive outcome of stem cell transplantation.

Intrathecal delivery

The patient is positioned in the curled up "foetal ball" position to open up the spinous processes. Under local anesthesia, a standard lumbar puncture procedure is performed at the L4-5 level. A 18G Touhy needle is inserted into the sub-arachnoid space. After ascertaining free flow of CSF, an epidural catheter or spinal needle is inserted into the space, far enough to keep 8-10 of the catheter in the space. The stem cells are then injected slowly through the catheter, keeping a close watch on the hemodynamics of

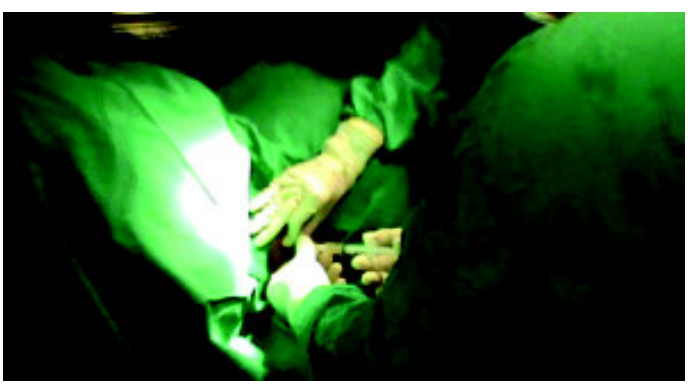

Figure 3: Intrathecal administration of stem cells

the patient. The cells are flushed in with CSF. The catheter is removed and a benzoin seal followed by a tight compressive dressing is given. This procedure is usually done under local anesthesia. Sedation with local anesthesia is given to children.

This method is minimally invasive and is the safest targeted mode of transplantation. In a study carried out by Callera et al, it was demonstrated for the first time that autologous bone marrow CD 34+ cells labelled with magnetic nanoparticles when delivered intrathecally, migrates into the injured site in patients with spinal cord injury.

Intravenous delivery

Intravenous delivery of stem cells is one of the most widely used routes of administration. It is safe, minimally invasive and has no ethical issues involved. Inspite of these advantages, it is not the most efficient method of transplantation. In various studies it has been seen that the cells administered via IV get trapped in organs (e.g. lungs) other than the target organ. They are also more susceptible to the host immune system.

Intra-arterial injection

Following revascularization surgery such as Carotid endartrectomy or Superficial Temporal artery to Middle Cerebral artery bypass, stem cells could be injected intra-arterially immediately after the completion of the revascularization procedure. The advantage of this approach is that the stem cells would directly reach the ischemic brain and also since the artery is already exposed no separate procedure needs to be done for the stem cell injection. The other method of intra-arterial injection would be via the endovascular interventional route. This is done by making a puncture in the femoral artery and negotiating a catheter to the arteries supplying the brain. The advantage of this is that it is a relatively non invasive procedure and the limitations of intravenous injection are avoided.

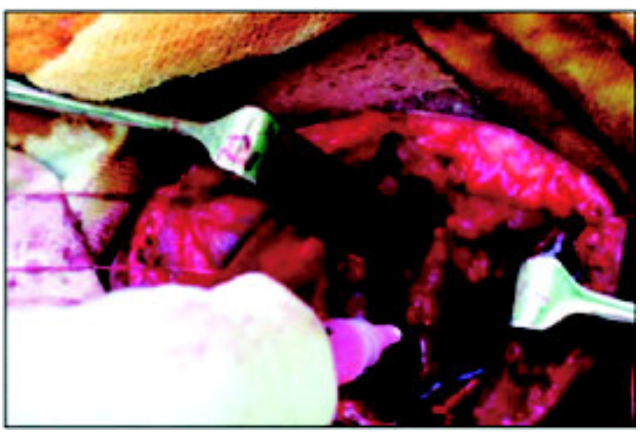

Figure 4: Intra-arterial Injection of stem cells into the carotid artery

Intraspinal transplantation

Direct implantation into the spinal cord may be done in different ways:-

a) Through a complete laminectomy from one level above to one level below the injury site so that there is sufficient access to the transplantation site.

b) Though a minilaminectomy and exposure of the spinal cord. Two injections are made on either side above the injury site and two injections are made below the injury site.

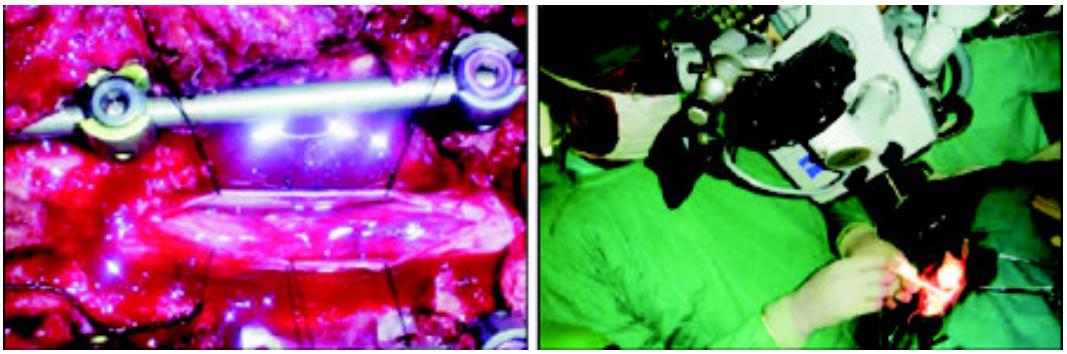

Figure 5: Instraspinal administration of stem cells

Stereotactic implantation into the brain

Cell transplantation for neurological conditions started with stereotactic implantation of fetal cells for Parkinson's disease. There are many stereotactic systems available all over the world however the two most popular ones are the Leksell Stereotactic system and the CRW Stereotactic system. The area where the tissue is to be transplanted is identified on the MRI scan and then using the MRI software the X, Y and Z coordinates are obtained. A small burr hole is drilled into the skull of the patient and the cells are transplanted at the desired location using the X, Y and Z coordinates. The entire procedure is done under local anesthesia. However, the clinical outcome of this method is not significantly different from non transplanted patients. It is also the most invasive method of transplantation and could result in secondary injury.

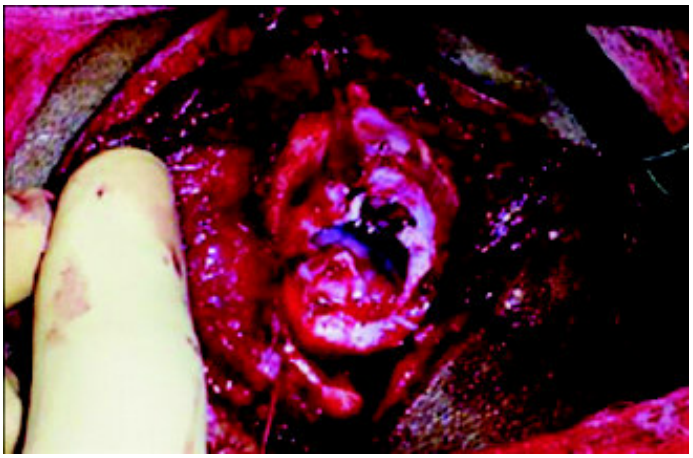

Figure 6: STA-MCA bypass

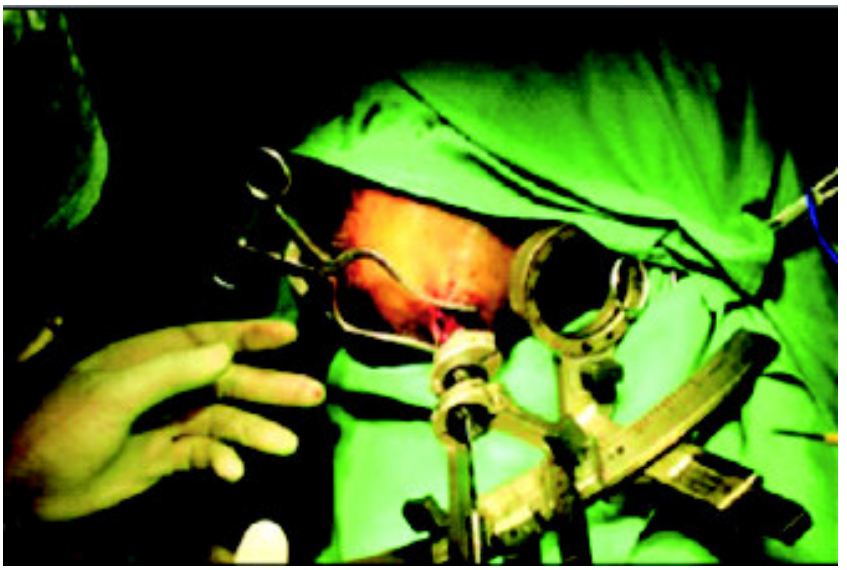

Figure 7: The Leksell Stereotactic frame for direct stem cell implantation into the brain

Intramuscular injection

In certain disorders, especially Muscular dystrophy, cells are also transplanted into the muscles. The points at which they are injected are termed as the "motor points". Motor point is the point at which the main nerve enters the muscle or, in case of deeply placed muscle, at the point where the muscle emerges from under covers of the more superficial ones. At these motor points, the area is cleaned and the cells diluted in CSF are injected with the 26G needle into the muscle at an angle (approx. 45 degrees).The piston/plunger of the syringe is slightly withdrawn to verify that the needle is not inside a blood vessel. Once the needle is removed, the site is immediately sealed with a benzoin seal.

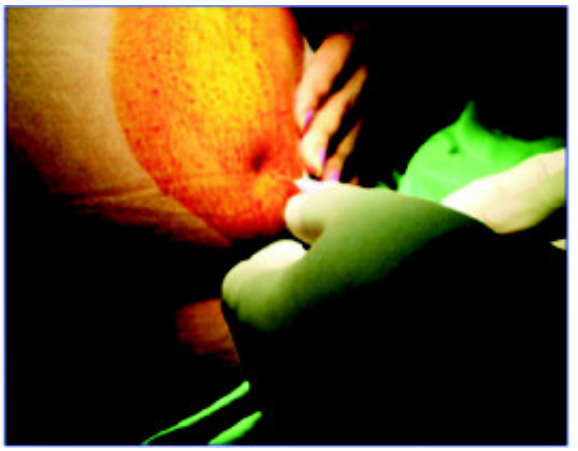

Figure 8: Intramuscular

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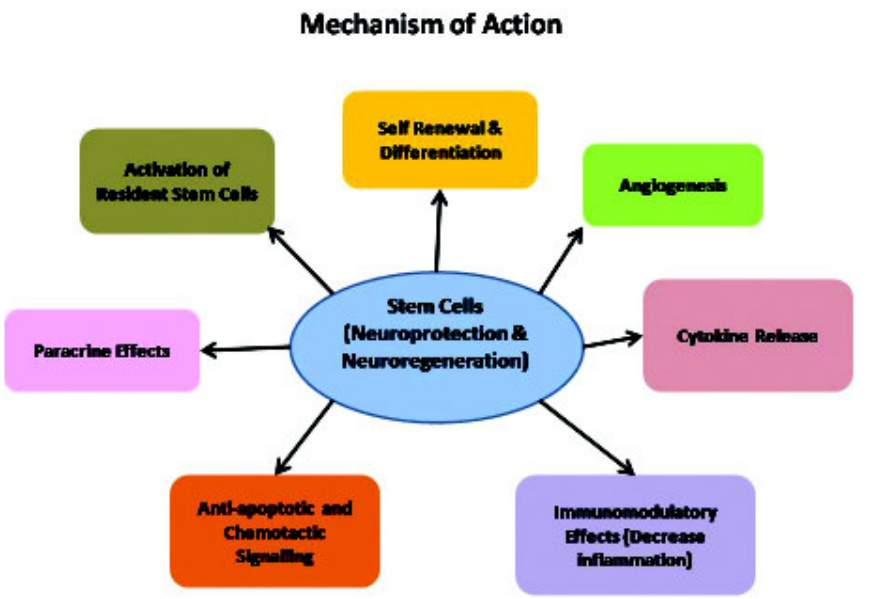
4. How do Stem cells work?

Stem cells are actively involved in the formation of new tissues and thereby can also promote repair and regeneration. Their role, in the normal wear and tear of the body, appears to be assistance of repair and maintenance of normal tissue structure and function. Cell-based therapy could therefore potentially be used to treat a wide array of clinical conditions caused due to cellular damage. The process of deciphering the underlying mechanism of action of stem cells is a continuous process. However, years of experiments and studies have put forward few theories which either individually or in combination carries out the repair of the damaged tissues. Few of the mechanisms have been discussed below.



Plasticity, Pluripotency and Production

Stem cells have remarkable property of plasticity wherein they can cross the lineage boundaries. They can differentiate into cells with function and phenotype other than theirs. In 1998, Ferrari et al. first reported that mouse bone-marrow-derived cells give rise to skeletal muscle cells when transplanted into damaged mouse muscle. Thereafter, transplanted bone marrow cells were reported to generate a wide

spectrum of different cell types, including hepatocytes, endothelial, myocardial, neuronal, and glial cells. Moreover, HSC can produce cardiac myocytes and endothelial cells, functional hepatocytes and epithelial cells of the liver, gut, lung, and skin. Mesenchymal stromal cells (MSC) of the bone marrow can generate brain astrocytes. Enriched stem cells from adult mouse skeletal muscle were shown to produce blood cells. In most of these plasticity studies, genetically marked cells from one organ of an adult mouse apparently gave rise to cell type characteristics of other organs following transplantation, suggesting that even cell types once thought to be terminally differentiated are far more plastic in their developmental potential than previously thought. A critical aspect of the observation of adult stem cell plasticity is that in order for plasticity to occur, cell injury is necessary. This suggests that micro-environmental exposure to the products of injured cells may play a key role in determining the differentiated expression of marrow stem cells.

The events underlying stem cell plasticity could relate to a variety of mechanisms such as dedifferentiation, trans-differentiation, epigenetic changes, and/or cell fusion. Rerouting of cell fate may result from the multistep process known as dedifferentiation where cells revert to an earlier, more primitive phenotype characterized by alterations in gene expression pattern which confer an extended differentiation potential. Molecular and epigenetic changes have shown to be involved in the process of dedifferentiated, possibly mediated by signals released after cellular injury.

Another mechanism put forward to explain, stem cell switch to a novel phenotype, is a process known as trans-differentiation. Cells may differentiate from one cell type into another within the same tissue or develop into a completely different tissue without acquiring an intermediate recognizable, undifferentiated progenitor state.

Trans-differentiation events are at times considered as misinterpretations caused by cell fusion resulting in nuclear reprogramming and changes in cell fate. Adult stem cells from bone marrow may be able to fuse with cells of the target organ. So far, bone-marrow-derived cells were shown to form fusion heterokaryons with liver, skeletal muscle, cardiac muscle, and neurons. There is evidence that such fused cells become mono-nucleated again, either by nuclear fusion or by elimination of supernumerary nuclei. Fusion and nuclear transfer experiments demonstrated that genes previously silenced during development could be reactivated by cytoplasmic factors modulating the epigenetic mechanisms responsible for the maintenance of a specific state of cell differentiation. Despite the limitation of the low frequency of this event and its dependence of the developmental stage of donor nuclei, cell fusion may be considered as a potential avenue for tissue repair. The physiological purpose of adult cell fusion is speculative. As outlined by Helen Blau, fusion could be a means by which cells 1) deliver healthy genetic material to dying cells (rescue function), 2) supply cells with new genes (repair function), or 3) correct genetically defective cells such as in muscular dystrophy (gene replacement).

Fusion could even be considered a basic mechanism for keeping the adult cell systems intact throughout our lifespan.

In addition to the aforementioned phenomena of cell fate switching, the presence of a rare population of pluripotent primitive stem cells may also explain the acquisition of an unexpected phenotype. Non-hematopoietic cell populations from bone marrow and umbilical cord blood were enriched by in vitro culture and demonstrated to have the potential to differentiate into derivatives of all three germline layers with meso-, endo-, and ectodermal characteristics. Known as multipotent adult progenitor cells (MAPC), these cells contribute to most, if not all, somatic cell lineages, including brain, when injected into a mouse blastocyst. Interestingly, while MAPC express Oct4, a transcription factor required for undifferentiated embryonic stem cells maintenance at levels approaching those of ESC, MAPC do not express two other transcription factors known to play a major role in ESC pluripotency, Nanog and Sox2. This particular expression profile may contribute to the fact that the use of ESC, but not MAPC, carries the risk of generating tumors. Thus, MAPC are a promising source of autologous stem cells in regenerative medicine. Their low tumorigenicity, high regenerative plasticity, and optimal immunological compatibility are essential assets for the successful transplantation of MAPC-derived tissue-committed cells without immune-mediated rejection.

The Paracrine Effect

Exploration of the various cellular mechanisms occurring (both during normal physiology as well as after tissue injury) in the process of stem cell renewal and differentiation, suggests that stem cell treatment or transplantation of stem cells remodels and regenerates injured tissue, improves function, and protects tissue from further insult. These have also led to phase I and II clinical trials regarding stem cell treatment for a variety of surgical diseases. Despite these encouraging advances, the mechanism of this protection is still not well-characterized. As discussed earlier, it was initially hypothesized that immature stem cells differentiated into the phenotype of injured tissue, repopulated the diseased organ with healthy cells, and subsequently improved function. But, recent research indicates that this stem cell-mediated protection may not have resulted from differentiation into the target tissue type. Instead, several lines of evidence suggest that stem cells may mediate their beneficial effects, at least in part, by paracrine mechanisms. The reasons for the above postulations are as follows:

First, studies demonstrate that donor stem cell engraftment and survival after transplantation is only 1-5% which is too few to be relevant therapeutically and influence directly organ function.

Second, stem cells have been shown to confer acute improvement in end organ function less than 72 hr after injury, precluding differentiation as a cause due to time required for meaningful differentiation and regeneration of these donor cells.

Third, and perhaps most importantly, in vitro and in vivo animal studies have revealed that much of the functional improvement and attenuation of injury afforded by stem cells can be replicated by cell free, conditioned media derived from stem cells. Taken together, these indirect and direct data suggest that stem cells may improve injured

organ performance and limit injury not via differentiation but rather via complex paracrine actions rather than an organogenetic role.

Though complete understanding of the mechanism of action of the stem cells is still sometime away, the following effects have been proposed.

Stem cells transplanted into injured tissue express paracrine signaling factors including cytokines and other growth factors, which are involved in orchestrating the stem cell-driven repair process through increasing angiogenesis, decreasing inflammation, preventing apoptosis, releasing chemotactic factors, assisting in extracellular matrix tissue remodeling and activation of resident/satellite cells which is discussed further in details.

Increased Angiogenesis

Stem cells produce local signaling molecules that may improve perfusion and enhance angiogenesis to chronically ischemic tissue. Although the particular growth factors contributing to this neovascular effect remain to be defined, the list includes vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (FGF2). VEGF is a strong promoter of angiogenesis. Chen et al. have recently shown that treatment with bone marrow stromal cells enhances angiogenesis by increasing endogenous levels of VEGF and VEGFR2. They previously demonstrated that administration of recombinant human VEGF165 to rats 48 hours after stroke significantly increased angiogenesis in the penumbra and improved functional recovery.

Hepatic Growth Factor (HGF) exerts beneficial effects on neovascularization and tissue remodeling, while FGF2 is involved intimately with endothelial cell proliferation and may be a more potent angiogenic factor than VEGF.

When exposed to either insult or stress, mesenchymal stem cells (MSC) in cell culture and in vivo significantly increase release of VEGF, HGF, and FGF2, which may improve regional blood flow as well as promote autocrine self survival. Increased perfusion due to the production of stem cell angiogenic growth factor has also been associated with improved end organ function. VEGF overexpressing bone marrow stem cells also demonstrates protection of injured tissue.

Thus, VEGF, HGF, and FGF2 may be important paracrine signaling molecules in stem cell-mediated angiogenesis, protection, and survival.

Decreased Inflammation

Stem cells appear to attenuate infarct size and injury by modulating local inflammation. When transplanted into injured tissue, the stem cell faces a hostile, nutrient-deficient, inflammatory environment and may release substances which limit local inflammation in order to enhance its survival. Modulation of local tissue levels of pro-inflammatory cytokines by anti-inflammatory paracrine factors released by stem cells (such as IL-10 and TGF-β) is important in conferring improved outcome after stem cell therapy.

Anti-Apoptotic and Chemotactic Signaling

Stem cells in a third pathway promote salvage of tenuous or malfunctioning cell types at the infarct border zone. Injection of MSC into a cryo-induced infarct reduces myocardial scar width 10 weeks later. MSCs appear to activate an anti-apoptosis signaling system at the infarct border zone which effectively protects ischemia-threatened cell types from apoptosis. Furthermore, expression profiling of adult progenitor cells reveals characteristic expression of genes associated with enhanced DNA repair, upregulated anti-oxidant enzymes, and increased detoxifier systems. HGF has been observed to improve cell growth and to reduce cell apoptosis.

Evidence also exists that both endogenous and exogenous stem cells are able to "home" or migrate into the area of injury from the site of injection or infusion. MSC in the bone marrow can be mobilized, target the areas of infarction, and differentiate into target tissue type. Granulocyte colony-stimulating factor (G-CSF) has been studied widely and promotes the mobilization of bone marrow-derived stem cells in the setting of acute injury. This homing mechanism may also depend on expression of stromal cell-derived factor 1 (SDF-1), monocyte chemo-attractant protein-3 (MCP-3), stem cell factor (SCF), and / or IL-8.

Beneficial Remodeling of the Extracellular Matrix

Stem cell transplantation alters the extracellular matrix, resulting in more favorable post-infarct remodeling, strengthening of the infarct scar, and prevention of deterioration in organ function. MSCs appear to achieve this improved function by increasing acutely the cellularity and decreasing production of extracellular matrix proteins such as collagen type I, collagen type III, and TIMP-1 which result in positive remodeling and function.

Activation of Neighboring Resident Stem Cells

Finally, exogenous stem cell transplantation may activate neighboring resident tissue stem cells. Recent work demonstrates the existence of endogenous, stem cell-like populations in adult hearts, liver, brain, and kidney. These resident stem cells may possess growth factor receptors that can be activated to induce their migration and proliferation and promote both the restoration of dead tissue and the improved function in damaged tissue. Mesenchymal stem cells have also released HGF and IGF-1 in response to injury which when transplanted into ischemic myocardial tissue may activate subsequently the resident cardiac stem cells.

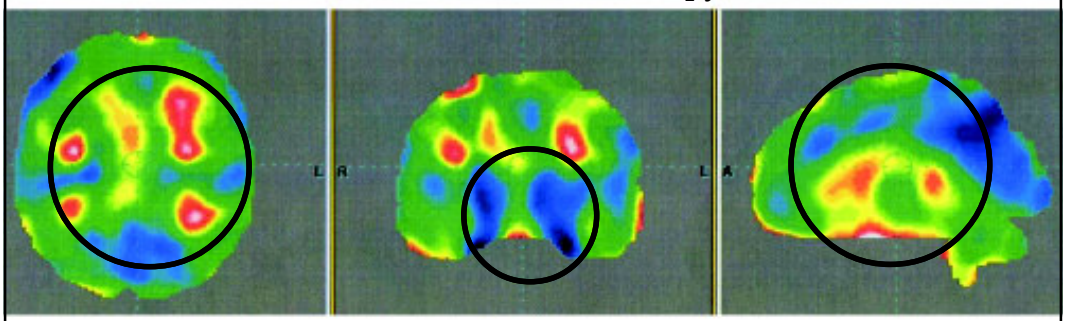
To sum up, although the definitive mechanisms for protection via stem cells remains unclear, stem cells mediate enhanced angiogenesis, suppression of inflammation, and improved function via paracrine actions on injured cells, neighboring resident stem cells, the extracellular matrix, and the infarct zone. Improved understanding of these paracrine mechanisms may allow earlier and more effective clinical therapies

Remyelination

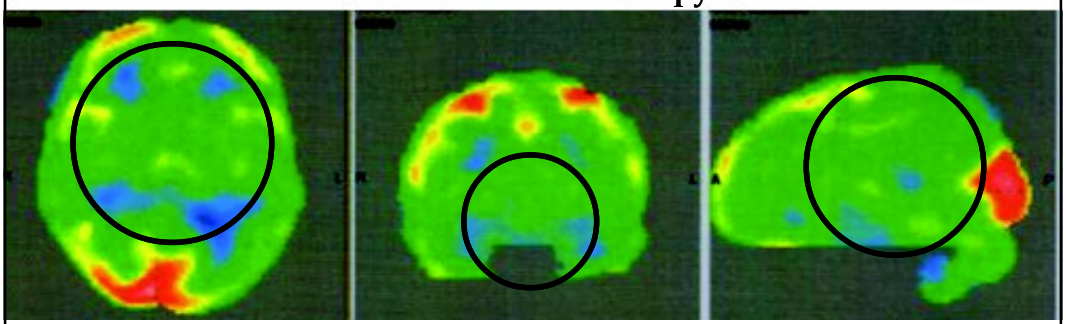
Remyelination involves reinvesting demyelinated axons with new myelin sheaths. Previous attempts aimed at regenerating myelin-forming cells have been successful but limited by the multifocal nature of the lesions and the inability to produce large numbers of myelin- producing cells in culture. Stem cell-based therapy can overcome these limitations to some extent and may prove useful in the future treatment of demyelinating diseases.

How Stem Cells Work in Autism

Before Stem Cell Therapy



After Stem Cell Therapy

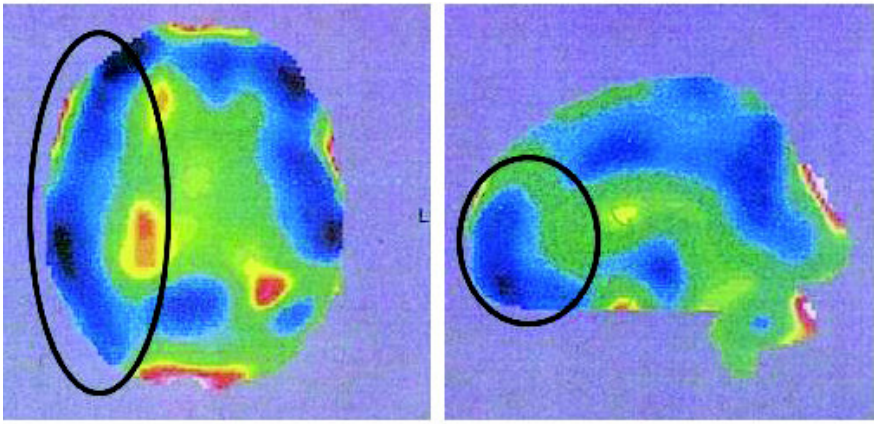


The above figure is a comparison of PET CT scans done before and after stem cell therapy. The red and the yellow areas denote hypermetabolism which have reduced to green which denotes near normal metabolism.

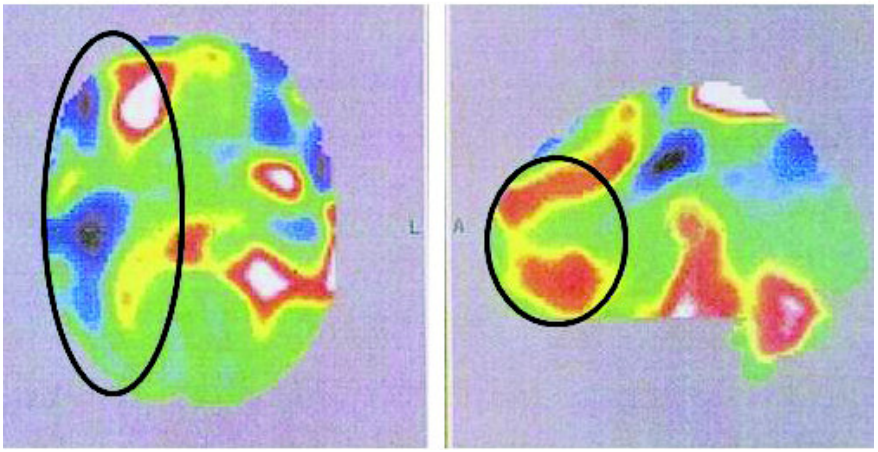
In autism, stem cells carry out the repair process by promoting angiogenesis, reversing the hypoperfusion by increasing the oxygen supply and balancing inflammation. These mechanisms may help in increasing the metabolic activity of the brain which can be seen in the PET CT scans.

How Stem Cells Work in Cerebral Palsy

Before Stem Cell Therapy



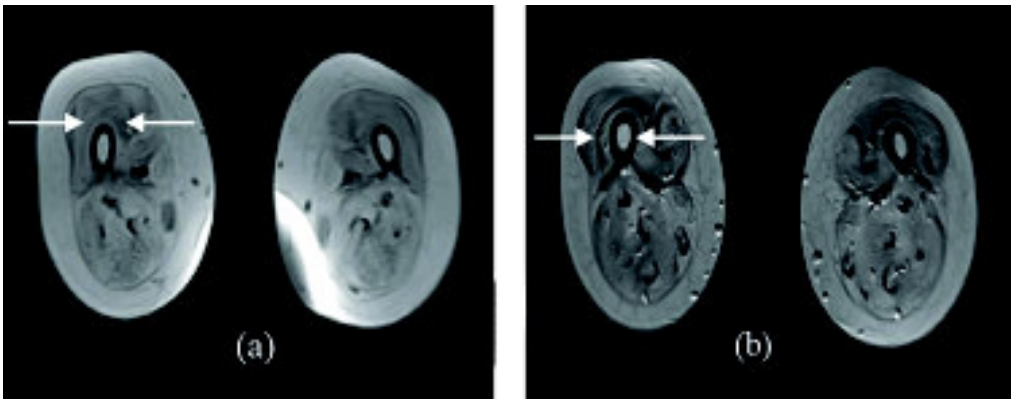
After Stem Cell Therapy



The above figure is a comparison of PET CT scans done before and after stem cell therapy. Post stem cell therapy there is reduction in the blue and the black areas which denote severely damaged areas and more green/red areas are seen indicating reduction in damage and improved brain function.

In Cerebral Palsy, stem cells reverse the brain damage by increasing the blood and oxygen supply through angiogenesis. They stimulate the endogenous cells and halt further cell death.

How Stem Cells Work in Muscular Dystrophy



Before Stem Cell Therapy **After Stem Cell Therapy**

The above figure is a comparison of MRI of vastus medialis and vastus lateralis of a patient with DMD done before and after stem cell therapy. The white arrows in (b) denote muscle regeneration

In Muscular dystrophy, the injected stem cells migrate to the damaged areas and differentiate into new cells. Through paracrine mechanism, they also stimulate the existing satellite cells and halt further degeneration of the muscles.

Recent studies have shown that remyelination can be accomplished by supplying demyelinated regions with cells like Schwann cells, oligodendrocyte lineage cells lines, olfactory ensheathing cells (OECs), embryonic stem cells and neural stem cells, and Adult bone marrow derived stem cells. The remyelinating effect of these cells may be via one or more mechanisms, including: the stem cells act as an immunomodulator by producing soluble factors; they carry out direct cell replacement by differentiating into neural and glial cells in the lesion; and they indirectly promote neural and glial differentiation of endogenous cells. Interactions with viable axons and supportive astrocytic responses are required for endogenous immature cells to fulfill their potential remyelinating capacity.

Contrary to the general expectations that stem cells would primarily contribute to formation of tissue cells for repair, other mechanisms such as paracrine effects and remyelinations appear to be important ways via which stem cells seem to exert their effect. More basic research to understand these mechanisms is underway throughout the world.

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5. Stem Cell Transplantation in Autism

Autism spectrum disorders (ASD) are pervasive developmental disorders characterized by impairments or delays in social interaction, communication and language, as well as by repetitive routines and behaviors. In a report by the Surgeon General, autism is described as a severe, chronic developmental disorder, which results in significant lifelong disability. It also states that autism has roots in both structural and functional brain abnormalities with genetic predispositions. They are called spectrum disorders because of the wide range and severity of symptoms. The prevalence of autism has increased radically over the few decades. In United States of America the cases have increased from 4 per 10,000 in 1989 to 67 cases per 10,000 in 2000. Currently the figure has exponentially increased to 1 in 68 children in USA and 1 in 250 in India.

Autism spectrum disorder is associated with known genetic causes in 10-15% of cases. The most common causes include fragile X syndrome (about 3%), tuberous sclerosis (about 2%), and various cytogenetic abnormal findings such as maternal duplication of 15q1-q13 (roughly 2%), and deletions and duplications of 16p11 (about 1%).

Like other complex neurodevelopmental disorders, ASD is thought to be the final common pathway of multiple etiological and neuropathological mechanisms, thus, complicating the search for autism-specific biological markers. As there are no definitive biological markers, diagnosis relies on the recognition of an array of behavioral symptoms that vary from case to case, that are increasingly heterogeneous and which overlap with those of other childhood neuropsychiatric disorders.

Pathophysiology:

Brain hypoperfusion and immune dysfunctions have been characterized as the two main brain pathologic alterations in autism cases.

Research on animal brain to study the etiology of autism has shown that a major dysfunction of the autistic brain resides in neural mechanisms of the structures in the medial temporal lobe, and, perhaps, more specifically the amygdaloid complex. Distinct patterns of memory losses and socioemotional abnormalities emerge as a result of extent of damage to the medial temporal lobe structures.

Functional brain imaging, such as positron emission tomography (PET), single photon emission computed tomography (SPECT) and functional MRI (fMRI) have added a new perspective to the study of normal and pathological brain functions. These imaging studies have shown bilateral hypoperfusion of the temporal lobes in autistic children. In addition, activation studies, using perceptive and cognitive paradigms, have also shown an abnormal pattern of cortical activation in autistic patients. This suggests that different connections between particular cortical regions could exist in autism. Further to this, Brain SPECT has established a positive correlationship between regional cerebral flow and development quotient (intelligence quotient) in the left laterotemporal and both dorso-medio-lateral frontal areas, and a negative one in the cerebellar vermis area. This goes to show that cerebral blood flow decrease in the temporal and frontal areas relates to the brain mechanism of autism as well as to intelligence levels.

Clinical symptoms

As with overall autism, symptom presentation is ambiguous among the very young. Children diagnosed with ASD suffer from problems with sensory integration, speech, and basic functions like toilet training, getting dressed, eating meals, brushing teeth, etc.

Many medical conditions can also accompany these symptoms, such as digestive problems, severe allergies, inability to detoxify, very high rate of infection, and vision problems. Some children with ASD display violent or self-harmful behaviors. IQs in children with this disorder range from superior to severely mentally retarded.

Conventional therapies

Autism, similar to other neurodevelopmental disorders, is incurable and requires chronic management. Since, it has become a prevalent dilemma in the present society; interventions to improve the quality of life of people with autism have increased considerably. But, the fact remains that individuals with ASD are among the most difficult and costly to treat.

Currently, the treatments available for autism can be divided into behavioral, nutritional and medical, there being no standard approach.

Recent data has shown evidence that early intensive behavioral intervention has improved outcomes.These interventions are designed to facilitate development and learning, promoting socialization, self awareness, reducing maladaptive behaviors and educating and supporting families. Nutritional interventions restrict allergy-associated dietary components, as well as to supplement minerals or vitamins which may be lacking. Autistic children tend to have problems with digestion, including food sensitivity - particularly to casein and gluten in dairy and wheat products. Medical interventions usually treat specific activities associated with autism. For example, serotonin reuptake inhibitors (SSRI's) such as fluoxetine, fluvoxamine, sertraline, and

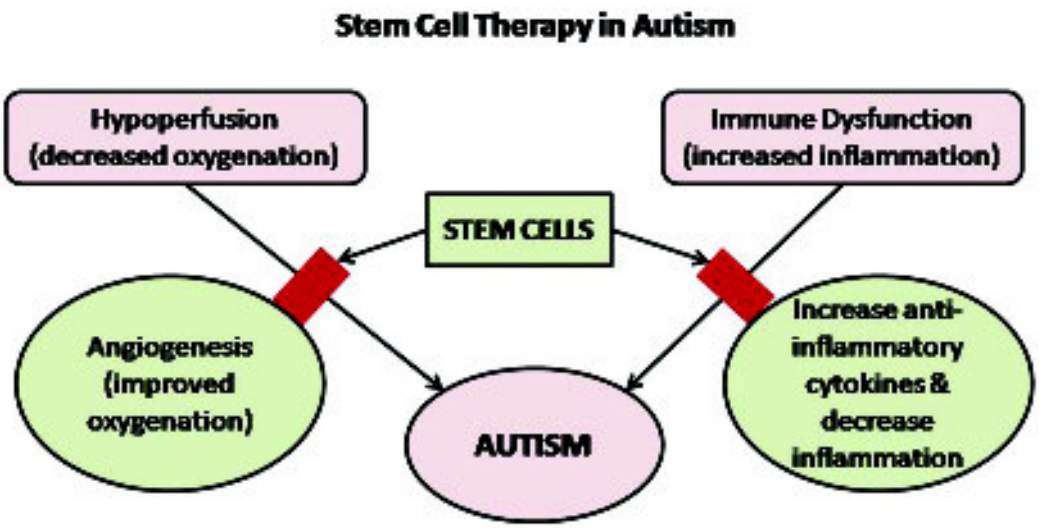
clomipramine, are used for treatment of anxiety and depression. Some studies have shown that SSRI's also have the added benefit of increasing social interaction and inhibiting repetitive behavior. Typical antipsychotic drugs such as thioridazine, fluphenazine, chlorpromazine, and haloperidol have been showed to decrease behavioral abnormalities in autism. Atypical antipsychotics such as risperidone, olanzapine and ziprasidone have also demonstrated beneficial effect at ameliorating behavioral problems. Autism associated seizures are mainly treated by administration of anticonvulsants such as carbamazepine, lamotrigine, topiramate, and valproic acid. Attention deficient/hyperactivity is treated by agents such as methylphenidate.

Other treatments include psychiatric care, neurodevelopmental therapies, and treatment for co-occurring medical conditions.

Other upcoming treatments include, Hyperbaric Oxygen therapy (HBOT), Relationship Development Intervention (RDI),Water Therapy or Aquatic Therapy, Floortime or Developmental Individual Difference Relationship Model (DIR Model), Hippotherapy ,The Handle Method, Neurofeedback, etc.

Summary of current clinical evidence of the role of stem cells in Autism

Though, autism is a very complex neurodevelopmental disorder, different studies have also tried understanding the basic pathophysiology of autism or, in simple terms, why autism develops and what happens in the brain. It is understood now, that the neural hypoperfusion and immune dysregulation are the two key pathologies associated with Autism. There is reduced blood flow supply to certain specific areas of the brain (mesial temporal and cerebellum), which in turn could be the cause of reduced functioning in this area. 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 stem cells having strong angiogenic potential which could facilitate counteractive processes of improving perfusion by angiogenesis and balancing inflammation by immune regulation would exhibit beneficial clinical effects in patients with autism. Other contributing effects of the 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.

Worldwide published scientific evidence on Stem Cell Therapy in Autism:

The first ever clinical study published in the world to give clinical evidence of the role of stem cells in autism, came out in August, 2013 from the NeuroGen Brain and Spine Institute, Mumbai, India. This is an open label proof of concept study of autologous bone marrow mononuclear cells (BMMNCs) intrathecal transplantation in 32 patients with autism followed by multidisciplinary therapies. All patients were followed up for 26 months (mean 12.7).The outcome measures used were Childhood Autism Rating Scale(CARS), Indian Scale for Autism Assessment(ISAA), Clinical Global Impression (CGI), and Functional Independence Measure(FIM/Wee-FIM) scales. Positron Emission Tomography-Computed Tomography (PET-CT) scan recorded objective changes. It was found that 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. On CGI-II 96% of patients showed global improvement. The efficacy was measured on CGI-III efficacy index. Few adverse events were reported, including seizures in three patients, but these were reversible and easily controlled with medications. The encouraging result of this leading clinical study provides future directions for application of cellular therapy in autism.

The second study to be published is by Shenzhen Beike Bio-Technology Co., China, which studied the safety and efficacy of human umbilical cord mesenchymal stem cells (hUC-MSCs) and human cord blood mononuclear cells (hCB-MNCs) transplantation in patients with autism. This study comprised of 37 subjects diagnosed with autism ,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. Treatment safety was evaluated with laboratory examinations and clinical assessment of adverse effects. They used the Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) scale and Aberrant Behavior Checklist (ABC) to assess the therapeutic efficacy at baseline (pre-treatment) and following treatment. They did not find any significant safety issues related to the treatment and no observed severe

adverse effects. 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 ($p < 0.05$).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.

Recently, in October 2014, Bradstreet et al, Ukraine 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 (ATEC) test and Aberrant Behavior Checklist (ABC) scores. Improvement was also seen in behavior, eye contact, appetite, etc

Other ongoing clinical trials worth mentioning, on similar lines, are being carried out, in Mexico, Greece and Ukraine.

A detailed analysis of the improvements seen after stem cell therapy:

Presented are the details of the improvements seen in 100 children with autism who underwent stem cell therapy. Out of these 91% showed clinical improvements.

Improvements in Autistic Children after Stem Cell Therapy can be broadly classified as:

A) **Clinical/Neurological improvements:**

These were reduction in abnormal stereotypical behavior, reduction in self stimulatory behavior, improvement in eye contact, attention span, speech, communication skills and social interactions (shown in graphs/figures).

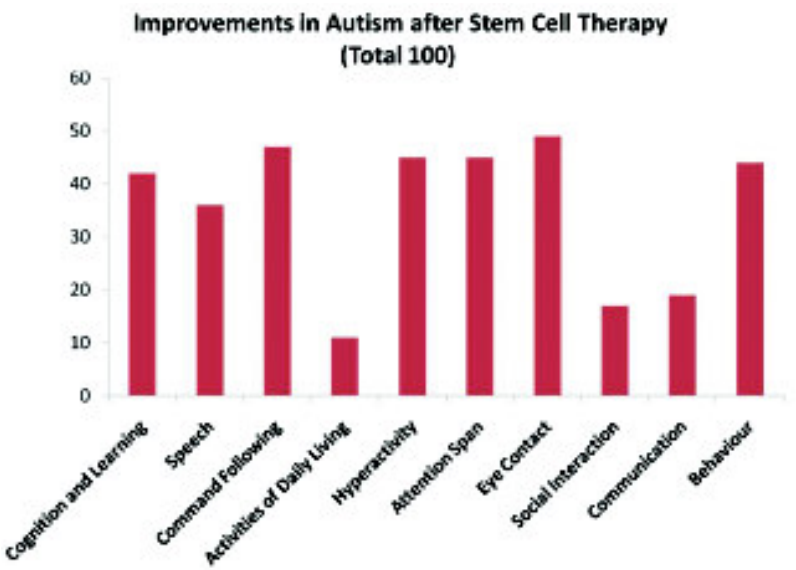


Figure 1: Graph representing symptomatic improvements in autism after stem cell therapy

Hyperactivity, eye contact and attention span:

Hyperactivity is one symptom, which improves most visibly. In 54% children, it was found that hyperactivity reduced significantly. This was , generally accompanied by improved eye contact along with improved attention span. This overall helped in training the child better. Child would now sit at one place for a longer time and respond to commands as well as teaching better. This overall resulted in enhanced school performance as well as understanding and cognition. It is also worth mentioning here that in those children who were on medications for hyperactivity, it was possible to reduce the dose of the medicines. This was done by the child's previous physician/ pediatrician. There was no consequent increase in hyperactivity.

Abnormal and Self stimulatory behavior:

Almost 52% of the children showed reduction in abnormal behavior. Parents found that hand flapping, unnecessary laughing and crying, use of abusive language reduced. This leads to the children becoming more manageable. While 31 % showed reduction in self stimulatory behavior, equal number showed reduction or stopping of self injurious behavior. Violent behavior and aggression also reduced.

Communication:

After stem cell therapy, it was observed that communication improved in many of the children. This was both verbal as well as non verbal. One child with autism, who was almost non verbal and was extremely hyperactive, once he calmed down after stem cell therapy, started gesturing his needs. He also started verbalizing in bisyllables and small sentences. His overall communication about his needs as well as his emotions to his parents and sister improved remarkably. Overall, improvement in verbal communication was also noticed in 23 % of the children, with respect to improved speech and language communication skills.

Social interaction:

Along with a reduction in hyperactivity, attention to surroundings and awareness about surroundings also increased. This lead to improved social interaction, along with an increase in initiative for different activities, which hitherto, was not observed in these children. Overall, 20% of the children improved in their social interaction skills.

B) Improvements in the Objective Assessment scales:

Changes on Objective Assessment scales:

All the children were scored on the ISAA (Indian Scale for Assessment of Autism) scale which quantifies the severity of autistic symptoms and enables the measurement of associated disability. The score was noted before and 6 months after stem cell therapy. There was a significant improvement in the scores after the stem cell therapy.

There were some patients who also showed a dramatic change on the severity category. What is remarkable and worth noting is that 3 of these children went from severe to moderate autism, 8 went from moderate to mild and 1 patient changed to the non autistic category.

Another scale used for monitoring the children is the Clinical Global Impression (CGI) scale. This is used as a clinical research tool to measure the severity of the illness along with the efficacy and response of the intervention/treatment in patients with autism. This scale also revealed improvement, when performed before and after the stem cell therapy, in terms of, the severity of illness and efficacy index. Thus, this indicated that the treatment was efficacious.

C) Objective improvements on SPECT/PET CT Scan Brain:

- Most of the children with autism, have grossly normal brain morphology on MRI Scans of the Brain. However SPECT/PET CT Scan of the brain ,which shows abnormalities in brain perfusion/metabolism is now emerging as a useful imaging technique to identify the areas which are affected as well as the severity of the damage.
- These imaging were done before the stem cell therapy and 6 months after the therapy. The clinical improvements as well as changes on the assessment scales, are also objectively corroborated by changes seen on the SPECT /PET CT scan of the brain.

An example below is a PET CT Scan brain of a child with autism before the stem cell therapy and 6 months after the stem cell therapy. 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

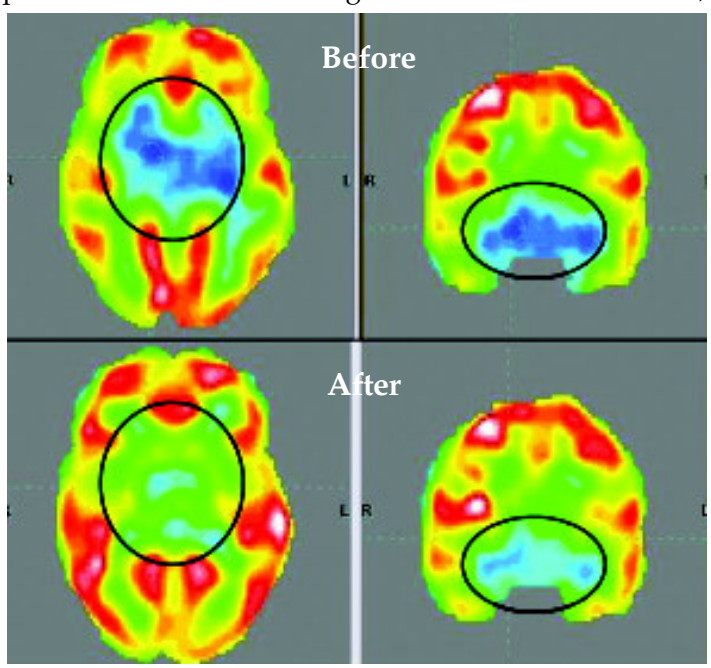


Figure 2. : Pre and post stem cell therapy PET CT scan of the brain. The areas of reduced metabolism and FDG uptake are seen in different shades of blue colour.The improved metabolism is depicted as an increased FDG uptake which is seen in this PET- CT scan as more areas of green color , six months after stem cell therapy.

parahippocampus (figure:PET CTScan).This correlates with reduction in hyperactivity, improved cognition, colour concepts, social interaction, awareness about surroundings, phonation, communication using non verbal means/gestures. Changes in objective assessment scales, such as, WeeFIM (From 58 to 65) and ISAA from 123 to 103) was also seen in the same child.

Adverse Events / Complications:

a) None of the children had neurological worsening or clinical deterioration.

b) There are some minor post procedure effects, such as headache or nausea, which occur in some patients but which settles in 2-3 days. This is part of the procedure of injecting into the spinal fluid. Its called a spinal headache.

c) Some temporary increase in hyperactivity may occur which settles over a period of time.

d) Epilepsy is the only significant adverse event that can occur. However we have noticed that it does not occur in patients who are already on anticonvulsant medications and also does not occur in those children who have a normal EEG before the treatment. However in those children whose EEG; show abnormal epileptic activity and who are not on anti epileptic medications seizures can occur. To prevent this it is recommended that an EEG be done before the stem cell therapy. If it is normal then there is nothing to worry about. If it is abnormal then it is recommended that antiepileptic medications be started before the stem cell therapy and be continued for a 6 month period. Those already on antiepileptic drugs should continue these as before and after the stem cell therapy. With this strategy the possibility of seizures or an increase in seizures is almost completely eliminated. We noticed this in 3 of the 6 children who had abnormal EEGs but were not on medications. However after following the above there has been no incidence of epilepsy in any of the children.

Conclusion :

Based on published literature and our own clinical experience we consider that Stem cell therapy is a safe and effective treatment option for children with Autism. Clinical improvements are seen in over 90% of those treated and include improvements in hyperactivity, communication, emotional responses, social relationships, behavior, speech, attention, eye contact and sensory problems. These clinical improvements are collaborated with objective improvements seen on PET CT Scans of the brain.

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6. Stem Cell Transplantation in Cerebral Palsy

Cerebral palsy (CP) is a non progressive encephalopathy with clinical syndrome of restricted movement and posture with numerous possible etiologies. Its development could be attributed to prenatal, perinatal or post natal factors. Evidence suggests that prenatal factors result in 70-80% of cases of cerebral palsy. Cerebral palsy (CP) is known to affect 2/1000 live-born children. The symptoms of CP vary in terms of severity. The main symptoms include muscle spasticity, muscle weakness, uncontrolled movements, impaired mobility, speech impairment and/or challenges in eating, dressing, bathing, etc depending on the area of the brain affected. Movement dysfunction is often accompanied by visual impairment, hearing loss, osteoporosis, learning disabilities, cognition impairment, behavioral issues and seizures. Risk factors for cerebral palsy include prenatal anemia, improper nutrition, infections, premature delivery, etc. Hypoxia and ischemia are also major risk factors prenatally and during delivery.

The conventional treatments available currently for CP are physical and behavioral therapy, Hyperbaric oxygen therapy (HBOT), Botulinum A toxin injection, surgical treatments, assistive devices, and management of associated conditions.

The prevalence of CP is increasing due to decrease in mortality of low birth weight infants and increase in the rate of CP in these children. Hence, establishing a standard therapeutic approach is the focus of researchers and clinicians all over the world. Although the available treatment options are helpful in managing the symptoms to some extent, none of them repair the damaged brain.

Pathophysiology of brain damage in cerebral palsy

Hypoxic ischemia is the most common cause of damage

Hypoxic ischemia is a common cause of damage to the fetal and neonatal brain leading to cerebral palsy and associated disabilities in children. The neuropathology underlying cerebral palsy mainly includes periventricular leukomalcia (PVL). PVL consists of diffuse injury in deep cerebral white matter, with or without focal necrosis and/or loss of pre-myelinating oligodendrocytes (pre-OLs), astrogliosis and microglial

infiltration. The vulnerability of these cells to damage depends on type of cells and stage of development at which the damage occurs. Oligodendrocytes (OLs) develop through a well-established lineage of OL progenitors to pre OLs to immature OLs to mature OLs. Loss of pre OLs in hypoxic ischemia may lead to deficiency of mature OLs resulting in myelination disturbance which leads to neuronal dysfunctions.

Microglial activation- a secondary effect to ischaemia : adding insult to injury!

The activated microglia secretes various cytokines, such as tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ), Interleukin -1 beta (IL-1β) and superoxide radicals, exerting a toxic effect on neurons and oligodendrocytes.

Connecting the pathophysiology of cerebral palsy and stem cells:

Stem cells stimulate the repair process by homing to the injured sites of the brain and carrying out regeneration. Injury to the brain at and around the time of birth, leads to the damage of the stem cells in the brain. This leads to loss of the inherent regenerative potential or resources (the neuronal progenitors or stem cell niches in the growing brain) of the brain. Stem cell therapy, hence, restores the lost cells and helps form newer cells and connecting cells. It may also support their survival by introducing other cell types able to restore missing enzymes to an otherwise deficient environment. Stem cells also reduce the levels of harmful chemicals, such as TNF-α, IL-1γ, IL-1β, IL-6 raised due to activation of cells forming a scar tissue in the brain, enhancing the endogenous brain repair. These cells also secrete neurotrophic factors and growth factors such as connective tissue growth factor, fibroblast growth factor 2 and 7, interleukins, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and basic fibroblast growth factor (bFGF) which are responsible for cell multiplication, protection of brain cells and angiogenesis/formation of new blood vessels, retrieving the lost tissue functions.

Recent advances in stem cell therapy provide the hope of developing more effective interventions in treating CP. Research has shown that bone-marrow-derived cells could develop into neural tissue. Woodbury et al. showed that adult rat and human bone marrow stromal cells can be induced to differentiate into neurons.

Various experimental studies have demonstrated that cell transplantation in the CP models lead to survival, homing and differentiation of cells into neurons, oligodendrocytes and astrocytes (support cells).

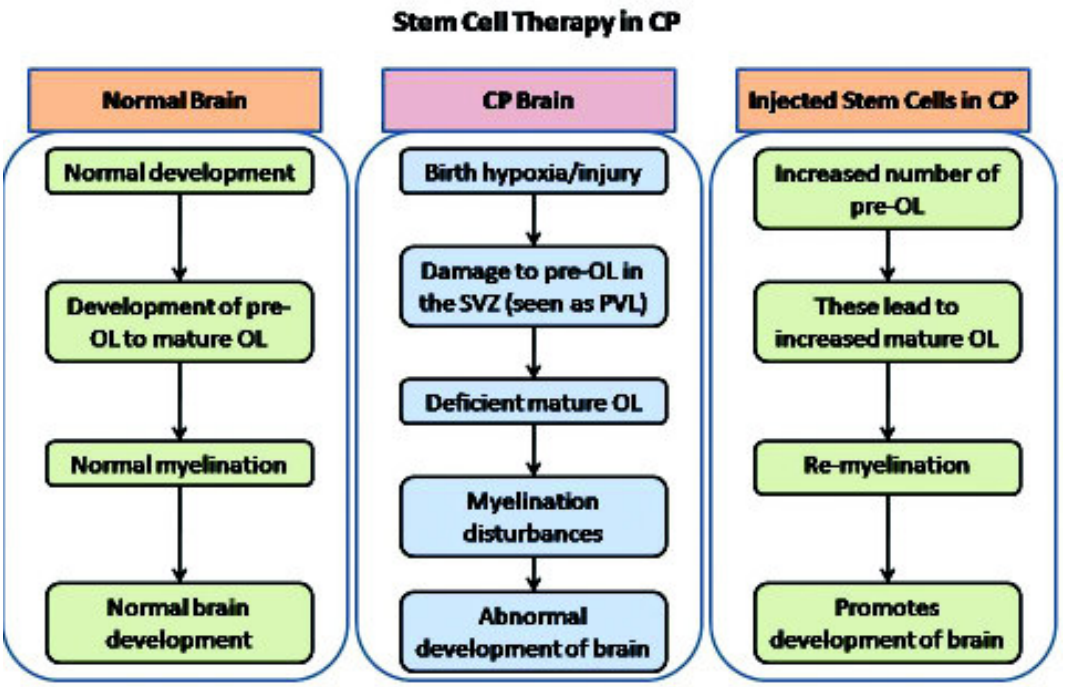
Mueller et al. (2005) demonstrated how human neural stem cells (hNSCs) replace the lost cells in a newborn mouse model of brain damage. Mice received brain parenchymal or intraventricular injections of hNSCs derived from embryonic germ (EG) cells. The stem cells migrated away from the injection site to the site of injury within the striatum, hippocampus, thalamus and white matter tracts and at remote locations in the brain. Subsets of grafted cells expressed neuronal and glial cell markers. It was found that

the complement of striatal neurons in brain-damaged mice was partially restored. Hence, they concluded that human EG cell-derived NSCs can engraft successfully into injured newborn brain, where they can survive and disseminate into the lesioned areas, differentiate into neuronal and glial cells and replace lost neurons.

Summary of current clinical evidence of the role of stem cells in Cerebral Palsy

Over the years, various types of stem cells and cellular elements have been used, via various routes of administration to stimulate neurogenesis in the damaged brain, especially in children. This intervention holds more logical attraction, since adaptive responses would be expected to be permissive in a growing brain.

Seledtsov et al, way back in 2005, carried out a controlled study which included 30 patients with severe forms of cerebral palsy. A cell suspension from immature nervous and haemopoietic tissues was injected into the subarachnoidal space of a recipient through a spinal puncture. One year after the treatment, activity of the major psychomotor functions in treated patients considerably surpassed the normal. Their findings suggested that cell therapy was an effective, safe and immunologically justified method of therapy for patients with cerebral palsy.



Similar route of stem cell transplantation (intrathecal) in 125 severely brain injured patients with cerebral palsy is a study done at the centre of immunotherapy, reported neurological improvement in 85% of the cases.

Recent work in the field of stem cells is seeing an increasing trend towards the use of either:

a) Allogenic/autologous cord blood derived cells

b) Autologous bone marrow derived cells.

The favoured route of transplantation, is biased towards safety, viz. either intravenous or intrathecal as opposed to direct stereotactic injection into the brain.

Ramirez et al presented their data based on parental observations and completed questionnaires concerning the responses of cerebral palsy children to the umbilical cord blood stem cells. Eight children (3-12 years of age) diagnosed with cerebral palsy underwent transplants with 1.5 million stem/progenitor cells (CD34+ and CD133+) which were purified and expanded from the American Association of Blood Banks (AABB)-certified human umbilical cord blood. According to parent tendered observational reports, none of the children had graft versus host reactions. All the eight children showed some improvement in mobility and/or cognitive function. Six children (75%) showed improvement in muscle tone, hip movement, leg movement, rolling to the side, sitting and standing balance by the end of six months.

The results demonstrated by Sharma et al agree with the above trend. Autologous mononuclear cells administered intrathecally in 20 CP children, with a mean follow up of 15 months ± 1 month, showed improvement in 85% cases. Improvement in muscle tone (15/20) and speech (10/20) as well as significant reduction in seizure frequency (2/20) patients and dystonic movements, improved limb strength (11/ 20) patients were observed. On FIM scale, 3 of them showed improved scores. Lee et al conducted a pilot study, wherein autologous cord blood (CB) cells were infused intravenously in 20 children with cerebral palsy. Out of them 11 were quadriplegics, 6 hemiplegics and 3 diplegics. On follow up after 6 months, diverse neurological domains improved in 5 patients (25%) as assessed with developmental evaluation tools as well as by fractional anisotropy values in brain MRI-DTI. The neurologic improvement occurred significantly in patients with diplegia or hemiplegia rather than quadriplegia.

Li et al carried out intravenous autologous BMSCs transplantation in an 11 year old CP boy. They carried out four infusions. Six months after the treatment he could walk better and his vision had significantly improved. These findings were further supported by electrophysiological examinations.

Hassan et al carried out a study on fifty two Egyptian patients with cerebral palsy who were divided into: group I (26 patients who underwent stem cell transplantation) and group II (26 patients who did not undergo stem cell transplantation). These

patients underwent intrathecal autologous bone marrow derived stem cell transplantation. On follow up of 1 year, statistically significant improvements were noticed in motor, independence and communication skills using Boyd's developmental progress scale and 100 points scale.

In a Chinese study, Yang et al carried out UC-MSC transplantation by intravenous infusion and intrathecal injections in twenty-five patients with CP. Six months after the transplantation, 22 of 25 cases subjectively had improvement in motor function. All patients objectively had improvement in scores of GMFM and BSS post-treatment.

Chen et al, assessed neural stem cell-like (NSC-like) cells derived from autologous marrow mesenchymal stem cells as a novel treatment for patients with moderate-to-severe cerebral palsy. 60 cerebral palsy patients were included in this study. (30 control group, 30 treatment group)

On a 6 month follow up, the GMFM scores in the transplantation group were significantly higher. All the 60 patients survived, and none of the patients experienced serious adverse events or complications. They concluded, that NSC-like cells are safe and effective for the treatment of motor deficits related to cerebral palsy.

Luan et al, treated 45 CP children by injecting neural progenitor cells (NPCs) derived from fetal tissue into the lateral ventricle. After 1 year, the developmental level in gross motor, fine motor, and cognition of the treatment group was significantly higher compared to the control group. No delayed complications of this therapy were noted.

Min et al, administered allogeneic umbilical cord blood (UCB) cells potentiated with recombinant human erythropoietin (rhEPO) in children with CP. In total, 96 subjects completed the study. Compared with the EPO (n = 33) and Control (n = 32) groups, the pUCB (n = 31) group had significantly higher scores on the GMPM and BSID-II Mental and Motor scales at 6 months. DTI revealed significant correlations between the GMPM increment and changes in fractional anisotropy in the pUCB group. 18F-FDG-PET/CT showed differential activation and deactivation patterns between the three groups. UCB cell treatment ameliorated motor and cognitive dysfunction in children with CP accompanied by structural and metabolic changes in the brain.

Clinical results of Stem Cell Therapy at NeuroGen BSI:

At Neurogen BSI, we have treated over 150 CP cases. In an analysis of 108 patients with maximum follow up of 4 years, stem cell therapy was found to be safe and effective in 92.6% of the patients. Improvements were in seen in symptoms like oromotor/speech, balance, trunk activity, upper limb activity, lower limb activity, muscle tone, ambulation and activities of Daily Living.

Out of the 108 patients, 15.74% showed significant improvements, 48.14% of patients showed moderate improvements and 28.7 % showed mild improvements.

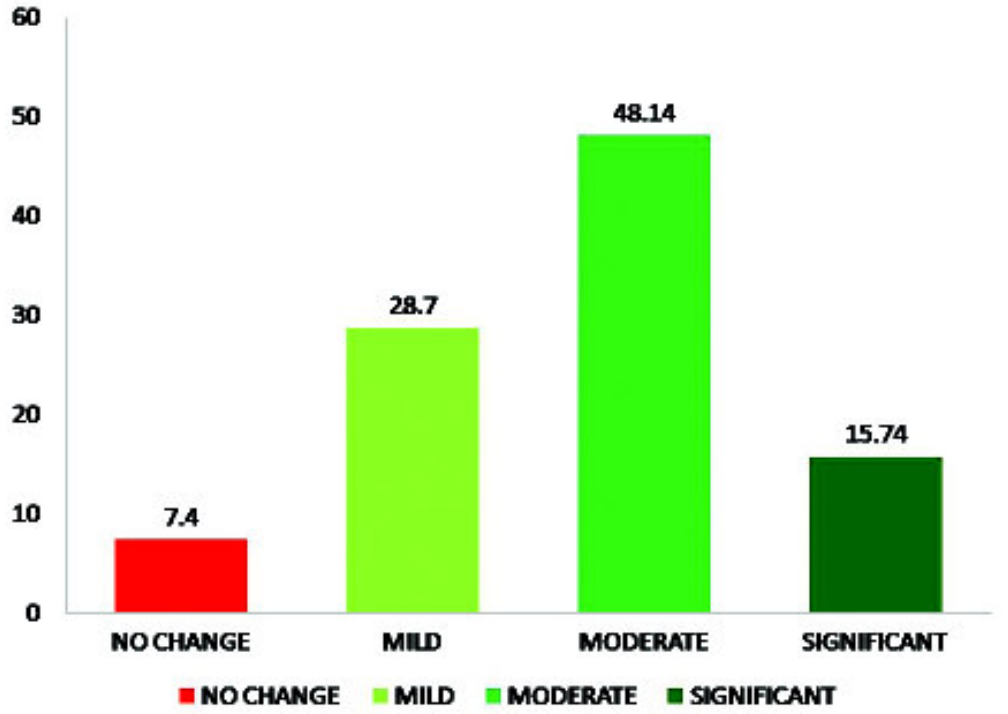


Figure 1: Graph representing improvements in Cerebral Palsy after stem cell therapy

Progressive improvements seen after stem cell therapy :

After stem cell therapy, immediate improvements were observed within a week in muscle tone, involuntary movements of the limbs, head control and drooling.

From 1 week to 3 months of intervention, improvement in voluntary control resulted in initiation of opening and closing of fingers and improved midline orientation. As the tone of the hypertonic muscles reduced, trunk control, sitting balance and gross motor movements of limbs also improved.. Many patients also showed improved oromotor activities.

From 3 months to 6 months of intervention, eye hand coordination was better due to improved head control and gross motor skills. Sitting balance improved further along with initiation of weight shifting while sitting. Maintaining upright position, dynamic trunk balance and weight bearing on legs improved. Initiation of steps while walking with support and/or assistive devices was also seen in patients who were not able to walk previously. Muscle tone and motor control and independence for daily activities improved.

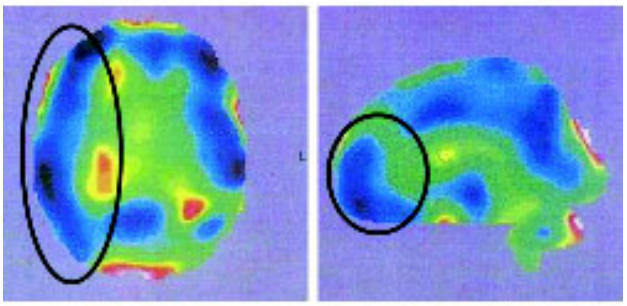
Cognitive skills and understanding improved progressively from one week to six months. Cooperation during therapy sessions was better due to which it was easier for the caregiver to handle the patient.. Cognition improved with respect to awareness, understanding, response time and command following.

Some patients were followed up even after six months, 1 year, 2 years, 4 years. These patients showed improvement in fine motor activities. Equilibrium reactions developed along with increase in dynamic balance. Speech started improving in the aspects of clarity, fluency and intelligibility. Individuals with monosyllable speech developed bisyllable speech, bisyllable improved to word formation and words improved to phrases. There was also gradual improvement in ambulatory status.

Objective improvements on PET CT Scan Brain:

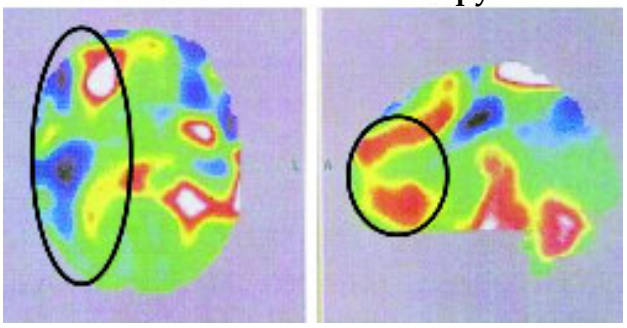
PET-CT scan was done to monitor improvements in the metabolic activity of the brain. The PET scan measures the 18-FDG uptake which correlates to the glucose metabolism in the brain. The damaged areas of the brain in CP are functioning low and any improvement in the functioning of these areas will lead to an increase in the FDG uptake. Previous studies in patients with CP have shown reduced metabolic activity in various areas of the brain depending on the individual case. A comparative scan performed before and after cell therapy demonstrated increased metabolic activity in frontal, parietal, temporal, basal ganglia, thalamus and cerebellar areas of the brain. The clinical and functional improvements correlated to the changes observed in the PET scan. Improved metabolism in frontal and temporal areas led to improvement in speech and memory. Improvement in basal ganglia led to improved voluntary movement and coordination. Improvement in parietal area led to improved awareness and improvement in cerebellum led to improved balance and fine motor coordination.

Before Stem Cell Therapy



Before stem cell therapy PET CT scans showing, blue/black areas as severe damage.

After Stem Cell Therapy



After stem cell therapy PET CT scan showing, reduced blue/ black areas and more green/ yellow/red denoting improved metabolic activity

Conclusion

Published international clinical work as well as our own experience clearly establishes the safety and efficacy of stem cell therapy for cerebral palsy. Improvements are seen in over 90% of the patients treated and these include oromotor , speech, balance , trunk activity, upper limb activity, lower limb activity, muscle tone, ambulation and activities of daily living. These clinical improvements are reflected in the objective improvements seen on the PET CT Scans of the brain done before and after the stem cell therapy.

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7. Stem Cell Therapy in Intellectual Disability

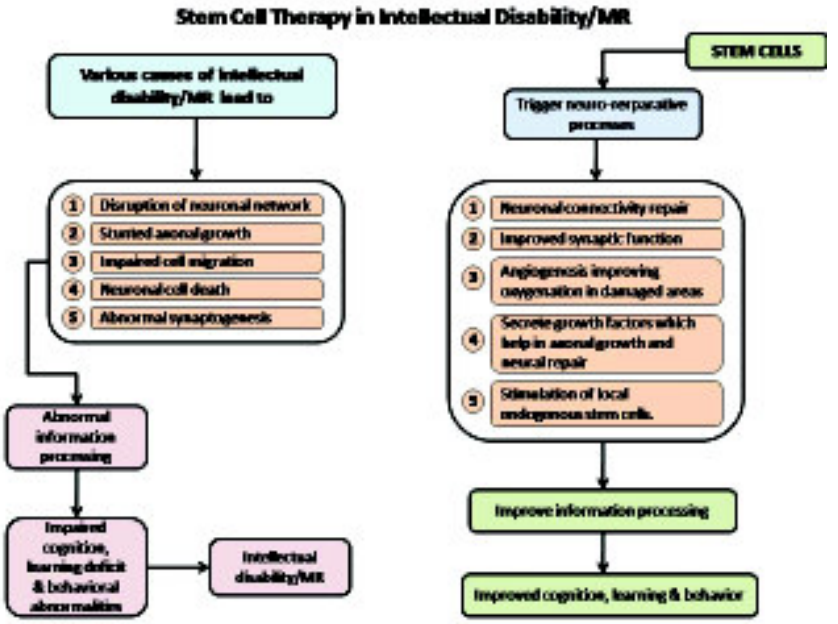
Intellectual disability (ID) in children is a heterogeneous group of disorders with varied causes. It is a developmental disability which first appears in children under the age of 18. It is defined as a level of intellectual functioning (as measured by standard intelligence tests) that is well below average and results in significant limitations in the person's daily living skills (adaptive functioning). Such skills include the ability to produce and understand language (communication); home-living skills; use of community resources; health, safety, leisure, self-care, and social skills; self-direction; functional academic skills (reading, writing, and arithmetic); and job-related skills. In Intellectual disability, the IQ score is below 70-75. About 30% of ID cases are caused by hereditary factors. Intellectual disability may be caused by an inherited genetic abnormality, such as fragile X syndrome. Fragile X, a defect in the chromosome that determines sex, is the most common inherited cause of Intellectual disability. Single-gene defects such as phenylketonuria (PKU) and other inborn errors of metabolism may also cause Intellectual disability if they are not discovered and treated early. An accident or mutation in genetic development may also cause retardation.

The common symptoms presented by children with Intellectual disability are impairment in adaptive functioning, continued infant-like behavior, failure to meet developmental milestones such as sitting, crawling, walking, or talking, in a timely manner, decreased learning ability and ability to think logically, trouble remembering things, failure to meet the intellectual development markers, inability to meet educational demands at school, lack of curiosity and difficulty in solving problems.

With increasing awareness, the prevalence of intellectual disability has risen considerably. Hence, to find a cure for it is the need of the hour. Currently, there is no treatment available for ID. But with correct support and teaching, most individuals can learn to do their daily activities. Management of the patient depends on the level of the child and the associated conditions such as epilepsy, hyperkinesis, behaviour problem and sensory handicaps. But these strategies do not answer the core neurological problems of ID.

Stem Cell Therapy for Intellectual Disability

In case of intellectual disability, any damage to the brain is a permanent and irreversible damage as the neurons of the brain, once damaged, cannot repair themselves on



their own. The underlying neuropathology of intellectual disability includes neuronal death along with disruption in neuronal networks, cell migration, cell multiplication, axon growth, brain plasticity, synaptogenesis, etc. Studies have shown that major defects are recorded in hippocampus and cerebral cortex areas of the brain which further lead to faulty information processing and consecutively affect the cognition and adaptive behavior.

To reverse the damage caused to the central nervous system, only a neurorestorative therapy like stem cell therapy would be beneficial. As discussed in the previous chapters, stem cells have a unique property of migrating towards the damaged areas on administration. They survive, migrate, proliferate and differentiate into the required cell types. They not only replace the dead cells but also stimulate the endogenous cells and prevent further damage. Their paracrine activities such as secretion of growth factors, angiogenesis, neurogenesis, immunomodulation, decreasing inflammation, etc also help in the repair process. This could help repair the disrupted neuronal networks in ID and hence improve the information processing.

Not many clinical studies have been carried out to study the effect of stem cell therapy in ID. But animal studies have shown that administration of stem cells may support the ability for structural brain repair as well as cognitive improvement in models with damaged brain. At Neurogen Brain Spine Institute, 18 patients with intellectual disability were administered with stem cell therapy. On a mean follow up of 6 months, 70% cases showed improvement in toilete training, 64.29% in home living, 60% in communication, 57.14% in self-care, 50% in cognition and social skills amongst others. However, the improvements recorded were minimal as majority of the patients belonged to the older age group. Hence, the results might vary when children are treated at an early age.

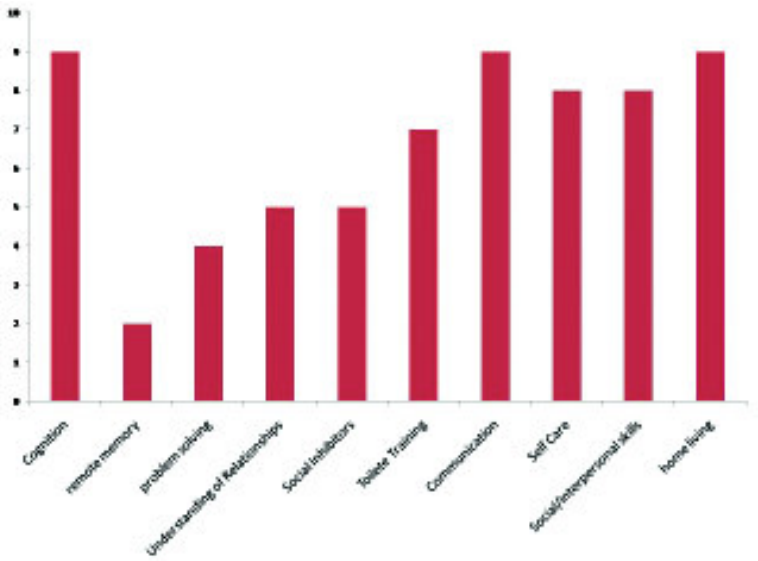


Figure 1: Graph representing improvements in intellectual disability after stem cell therapy (N=18)

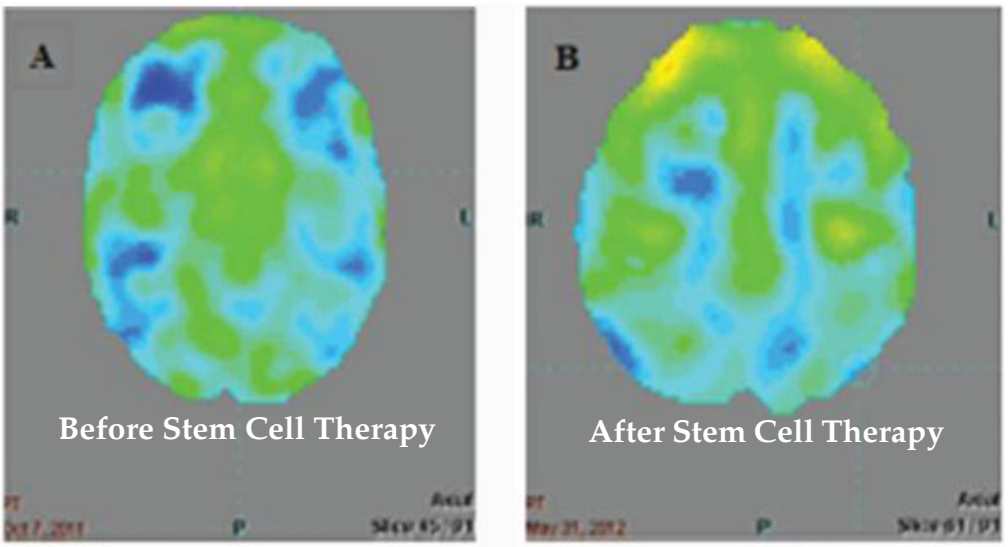


Figure 2: PET-CT scan images pre stem cell therapy (A) and post stem cell therapy (B) showing the increased FDG uptake in the frontal, parietal, temporal, occipital, mesial temporal structures bilaterally.

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8. Stem Cell therapy in Muscular Dystrophy

Muscular dystrophy (MD) is a group of genetic disorders that weaken the muscles. Each type of muscular dystrophy is associated with a distinct genetic mutation. The nature of the gene mutation and location of the chromosome determines the characteristics of the muscular dystrophy and their inheritance. But in general they are characterized by progressive wasting and weakness of the skeletal muscles. Most types of MD are multi-system disorders with manifestations in the heart, endocrine glands, skin, eyes, gastrointestinal and nervous systems.

Muscular dystrophy is broadly classified into nine types including Duchenne, Becker, limb girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal and Emery-Dreifuss. Out of these Duchenne, Congenital and Emery-Dreifuss muscular dystrophy are commonly observed in children. They are classified based on the gene mutation, age of onset of the symptoms etc.

Duchenne muscular dystrophy (DMD)

DMD is one of the most common childhood forms of muscular dystrophy affecting mainly boys. It is caused due to a mutation in the dystrophin gene, located on the X chromosomes (Xp21) in humans. The protein dystrophin, coded by the dystrophin gene is an important structural component of the muscle providing structural stability to the dystroglycan complex (DGC), located on the cell membrane. Absence of dystrophin allows excess calcium to penetrate the sarcolemma (cell membrane). In a complex cascading system, increased oxidative stress within the cell damages the sarcolemma, and eventually results in the death of the cell. Muscle fibers undergo necrosis and are ultimately replaced with adipose and connective tissue.

The characteristic features of DMD are muscle wasting beginning from the legs and pelvis, then progressing to the shoulder and neck muscles, followed by loss of arm muscles and respiratory muscles. Calf muscle enlargement (pseudohypertrophy) and a positive Gowers' sign are commonly observed. Cardiomyopathy is also common, but the development of congestive heart failure or arrhythmias (irregular heartbeats) is occasional. Affected children usually tire more easily and have less overall strength than their peers.

Common symptoms of DMD are frequent falls, fatigue, weakness of all antigravity muscles, equinnus gait, waddling gait (increased lumbar lordosis), swelling (pseudohypertrophy) of calf muscles.Tightening of achilles tendon and muscles of the thigh impair functionality because the muscle fibers shorten and fibrosis occurs in connective tissue.

Progressive respiratory muscle weakness and/or cardiomyopathy is the cause of mortality in these children. The life span ranges from 17 years to 22 years.

Becker Muscular Dystrophy (BMD)

BMD is a less severe form of Duchenne muscular dystrophy, but with a later, and much slower rate of progression. The genetic defect lies in the same gene/protein (dystrophin), however, there is some amount of protein formed, which makes the severity lesser and progression slower. Noticeable signs of muscular dystrophy also include the lack of pectoral and upper arm muscles, especially when the disease is unnoticed through the early teen years (some men are not diagnosed with BMD until they are in their thirties). Muscle wasting begins in the legs and pelvis (or core), then progresses to the muscles of the shoulders and neck, followed by loss of arm muscles and respiratory muscles. Calf muscle enlargement (pseudohypertrophy) is quite obvious. Cardiomyopathy may occur, but the development of congestive heart failure or arrhythmias (irregular heartbeats) is rare.

Congenital muscular dystrophy (CMD)

Congenital muscular dystrophy is a group of clinically and genetically heterogeneous neuromuscular disorders. It is a slow progressing disorder with onset of symptoms since birth. CMD results from mutations in a variety of different genes, including those encoding the laminin 2 chain, fukutin-related protein. Classification of the CMDs has become increasingly complex, and a wide spectrum of clinical features is now apparent. Patients present at birth, or within the first few months of life, with hypotonia, muscle weakness and often with joint contractures. Serum creatine kinase (CK) levels may also be markedly elevated in some cases. A major distinction between the various forms is the involvement of the central nervous system (CNS) which may include white matter abnormalities, structural changes, mental retardation and involvement of the eyes. Early and severe rigidity of the spine, distal joint laxity, muscle hypertrophy and respiratory insufficiency are also features of note in distinct entities.

Emery- Dreifuss Muscular Dystrophy (EDMD)

Emery-Dreifuss muscular dystrophy (EDMD) is a rare form of dystrophy which is benign in nature. The types are distinguished by their pattern of inheritance: X-linked, autosomal dominant and autosomal recessive. The genes responsible for EDMD encode proteins, emerin and the lamins A and C, which are associated with the nuclear envelope

The onset of the disorder is in the early childhood and its progression is relatively slow. It is characterized by early contractures mostly involving the elbows, ankles, and neck; progressive muscle wasting and weakness beginning in the upper arms and lower legs and progressing to muscles in the shoulders and hips and cardiomyopathy Although the three types have similar signs and symptoms, researchers believe that the features of autosomal dominant Emery-Dreifuss muscular dystrophy are more variable than the other types. A small percentage of people with the autosomal dominant form experience heart problems without any weakness or wasting of skeletal muscles.

Limb Girdle Muscular dystrophy (LGMD),Oculopharyngeal muscular dystrophy (OPMD), Myotonic dystrophy (DM),Facioscapulohumeral muscular dystrophy (FSHD), Distal Muscular dystrophy (DD) are some of the other common types of MD, which though have their routes in childhood, manifest in early or late adulthood.

Diagnosis of Muscular dystrophy

Diagnosis of muscular dystrophy is based upon a clinical assessment (combination of a characteristic clinical presentation), immunohistochemistry tests, muscle biopsy,electrophysiological tests,genetic work up etc.

A typical clinical presentation of slow or late achievements/development of motor milestones, calf pseudohypertrophy and increased level of creatine kinase (CPK) are indicators of high suspicion of muscular dystrophy. An electromyography (EMG) shows electrical activity suggestive of a primary muscle pathology/myopathy.

A muscle biopsy followed by immunohistochemistry or immunoblotting generally confirms muscular dystrophy. It may also be able to indicate the type of MD.

Genetic test (from blood test) confirms the type of muscular dystroph and also demonstrates the various deletions depending on the type of MD such as that of dystrophinopathy, sarcoglycan, dysferlin, etc. The above test, apart from elucidating the nuances of genetic defect, also helps in revealing the possible genetic inheritance pattern.

Conventional treatment

The main aim of the treatment for muscular dystrophy is to control its symptoms and maximize the quality of life of the children making them more independent. Although, there is no permanent cure for muscular dystrophy, physiotherapy, assistive devices such as braces, wheelchair etc, orthopedic management and steroids are currently used for the management of the disorder. These therapies work by strengthening the muscles and increasing strength. They are designed to help prevent or reduce deformities in the joints and the spine and to allow people with muscular dystrophy to remain mobile as long as possible. Prednisone (prednisolone) and deflazacort are the two types of steroids that are mainly used in DMD but their prolonged use can weaken bones and increase fracture risk.

Worldwide scientists, patient and parent groups and pharmaceutical companies, together are working on various molecules and drugs, which could:

a) Facilitate reduction in fibrosis, in the muscles of children and patients with muscular dystrophies

b) Target various antioxidants and scavenging molecules

c) The ultimate horizon/destination - gene therapy and exon skipping. Though, this theoretically could be the answer for genetic disorders, all stakeholders mentioned above, agree that it is possible but needs more research and may take a few years before it becomes a standard therapy.

d) Hence, therapies that can slow the progression of the disease are the focus now!! Therapies that can restore muscle stem cell regeneration are a feasible option. Satellite muscle stem cell regeneration in muscular dystrophy patients and especially in DMD boys is suppressed. This means i) that the boys' muscle cells are weakened and die more quickly than they should, and ii) that they are not replaced adequately. Intermediate therapies, which stimulate muscle regeneration, must be able to address these functions of this disease.

Sacco et al, in their research have quoted "DMD progression, although initiated and driven by dystrophin deficiency, is ultimately a stem cell disease".

Summary of current clinical evidence of the role of various types of stem cells in Muscular Dystrophy in children

Stem cell therapy is one of the evolving treatments showing promising results for muscular dystrophy. Animal studies have suggested that myoblast transplanted in the damaged muscles give rise to dystrophin expressing myofibres.

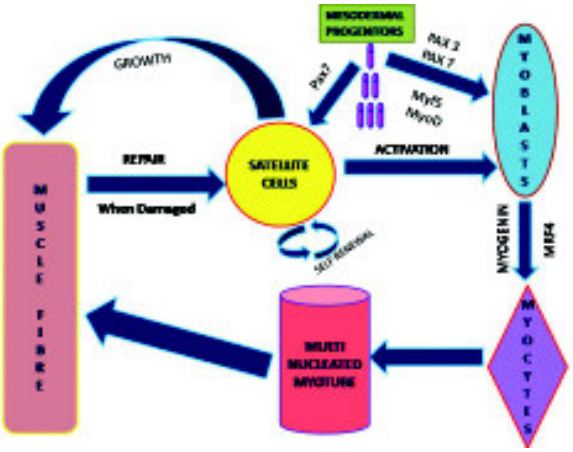
Further research has also been carried out to isolate and study different types of cells which include mesangioblasts, muscle-derived stem cells (MDSC), blood- and muscle-derived CD133+ cells, bone marrow derived stem cells, side population cells, umbilical cord blood cells etc.

Results of few studies are mentioned below.

Huard et al in their study demonstrated that transplanted myoblasts from immunocompatible donors, with simultaneous immunosuppression, showed some improvement in muscle strength and muscle positivity for dystrophin. However, this was found to be decayed over time and antibodies against dystrophin gene were formed.

Gussoni et al showed that donor myoblasts persist after injection; however, their microenvironment influenced whether they fuse and express dystrophin. They also showed the presence of bone marrow-derived donor nuclei in the muscle of a patient documenting the ability of exogenous human bone marrow cells to fuse into skeletal

muscle and persist up to 13 years after transplantation.



Similarly Skuk et al, through their trial on three Duchenne muscular dystrophy (DMD) patients concluded that significant dystrophin expression could be obtained in the skeletal muscles of following specific conditions of cell delivery and immunosuppression after myogenic cell injection.

However, Mendell et al reported that myoblasts transferred once a month for six months failed to improve strength in patients with Duchenne muscular dystrophy

All the above studies were carried out with immunosuppression as Trembley et al in their study presented that myoblast transplantations without immunosuppression trigger a humoral immune response of the host. Antibodies fix the complement and lyse the newly formed myotubes suggesting that myoblast transplantations, as well as gene therapy for DMD, cannot be done without immunosuppression.

Though transplantation of myoblasts can enable transient delivery of dystrophin and improve the strength of injected dystrophic muscle, this approach was seen to have various limitations, including immune rejection, poor cellular survival rates, and the limited spread of the injected cells. It was thought that isolation of muscle cells that could overcome these limitations would enhance the success of myoblast transplantation significantly. The development of muscle stem cells for use in transplantation as treatment for patients with muscle disorders was thought to be an attractive proposition in the early 2000s.

However, all the publications reviewed here point towards some anatomic reconstitution of the dystrophin in the muscles, but fail to impress on the grounds of very mild functional improvement. Hence, other sources of adult stem cells have been explored, such as cord blood cells and bone marrow derived cells.

In the first case of prospective clinical transplantation reported by Zhang et al in 2005, it was demonstrated that allogenic cord blood stem cell transplantation reduces the serum creatine phosphokinase levels which slow down the necrosis of muscle cell. Hence, proving to be advantageous for the DMD patients.

Torrente et al (2007) tested the safety of autologous transplantation of muscle-derived CD133+ cells in eight boys with Duchenne muscular dystrophy. Stem cell safety was tested by measuring muscle strength and evaluating muscle structures with MRI and histological analysis. No local or systemic side effects were observed in all treated DMD patients. Treated patients had an increased ratio of capillary per muscle fibers

with a switch from slow to fast myosin-positive myofibers.

Yang et al in 2009 investigated the feasibility of double transplantation of BMSC and CB-MSC in progressive muscular dystrophy (PMD). It was found to be a convenient, safe and effective treatment. 82.9% cases out of 82 cases showed a positive outcome in a follow up period of 3-12 months. Activity of daily living scale (ADL) in 72 patients (87.8%) increased as compared with pre-treatment ($P < 0.01$). Reduction in blood parameters such as LDH levels creatine kinase was also observed.

Autologous bone marrow derived cell transplantation, intrathecally and intramuscularly, is a safe and effective option for slowing down deterioration and degeneration in progressive muscular dystrophy. Combinatorial action of different cellular components of bone marrow enhances satellite muscle cell stimulation, regeneration and helps reduce fibrosis in the muscle tissue. Sharma et al, demonstrated that the administration of autologous bone marrow-derived mononuclear cells in muscular dystrophy was safe and improves their quality of life. On a mean follow-up of 12 ± 1 months, overall 86.67% cases showed symptomatic and functional improvements, with six patients showing changes with respect to muscle regeneration and a decrease in fatty infiltration on musculoskeletal magnetic resonance imaging and nine showing improved muscle electrical activity on electromyography. Fifty-three percent of the cases showed an increase in trunk muscle strength, 48% showed an increase in upper limb strength, 59% showed an increase in lower limb strength, and approximately 10% showed improved gait.

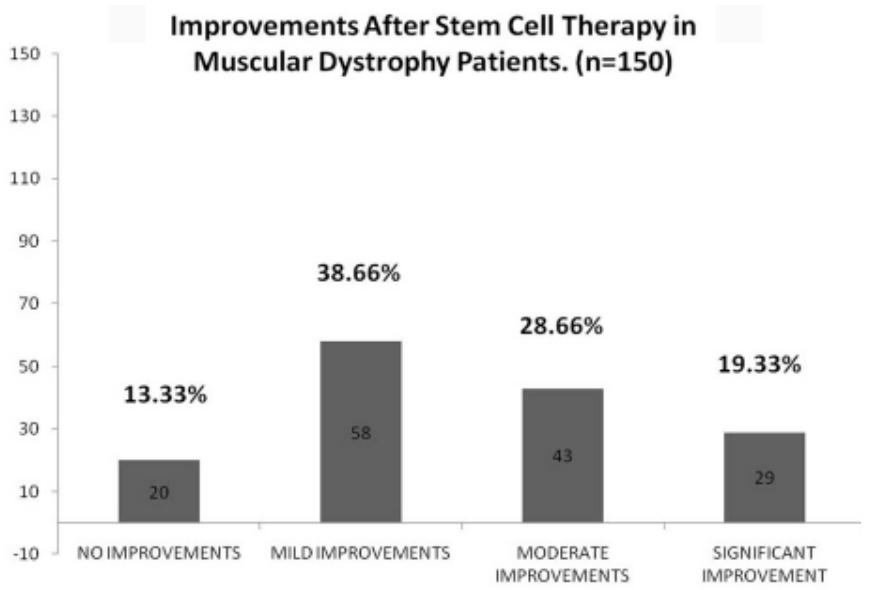


Figure 1: Graph showing improvements in muscular dystrophy patients after stem cell therapy (ref: Sharma et al. Cell Transplantation. 2013 Vol. 22, Supplement 1, pp. S127-S138)

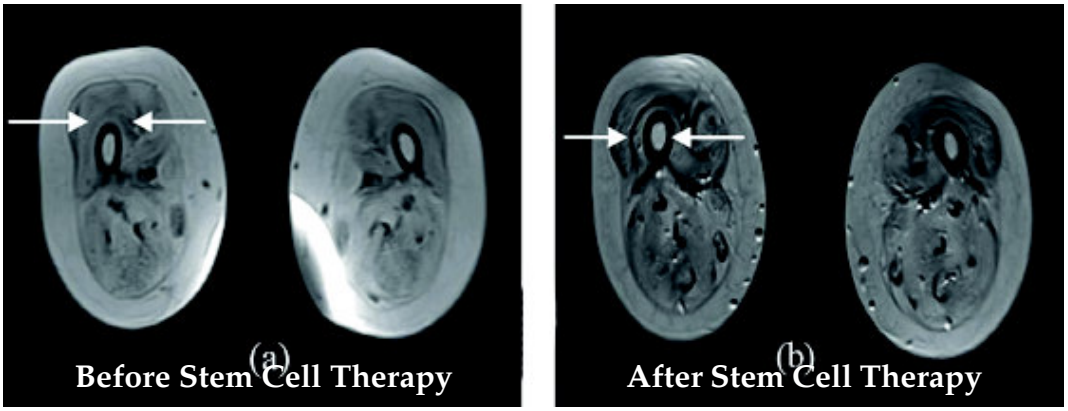


Figure 2: Pre and Post MRI scans. (a) MRI done before stem cell therapy (b) MRI done after stem cell therapy wherein white arrows denote muscle regeneration

At the NeuroGen Brain & Spine Institute, Mumbai out of 332 muscular dystrophy patients who underwent intrathecal autologous bone marrow derived mononuclear cell transplantation. 93.98% showed improvements in symptoms such as hand function, balance, stamina, trunk activation, standing and ambulatory status. 42.77% showed significant improvement, 36.14% showed moderate improvement while 15.06% showed mild improvement.

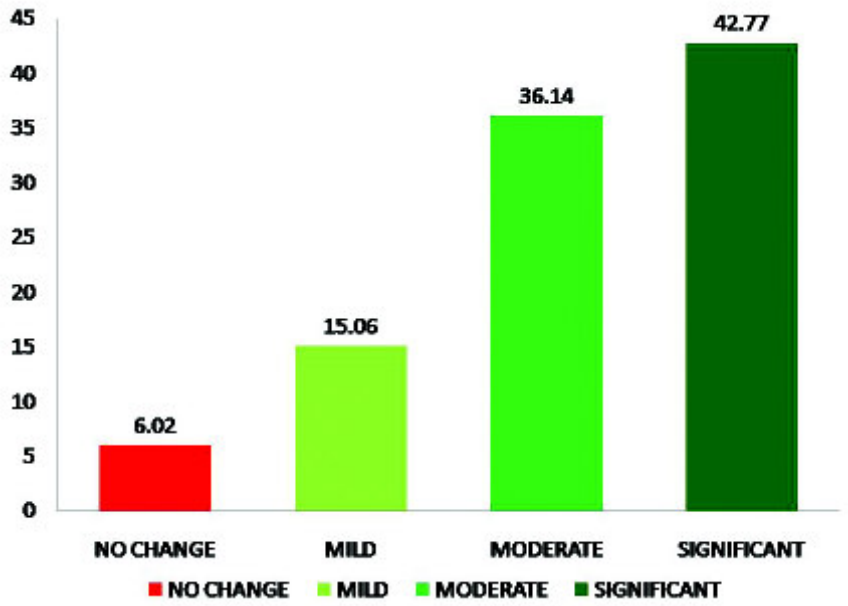


Figure 3: Graph representing improvements in muscular dystrophy after stem cell therapy

Conclusion

Research being done all over the world long with our own large clinical experience shows that stem cell therapy is a viable treatment option for muscular dystrophy and in particular for Duchene muscular dystrophy. Clinical improvements occur in over 90% of the patients and these include improvements in ambulatory status, hand functions, balance, stamina, fatigue, trunk activation and standing. Objective Improvements are also seen on muskuloskeletal MRIs of the limbs as well as on EMGs.

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9. Stem Cell Therapy in Spinal Cord Injury

Spinal cord injury is a devastating event that occurs suddenly and whose consequences range from minimal symptomatic pain to a tragic quadriplegia. If cervical spinal damage is severe, quadriplegia results, whereas an injury to the thoracic or lumbar spine leads to paraplegia.

In children, SCI is relatively a rare condition as compared to its prevalence in the adults. Nevertheless, up to 5% of spinal cord injuries occur in children. Diagnosis and treatment of SCI in children is challenging due to their age and behavioral differences. The type of SCI in children are different from that of adults as the anatomy and the mechanics of the spine varies until the child reaches 8-10 yrs of age. The primary causes of injury are birth related injuries, child abuse, falls or motor vehicle collisions. Young children also seem more vulnerable to post infectious or in?ammatory cervical spine issues.

Treatment

The acute management of the child with spinal injury requires rapid restoration of airway, breathing, and circulation. The child should be immobilized supine in a hard collar and on a fracture board. A full spine survey should be performed to eliminate possibilities of fracture and or dislocation. If there are no fractures or dislocation, a CT is performed to rule out an occult fracture, followed by an MRI. Good quality flexion-extension films are also obtained to rule out overt ligamentous instability. Children with severe spinal cord injuries diagnosed within 8 h of injury are administered a 24-hour course of methylprednisolone.

Early surgical intervention is required with definite spinal cord compression and progressive worsening of neurological deficits secondary to a fracture, epidural hematoma or extruded disc causing compression. Delayed or a planned intervention is advised for correction of spinal instability or kyphosis/scoliosis. Reduction of the fracture segments and fixation using spinal instrumentation is then performed according to the type of lesion.

Despite the surgery, many patients are left with neurological deficits, which may recover to some extent with regular rehabilitation.

Summary of current clinical evidence of the role of stem cells in spinal cord injury

Many strategies focusing on neuroprotection or axonal regeneration have been carried out to improve functional recovery after SCI. They work by modifying the injured environment to be beneficial for repair, by replacing lost cells, stimulating and guiding axonal growth or boosting remyelination.

McDonald et al first put forth the concept of use of stem cells for disorders other than haematopoietic. This has paved the way for a whole new area of regenerative medicine in neurological disorders.

Since 1998, this area has burgeoned and innumerable types of stem cells, with equally numerous routes of administering these have been extensively explored. However, before venturing into how stem cell therapy for spinal cord injury has evolved, it is imperative that we understand what is it that we are venturing out to achieve.

This has been very simplistically explained by McDonald as follows:

> ***1) It is not necessary to cure a nervous system injury, and also 2) a disproportionate return of function can result from a small degree of regeneration.***

It is now understood that substantial loss of spinal cord tissue, particularly gray matter, does not preclude near-normal long-tract function. A continuous cross-talk between the laboratory and clinic is mandatory for reaching readily achievable goals that improve quality of life.

Hence, in many of the reports published in various journals all over the world, apart from outcomes of motor and sensory improvement, emphasis has been given on functional improvement and the improvement in the quality of life.

One of the earliest (Rabinovich et al, 2003) stem cell transplants for neurological disorders (spinal cord injury) reported was using minimally manipulated cells from fetal nervous and haemopoietic tissues (gestational age 16-22 weeks). These were implanted subarachnoidally into 15 patients (18-52 years old) suffering from traumatic spinal cord injury (SCI) at cervical or thoracic spine level. Following cell transplantation, six patients showed improvement in their neurological status from A to C grade of SCI, exhibiting incomplete restoration of both motor and sensory function. The status of other five CT-treated patients was reported to be SCI grade B and was characterized by appearance of contracting activity in some muscles and incomplete restoration of sensitivity. The remaining four patients did not exhibit any clinical improvements. No serious complications of CT were noted. The results suggested a clinical relevance of the CT-based approach to treating severe consequences of SCI.

> ***Due to various ethical and medical concerns over embryonic and fetal stem cells, adult stem cells (bone marrow, olfactory ensheathing, etc.) have been tried extensively.***

Olfactory ensheathing cells' are being persistently pursued, though publications of their results have been sporadic.2005, 2008, 2010 and very fresh results (2014) from Prof. Geoffrey Raisman's research, has kept the interest in these cells alive!

In a Chinese language article, Zhou et al, (2004) briefly reported 70 cases following bone-marrow stem cell (BMSC) transplantation. 37 of these were SCI patients. After cell transplantation, the authors reported improved sexual function, sensation and functional improvement in five cases.

Peripheral macrophages have also shown to synthesize nerve growth factor after peripheral nerve damage and eliminate myelin which inhibits neural regrowth. Knoller et al transplanted SCI patients with incubated autologous macrophages. Out of the 8 patients treated in this study, 3 patients showed improvements of motor and sensory functions without any critical complications.

Pioneering and trendsetting paper by Park et al (2005) is one of the earliest reports of intrathecal autologous bone marrow cell transplantation in conjunction with the administration of granulocyte macrophage-colony stimulating factor (GM-CSF). This therapy was carried out in six complete SCI patients. The follow-up periods were from 6 to 18 months, depending on the patients. Sensory and motor recovery was noticed between 3 weeks to 7 months. Four patients showed neurologic improvements in their American Spinal Injury Association Impairment Scale (AIS) grades (from A to C). One patient improved to AIS grade B from A and the last patient remained in AIS grade A. No immediate worsening of neurologic symptoms was found. Radiological changes on MRI were also noticed. Serious complications increasing mortality and morbidity, however, were not found.

Syková et al in two separate publications in the same year (2006) have reported comparative results of intravenous infusion versus intra-arterial infusion of autologous BMMC in SCI. Both the papers reveal that patients receiving intra-arterial transplants show more improvements as compared to those receiving the intravenous transplants. In a novel method, using combination of BM mesenchymal stem cells (MSC) and patient's autoimmune T cells, Moviglia et al (2006) demonstrated the regeneration phenomenon based on the controlled inflammatory activity at the injured site. Both patients showed motor and sensory recovery with no adverse effects.

A series of publications exploring autologous BMSC transplantation either direct injection into the injury site or intrathecal delivery have, from Russia, Brazil, Mexico, Korea, China, India have come out in the last 10 years. These studies mainly address the safety aspects of autologous stem cells being delivered locally, so that the blood brain barrier can be bypassed, perhaps yielding more efficacious results.

Since, direct injection into the spinal cord is a complex process due to the formation of adhesions around the spinal cord as well as shriveling of the cord post injury, injecting BMSCs into CSF through the lumbar puncture, could be a more safe and efficacious procedure.

In 2008, H. Deda et al used autologous bone marrow derived hematopoietic progenitor stem cells to treat 9 patients with chronic complete SCI. Post transplantation all patients' movements and sensations were improved. All patients showed improved ASIA grade.

Another interesting study, by Geffner et al employed the strategy of administering bone marrow stem cells (BMSCs) via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenous. Comprehensive evaluations demonstrated improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring. Significant changes in bladder function were observed following BMSCs administration.

In 2009, Cristante et al. reported the use of peripheral stem cell delivered intra-arterially in 39 patients of chronic SCI. SSEP evaluation after 30 months of cell transplantation showed improved latency in 66.7% of patient's evaluation.

Pal et al. reported 30 patients with subacute or chronic SCI who received autologous BMSCs intrathecally, and only incomplete SCI patients (16.7%) were seen to have improved functionally without neurological or electrophysiological improvements. While another large-scale clinical trial in India (Kumar et al, 2009), consisting of 297 patients with chronic SCI, treated similarly, reported neurological improvements (32.6%) after a follow up of 3 months.

The proof of concept, of intrathecal stem cells reaching the site of injury came from, Callera et al who carried out a study wherein 10 patients received their own CD34+ cells labeled with nanoparticles via lumbar puncture and 6 patients received magnetic beads without stem cells. On follow up the CSF was assessed for presence of cellular components. MRI done 20 and 35 days after transplantation showed that the magnetically labeled CD34+ cells were visible at the lesion site in 5 patients out of 10. These signals were not visible in the control group.

The advent of 2010 has seen the emergence of newer sources of adult stem cells, mesenchymal stem cells, re-emergence of olfactory ensheathing cells and a combination of stem cells with various drugs.

T. E Ichim et al reported intrathecal administration of allogeneic umbilical cord blood ex-vivo expanded CD34 and umbilical cord matrix Mesenchymal Stem Cells, performed at 5 months, 8 months, and 14 months after spinal cord injury. Cell administration was found to be well tolerated with no adverse effects. Neuropathic pain subsided from intermittent 10/10 to once a week 3/10 VAS. Recovery of muscle, bowel and sexual function was noted, along with a decrease in ASIA score to "D".

O.S Abdelaziz presented a trial of 30 patients having chronic traumatic dorsal spinal cord injury in which 20 patients were administered autologous adult bone marrow mesenchymal stem cell through open surgical intraparenchymal and intralesional injection into the site of cord injury. The treatment was followed by monthly intrathecal injection of stem cells through lumbar or cisternal punctures. Clinical improvement

was observed in 6 out of 20 patients. This study reports that short duration of injury and small cord lesions correlated with good outcome.

Lima et al. carried out a clinical trial in Portugal where in 20 patients who sustained a traumatic SCI underwent OECs transplantation. They found some neurological, functional, electrophysiological and urodynamic improvements in all the patients. In a larger study, Huang et al transplanted 108 SCI patients with OECs. They were divided into group A (n = 79) who were given sufficient rehabilitation and group B (n = 29) with insufficient rehabilitation. On follow up, these patients showed changes in ASIA scale, walking ability, sexual functions. Comparing group A with group B, the increased scores in terms of motor, light touch, and pin prick were remarkably different. 29 out of 31 showed improvement in EMG examinations while 28 showed improvement in PVSEP

Saberi et al enrolled 33 SCI cases to study the safety of intramedullary Schwann cell transplantation. After a 2 year follow up, there were no tumor formations or other adverse events recorded. Similarly in China, Zhou et al injected 6 SCI patients with Schwann cells. On follow-up motor and sensory functions of all the patients improved with improvement in ASIA and FIM. Apart from this, there was improvement in spasticity and bladder bowel function was also observed.

In 2011, Ra et al studied 8 SCI patients who underwent intravenous administration of autologous adipose tissue-derived mesenchymal stem cells and found that hAdMSCs were safe and did not induce tumor development.

In 2012, Park et al carried out a study on 10 SCI patients who underwent intramedullary direct MSCs transplantation into injured spinal cords. 6 of the 10 patients showed motor power improvement of the upper extremities at 6-month follow-up, 3 showed gradual improvements in activities of daily living, and changes on magnetic resonance imaging such as decreases in cavity size and the appearance of fiber-like low signal intensity streaks. They also showed electrophysiological improvement.

Frolov et al injected 20 cases of cervical SCI with autologous hematopoietic stem cells. After 1 year, improved motor and somatosensory evoked potentials were recorded.

Clinical results of Stem Cell Therapy at NeuroGen BSI:

Sharma et al administered 110 thoracolumbar SCI patients with autologous bone marrow derived mononuclear cells, intrathecally. On a mean follow up of 2 years ± 1 month, overall improvement was seen in 91% of patients, including reduction in spasticity, partial sensory recovery, and improvement in trunk control, postural hypotension, bladder management, mobility, activities of daily living, and functional independence. A statistically significant association of these symptomatic improvements with the cell therapy intervention was established. Some patients showed a shift on the ASIA scale and changes in electrophysiological studies or functional magnetic resonance imaging. No major side effects were noted.

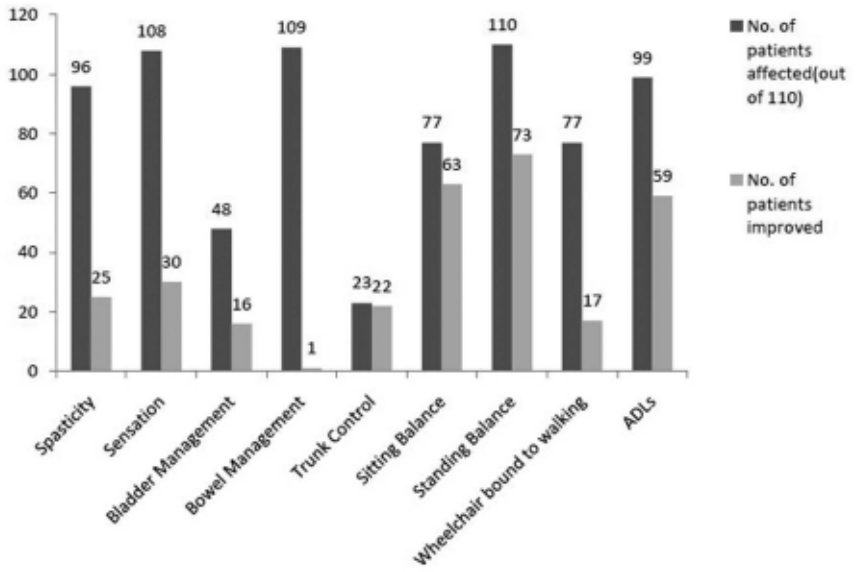

Figure 1: Graph representing improvements in thoracolumbar SCI after stem cell therapy (ref: Sharma et al, Journal of Neurorestoratology. 2013;1:13-22)

In another study, they carried out a detailed analysis of 50 chronic cervical SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells followed by neurorehabilitation. On a mean follow up of 2 years ±1 month, 37 out of 50 (74%) showed improvements. Sensation recovery was observed in 26%

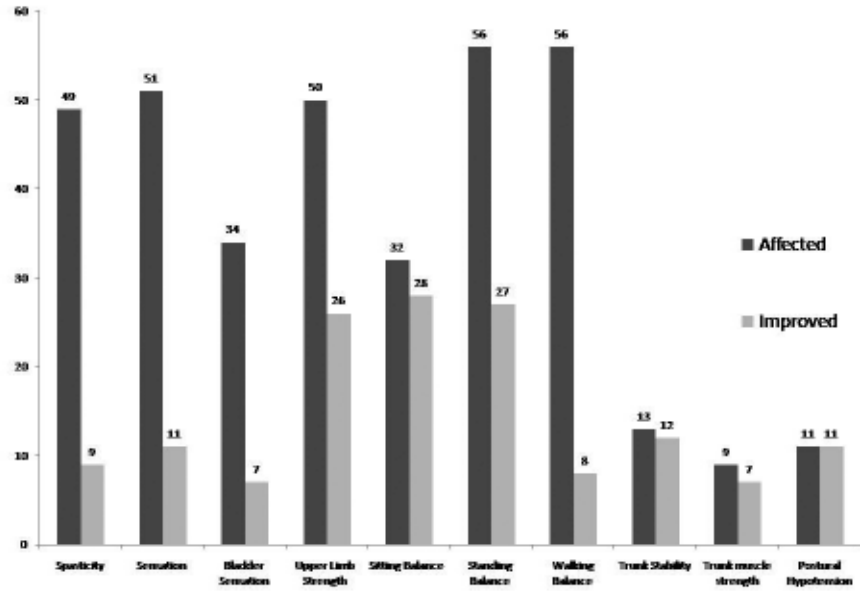

Figure 2: Graph representing improvements in cervical SCI after stem cell therapy. Ref: Sharma et al, J Neurol Disord 2013; 1: 138.

cases, improved trunk control in 22.4%, spasticity reduction in 20% and bladder sensation recovery in 14.2%. All the 50 cases had improvement in postural hypotension. 12.24% wheelchair bound patients started walking with assistance. Functionally, 20.4% patients showed improved ADLs and 48% showed a positive. No major side effects were noted in the duration of 2 years in both the studies. A better outcome was observed in thoracolumbar injury as compared to the cervical injury suggesting that the level of SCI greatly influences the recovery of the patient. Both studies demonstrated statistically significant clinical and functional outcome.

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10. How Rehabilitation Augments the Benefits of Stem Cell Therapy

Neurorehabilitation is the clinical subspecialty that is devoted to the restoration and maximization of functions that have been lost due to impairments caused by injury or disease of nervous system. The goal of neurorehabilitation is to help patients with impairments and disabilities and to make them functionally independent, which requires team of rehabilitation specialists, such as nurses, physical therapists, occupational therapists, speech therapist, psychologist and others.

Figure 1: A pediatric Rehabilitation centre

Importance of Rehabilitation:

The rehabilitation team has a role to set short term goals (generally considered to be two to three weeks) and long term goals (longer than 3 weeks) which should be objective, measureable and time limited. Neurorehabilitation team has an

understanding of neural regulation of movement patterns. A framework for typical motor behaviour is necessary to understand how motor behaviour is altered in persons with neurological dysfunction and how plastic properties of nervous system interact to produce change. Motor control is the study of how an individual controls movements already acquired. Neuroplasticity is defined as brain's ability to adapt or use cellular adaptations to learn or relearn functions which are previously lost as result of cellular death by trauma or disease at any age. Neuronal sprouting is thought to be primary mechanism, allowing injured neurons, to reconnect in new ways and allowing intact undamaged neurons to form new connection and to enhance function. Motor learning will continue throughout life as long as environment asks for change and CNS has pliability and desire to learn. The rehabilitation team promotes this learning and facilitates neural plasticity.

The philosophic foundation of rehabilitation team is to promote purposeful activity thereby preventing dysfunction and eliciting maximum adaptation. These goal-oriented activities are meant to be culturally meaningful and important to the needs of patient and their families. Activities include daily life and work skills, exercise, recreation and crafts. Exercise tasks in animal models, have shown that specifically skilled type of exercises lead to increased angiogenesis in damaged cortical areas whereas unskilled activities did not show this positive change. It is believed that in humans too rehabilitation techniques would enhance neuroplastic changes.

How rehabilitation augments the effects of stem cell therapy

The concept of Neuro Regenerative Rehabilitation Therapy (NRRT) at NeuroGen promotes a multidisciplinary and holistic approach to bring about recovery of neural function with a close integration of Neuro regenerative (including stem cell therapy), Neuro protective (medications) and neurorehabilitative therapies (physical / occupational / speech/ psychological). Thus, it combines the best neurobiological repair technologies and neurorestorative techniques. The rehabilitation protocol is then individualized to the specific requirements of each patient emphasizing on functional recovery and independence in ADL. The rehabilitation team sets up goals and the injected stem cells from within the body help in achieving those goals. Studies have shown that exercise induces mobility in the injected stem cells, thereby enhancing the achievable outcomes. Hence, neurorehabilitation appears to work complimentarily with stem cells therapy.

> Studies have suggested that the combination of bone marrow stem cell therapy and exercise training result in significant functional improvement in neurological disorders.

Neurorehabilitation facilitates neural plasticity and improves neural connectivity. It stimulates neurons to function at their optimum capacity. It also activates the local resident stem cells to help repair the damaged areas. Similarly exercise also stimulates the injected stem cells and guides them towards their targeted functions. It helps the regenerated cells to gain maximum function. Neurorehabilitation has also been

postulated to release growth factors, improve oxygenation and increase blood supply. Thus, the synergistic effect of stem cell therapy and neurorehabilitation brings about maximum benefits.

Physical therapy

As an important member of rehabilitation team a, physical therapist has a crucial role to play which includes, bed mobility, ambulation and transfer activities like ,transfers from bed to chair or from chair to commode or from wheelchair to car and so on. Their assessments emphasize measures of voluntary movement, sensory appreciation, ROM, strength, balance, fatigability, mobility, gait and functional status.

Practices in Physical Therapy includes:

1. Therapeutic exercise and re-education.
2. Neurofacilitation techniques.
    i) Proprioceptive neuromuscular facilitation
    ii) Bobath
    iii) Brunnstrom
    iv) Rood
3. Motor skills learning.
4. Task-oriented practice.
5. Forced use.
6. Massed Practice.
7. Biofeedback.
8. Virtual environment training.
9. Musculoskeletal techniques.
10. Electromyogram-triggered neuromuscular stimulation.
11. Orthosis and assistive devices.

Figure 2: Rolling exercise

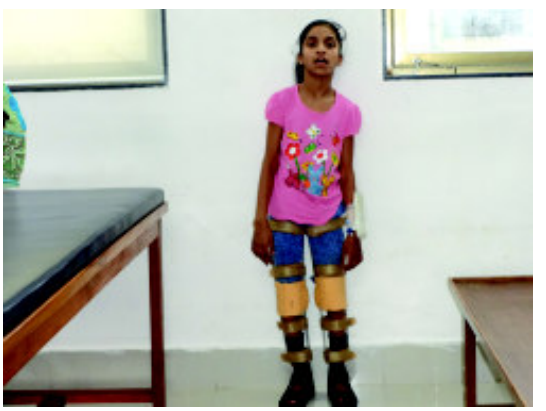

Figure 3: Standing using assistive devices

Occupational Therapy

Occupational Therapists bring expertise to the rehabilitation team in enhancing the independence and personal satisfaction of patients in their activities of daily living (ADL), community and leisure activities, social integration, and work performance. They play integral part in evaluating the need for a range of assistive devices and training patients to make them independent in eating, dressing, bathing combing and other ADL.

In the patient's home and workplace, the therapist provide grab bars, rails, ramps, environmental controls, computer interfaces, architectural changes such as widening a doorway to allow wheelchair access and emergency remote-control calling systems. Along with the physical and recreational therapist, occupational therapist seek out the environmental, personal, and activity-specific equipment and technologies that enhance the quality of life of patients

Success in retraining during rehabilitation depends on diverse variables that include the characteristics of a task, changing contexts and environments when performing a task, psychological reinforcements including positive contextual factors like motivation, attention, memory for carryover of what is taught and negative contextual factors like environmental distractions, anxiety, sleep deprivation and family support play a significant role.

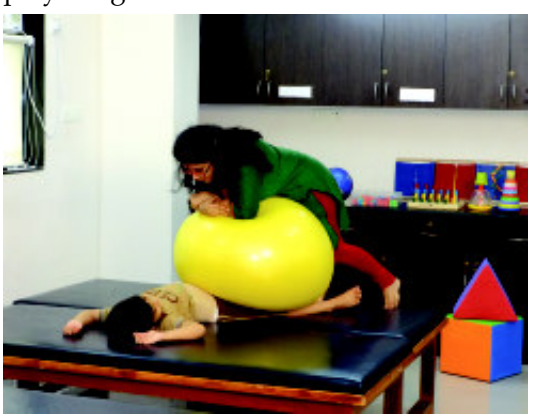

Figure 4: Deep Pressure

Figure 5

Psychology

The word psychology is derived from the Greek words Psyche (which means soul) and logos (which means study). Hence, psychology could be defined as a "study of the soul". However, today it is defined as the scientific study of the behaviour of individuals and their mental processes (American Psychological Association). Neuropsychological testing and evaluation is to identify the pattern of cognitive, behavioural, and emotional strengths and weaknesses and to provide specific treatment recommendations or clarify diagnostic questions. The domains and tests specified

Figure 6: Cognitive rehabilitation

Psychological Counseling:

The purpose of counseling is to broadly empower the client to cope with life situations, to reduce emotional stress, to engage in growth producing activity, to have meaningful interpersonal relationships and to make effective decisions. Counseling increases the control over present circumstances and enhances present and future opportunities.

There are several main broad systems of psychotherapy:

i) **Psychoanalytic:** It encourages the verbalization of all the patient's thoughts, including free associations, fantasies, and dreams, from which the analyst formulates the nature of the unconscious conflicts which are causing the patient's symptoms and character problems.

ii) **Behaviour Therapy:** This focuses on changing maladaptive patterns of behavior to improve emotional responses, cognitions, and interactions with others.

iii) **Cognitive Behavioural Therapy:** Seeks to identify maladaptive cognition, appraisal, beliefs and reactions with the aim of influencing destructive negative emotions and problematic dysfunctional behaviours.

iv) **Psychodynamic:** Primary focus is to reveal the unconscious content of a client's psyche in an effort to alleviate psychic tension.

v) **Existential Therapy:** This is based on the existential belief that human beings are alone in the world. This isolation leads to feelings of meaninglessness, which can be overcome only by creating one's own values and meanings.

vi) **Humanistic:** The task of Humanistic therapy is to create a relational environment where this the self-actualizing tendency might flourish.

vii) **Transpersonal Therapy:** Addresses the client in the context of a spiritual understanding of consciousness.

ix) **Body Psychotherapy:** Addresses problems of the mind as being closely correlated with bodily phenomena, including a person's sexuality, musculature, breathing habits, physiology etc. This therapy may involve massage and other body exercises as well as talking.

Play Therapy, Gestalt Therapy, Rational Emotive Behaviour Therapy, Solution based therapies and Reality Therapy some other forms of psychotherapy.

Speech therapy:

Speech therapy focuses on receptive language, or the ability to understand words spoken and expressive language or the ability to express. It also deals with the mechanics of producing words, such as articulation, fluency and voice. Speech therapy also deals with rehabilitation of language in children who do not speak congenitally due to hearing impairment, mental retardation, autism or attention deficit hyperactivity disorder.

Speech and language therapy is beneficial in neurogenic disorders of non - progressive and progressive origin.

i) **Aphasia:** Aphasia is defined as loss of reception or expression of language as a result of brain stroke. It can be classified as Broca's aphasia (patient presents with intact comprehension with affected expression), Wernicke's (patient presents with affected comprehension with jargon speech), Anomia or nominal aphasia (patient presents with naming difficulties).

   Recovery from aphasia depends on many prognostic factors like age, site and extent of lesion, concomitant problems and time lapsed between the stroke and initiation of therapy. Rehabilitation in aphasia focuses on the following:

   a) Improving auditory comprehension using pointing tasks "point to the spoon".
   b) Encouraging verbal utterances voluntarily.
   c) Improving sentence formation.
   d) Improving naming

   A study done on aphasics concluded that combination of two inout channels - auditory plus visual, auditory plus gestural may facilitate better comprehension and performance by the patient

   Many of the cases of do not improve with traditional speech and language. In such cases, nonverbal modalities can be used to augment or alternate patient's communication. The most commonly used AAC are communication boards, gestures and use of written modality.

   According to Collins (1986), severly aphasic patient may rely more on pictures for basic need that cannot be readily expressed by pointing or natural gesturing (as cited in Davis,2000) (5)

ii) **Dysarthria:** The literal definition of dysarthria is disordered utterance (dys means disordered or abnormal; arthria means to utter distinctly). A more comprehensive definition is that dysarthria is the impaired production of speech because of disturbances in the muscular control of the speech mechanism (as cited in Freed, 2000). Dysarthria can be classified as spastic dysarthria (due to upper motor

neuron lesion), flaccid dysarthria (due to lower motor neuron involvement), ataxic dysarthria (due to cerebellar involvement), hypokinetic and hyperkinetic dysarthria (due to basal ganglionic involvement) and mixed dysarthria.

Common causes of dysarthria are stroke, motor neuron disorder, multiple sclerosis, head injury and Parkinson's disease to name a few.

Most of the patients with dysarthria present with inability to produce sounds clearly, reduced loudness and monotonous or robotic speech. In cases of flaccid and spastic dysarthria, oro - motor structures and functions are restricted.

Treatment of dysarthria depends on the severity of speech problem. Speech and language pathologist aim to improve speech intelligibility (overall clarity of speech) by:

a) PNF (proprioceptive and neuromuscular facilitation).
b) Improving loudness levels.
c) Improving articulatory precision by using exaggerated consonants.

iii) **Apraxia:** According to Darley (1969), apraxia is an articulatory disorder resulting from impairment, as a result of brain damage of the capacity to program the positioning of speech musculature and the sequencing of muscle movement for the volitional production of phonemes. No significant weakness, slowness, or incoordination in reflex and automatic acts is seen (as cited in Freed, 2000). Treatment of apraxia of speech involves phonemic drills, giving proprioceptive and kinesthetic cues to the patients. MIT (melodic intonation therapy) is another technique used (as cited in Freed, 2000).

Darley (1975) stated that the goal of treating apraxia of speech is to help patients relearn the motor sequences needed to produce phonemes accurately.

iv) **Dysphagia:** Dysphagia means disordered swallowing. Swallowing disorders occur in all age groups from newborns to the elderly, and can occur as a result of CVA, presence of tumors and/ or progressive neurologic conditions. Swallowing consists of 4 stages namely oral preparatory, oral, pharyngeal and esophageal stage. Depending upon the stage affected, a swallowing therapist needs to make a judgement on the treatment modality.

A swallowing therapist aims to work on:

a) strengthening the oral and pharyngeal structures for swallowing.
b) modify the bolus in order to facilitate adequate swallowing.
c) recommend postures and maneuvers like chin tuck/ chin down postures according to the nature of disorder. During swallowing therapy, the therapist should ensure airway safety and rule out any silent aspiration. Children with autism, cerebral palsy, hearing impairment or mental retardation present with either absence of speech or deficient speech and language skills as compared to their age. The main aim of the speech therapist

is to bridge the gap between the chronological age and the language age of the child. The speech and language pathologist tries to explore the areas which the child would respond in and facilitate communication within child's impairment. Most widely used techniques for language learning are repetitions, modeling utterances, expanding a topic and role play. However, children with higher grade of severity may have to rely on alternative and augmented communication (AAC) in order to reduce the communicative burden on the caregivers.

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11. What are the Complications of Stem Cell Therapy?

Stem cell therapy is an exciting research area and it offers potential treatment for several developmental, traumatic and degenerative neurological diseases for which there is currently no cure. A lot was expected from this research and very intensive work has gone behind elucidating the pathways of neuronal development and differentiation. But, like any therapeutic modality, cellular therapy is also associated with some minor and major complications. The occurrence of these complications depends upon the type of cells used and the route of administration. Therefore, we describe the complications as cell related adverse events and procedure related adverse events.

Cell related adverse events:

Cell related adverse events depend on the type of cell, potency of cell, source or origin of cell, cultured or uncultured and cell processing. Here we describe the most studied stem cell types.

i) Embryonic Stem Cells
ii) Adult Stem Cells
iii) Umbilical Cord Stem Cells
iv) Induced Pluripotent Stem Cells

Below are the major cell related adverse events reported with different cell types.

It is important to note that not all the complications are associated with all cell types.

There are some adverse events like teratomas which have been reported only with the use of embryonic stem cells.

(1) Tumorogenecity/ Teratomas

Embryonic Stem Cells

Apart from ethical problems related to human embryonic stem cell derivations, nude mice experiments for various disorders, including brain injury, brought out the problem of teratoma formation after embryonic stem cell transplantation. To achieve human

embryonic stem (ES) cell-based transplantation therapies, allogeneic transplantation models of nonhuman primates have been useful. A model based on cynomolgus ES cells genetically marked with the green fluorescent protein has been described by researchers from Jichi Medical Centre, Japan. Primates provide a close mammalian representation to the humans. The cells were transplanted into the allogeneic fetus because the fetus is supposed to be immunologically premature and does not induce immune responses to transplanted cells. In addition, fetal tissue compartments are rapidly expanding, presumably providing space for engraftment.

However, the researchers found that 3 months after transplantation, a fluorescent teratoma, which was obviously derived from transplanted ES cells, was found in the fetus. Hence, it was understood that, though the transplanted cynomolgus ES cells can engraft in allogenic fetuses, the cells may, however, form a tumor if they "leak" into an improper space, such as the thoracic cavity. Another mammalian model, a rhodopsin-knockout mice, was used to determine whether transplantation of embryonic stem (ES) cells into its subretinal space had a tumorigenic effect.

Mouse ES-cell-derived neural precursor cells carrying the sequence for the green fluorescent protein (GFP) gene were grafted subretinally into the eyes of rhodopsin-/ - mice, whereas control animals underwent sham surgery. Eyes were retrieved after 2, 4, and 8 weeks after cell injection or sham surgery for histologic analysis. Grossmorphologic, histologic, and immunohistochemical analysis of eyes at 2 and 4 weeks after engraftment exhibited no morphologic alterations, whereas neoplasia formation was detected in 50% of the eyes evaluated at 8 weeks after engraftment. Since, the neoplasias expressed differentiation characteristics of the different germ layers, they were considered to be teratomas. The resultant tumor formation affected almost all layers of the eye, including the retina, the vitreous and the choroid. Hence, it has been established in many mammalian models that although ES cells may provide treatment for degenerative disease in the future, their unlimited self renewal and high differentiation potential poses the risk of tumor induction after engraftment. Though clinical studies on use of ES cells in humans are not available, however, cell lines studied shows that human ES lines with submicroscopic genetic abnormalities can display altered growth and differentiation properties suggestive of premalignant change. In other words, a normal karyotype is not necessarily a guarantee of a normal genetic makeup within a cell line. One of the "major challenges to the field" is developing techniques that can detect rare, abnormal cells, particularly if the transformations are not due to changes in gene sequence. Thus, a lot of caution and diligent research will be required before using various human ES cell lines for cell transplantation as a therapeutic option for patients with degenerative disease.

In the literature review, so far, we have not come across any reported complication, such as tumorogenecity, for treatment of neurological diseases using autologous adult stem cells,. None of the published human case reports with autologous bone marrow stem cell transplantation have reported any teratomas.

(2) Seizures

Seizure is one of the possible adverse events of autologous BMMNCs intrathecal transplantation. Earlier bone marrow transplantation in children with leukemia has exhibited epilepsy as adverse event post transplantation. A case series of autologous BMMNCs transplantation in stroke also reported one patient who developed seizures post transplantation. Seizures could be hypothesized to arise post transplantation due to increased production of Brain derived Neurotrophic factor (BDNF), Vascular endothelial growth factor (VEGF) and Nerve growth factor (NGF) by BMMNCs. However the exact mechanism remains unknown. Also these disorders present with seizures as a co-morbidity. Sharma et al, in their study, evaluate seizures as an adverse event of cellular therapy and effect of prophylactic antiepileptic regimen to prevent this adverse event. Children with pediatric neurodevelopmental disorders that underwent cellular therapy were included in the analysis. Seizures were considered as an adverse event if the frequency or intensity of the preexisting seizures increased after cellular therapy or if there were new onset seizures observed. Seizures occured as an adverse event in 6% of the patients, all of them except for one showed an abnormal epileptogenic focus in the EEG. For one patient this investigation was unavailable. Some of these patients also developed new onset of seizures. After starting the prophylactic antiepileptic regimen incidence of seizures as an adverse event reduced to 2% and none of the patients exhibited new onset seizures. In conclusion seizures were observed as an adverse event of cellular therapy, which can be controlled and reduced with the use of prophylactic antiepileptic regimen. Abnormal epileptogenic focus on an EEG is a strong predictor of seizures as an adverse event and an EEG screening of these patients before cellular therapy is recommended.

Table 1. Incidence of Seizures as an adverse event of cell therapy and its prevention by anti-epileptic prophylactic regimen

Population	Without antiepileptic prophylactic regimen		With antiepileptic prophylactic regimen	
	Sample size	Percentage of patients that developed seizures as an adverse event	Sample size	Percentage of patients that developed seizures as an adverse event
Autism	50	3 (6%)	50	0 (0%)
Cerebral Palsy	58	3 (5%)	63	2 (3%)

(3) Immunogenicity:

a) Autologous: Autologous adult stem cells, which are not modified or cultured, have not been associated with any cell related adverse events. Also, there is minimal risk of immunological reactions.

b) Allogenic: These may be associated with immunological reactions.

Hence, as of date, autologous adult stem cells appear to be a relatively safe and reasonably efficacious option for therapeutic use in neurological disorders.

Procedure related adverse events: Procedure related adverse events depend on the route of administration of stem cells. Here are some minor adverse events related to intrathecal administration, as our team is most experienced with this route of administration.

(1) Local Infection either at the bone marrow aspiration site or the CSF injection site or a more severe meningitis is always a possibility after stem cell implantation. However, at the NeuroGen Brain and Spine Institute where over 2000 stem cell implants have been done there has not been any case of local or meningeal infection. None of the other papers reviewed have reported any very serious infection leading to any morbidly or mortality.

(2) Spinal Headache: This is a frequent post treatment symptom which occurs in almost one fourth of all patients (low pressure post spinal headache). Once it comes on, this headache is very severe, but is self limiting and resolves in 3 days. The headache is worse on sitting up. The methods to prevent this are making the patients lie in bed (preferably, head low position) for at least a day after the implantation, drinking lots of fluid, the application of a lumbosacral belt (to act as a binder to raise the intracranial pressure) and the use of analgesics. It is our observation that by keeping the lumbar dressing at the lumbar puncture site on for about 5-6 days the incidence of the spinal headache is reduced.

Giddiness, vomiting and neck pain are some other occasionally occurring adverse events. But these are usually always self limiting and respond to medical management and rest.

Similarly, other surgical methods, such as intraspinous, intracerebral, intrarterial and intravenous injections have possibilities of side effects or complications, specific to the respective procedures.

It is beyond the scope of this book to describe the adverse events associated with all other types of stem cells, though umbilical cord stem cells may be associated with immunological reactions and infections. Induced Pluripotent Stem Cells (IPSCs) have not reached clinical applications due to associated complications of genomic instability, viral vector infections and mutagenesis.

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12. Ethics and Regulations Involved for Stem Cell Therapy

Stem cell research holds an incredible promise to treat persons with devastating disorders. Human stem cell therapy (HSCT) is a controversial theme in the religious, political, legal, ethical and scientific worlds. It has been riddled with ethical questions, in part because the predominant methods being used to derive or attempt to derive human embryonic stem cells require destruction of the embryo. Although there are ethical issues surrounding the collection and use of somatic (adult) stem cells from aborted fetuses and umbilical cord blood, the most intense controversy to date has been focused on the source of human embryonic stem (hES) cells. However, the ethical issues not only involve the embryos but also the creation of chimeras, oocyte harvesting. Other important ethical issues have arouse involving the informed consent of both donors of gametes and embryos as well as recipients of stem cells and stem cell products. Commercialization of the process and conduction of research have also raised concern.

Ethical issues exist in this field because the science behind human stem cell research is very new and many rules and guidelines regarding this specific area have yet to be created. The public opinion about embryonic stem cell research is divided between those that support the research and those that are highly influenced by religious groups that are against the research. Kiessling and Scott had once quoted "Scientific ignorance is the driving engine of the anti embryonic stem cell movement"

Embryonic Stem Cells

Different religions have different ideas about beginning of life and about the moral status of a blastocyst, of an embryo and of a fetus. Hindus and Buddhists believe that embryonic stem cell therapy is seen as a positive action by them as the embryonic cells used come either from leftovers of in vitro fertilized embryos or from natural abortions, and it is used in behalf of helping diseased and injured people. Even Islamic laws allow fertilization in vitro for infertile couples and those couples can either throw the unused fertilized embryos away or donate them to research, and Islamic scholars are for the donation of unused fertilized embryos to research. While, Roman Catholics are against the use of embryonic stem cells for research. They believe that full moral status is acquired at conception. According to Christian beliefs, a blastocyst

is already a living human being that should not be destroyed on behalf of therapeutic meanings, even when most of the blastocysts come from leftovers of in vitro fertilizations, which otherwise are destroyed, and even when this therapy could represent the cure of many life threatening diseases.

In view of addressing these ethical issues associated with human embryonic stem cell research, professional groups have issued guidelines for the ethical conduct of this research and its management. There are different kinds of ethical committees throughout the world. The private companies have set up Bioethics Committees and most universities have an Institutional Review Board or a Research Ethics Committee and they are a very important part of any research projects and most scientists welcome their recommendations. The committees use the Helsinki Declaration and the Nuremberg Code as guidelines when they decide whether a problem is ethically right to treat with stem cell therapy. The Helsinki Declaration which is developed by The World Medical Association (WMA) "is a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data."

Systematic data regarding these efforts should be collected in order to enhance the likelihood that they meet their ethical goals.

iPS cells (induced pluripotent stem cells)

The countless discussions over the ethical issues of embryonic stem cell research can be avoided with the introduction of iPS cells. These cells do not involve embryos or oocytes. As compared to oocyte donation, there are very few concerns involving risks to the donors as a skin biopsy which is done to obtain somatic cells is relatively non-invasive. No ethical issues are raised either for the donation of materials to derive iPS cells or their derivation. The President's Council (USA) on Bioethics called iPS cells "ethically unproblematic and acceptable for use in humans".

Umbilical Cord stem cells

The ethical issues of cord blood banking remain the same as for any tissue bank for allogenic research or transplantation. This issue has been tackled in the European group on Ethics in Science and New technologies (EGE) Opinion no. 11 on the ethical aspects of tissue banking (21 July 2001). The ethical values highlighted in this opinion are as follows: body integrity, respect of privacy and confidentiality of data, promotion of solidarity, fairness of access to healthcare and information and consent of the donors. The process of umbilical cord blood banking should comprise of a detailed consent which explains the couple or the woman clearly, the prospective new treatments. It should be stressed that these treatments are still at the experimental stage. Tissue bank activities should be principally reserved to non-profit making organizations or public health institutions. All private and public tissue banks should be monitored for quality standards and measures. These guidelines are based on the principle of respect for human dignity and integrity which asserts the principle of

non commercialization of the human body; principle of autonomy or the right to self-determination on the basis of full and correct information; principles of justice and solidarity, as regards to fair access to healthcare services; principle of beneficence, or the obligation to do good, especially in the area of health care; principle of non-maleficence, or the obligation not to harm, including the obligation to protect vulnerable groups and individuals, to respect privacy and confidentiality; and principle of proportionality which implies a balance between means and objectives. There are also a few value conflicts regarding the banking of umbilical cord blood. The values of freedom and free enterprise may conflict with the principles of solidarity and justice, according to which access to healthcare should be on an equitable basis and based on realistic needs, as well as with the principle of protection of vulnerable groups.

Informed Consent

Informed consent is a critical part of any research project. It is the process in which a participant/patient provides consent to participate in a research project after being fully informed of its procedures, benefits and risks. After completely understanding the information about the project, the participant/patient gives full and conscious consent for the physician/scientist to continue with the procedure. The consent is obtained after providing all the information to the patient in comprehensible non-medical terms (preferably in the local language) about the diagnosis; nature of treatment; risks involved, prospectus of success, prognosis if the procedure is not performed and alternative treatment. The three major aspects of the informed consent are information, voluntariness and capacity. In accordance with the observations of the Supreme Court, the National Commission of India stated that all information would imply adequate information to enable the patient to make a balanced and conscious judgement about whether or not to be a part of the trial or treatment.

Current ethical basis for using adult stem cells

The ethical basis of offering stem cell therapy as a treatment option is based on the Paragraph no. 32, World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subject. This declaration states that "In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physicians judgment if offers hope of saving life, reestablishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published."

Some other aspects of the stem cell therapy ethical debate that need further discussion are as follows:

(1) That there is a need to make a precise distinction between embryonic stem cells and adult stem cells. While stringent regulations for embryonic stem cell research are completely justified the same are not needed for adult stem cell research.

(2) That there is a need to look at the whole issue from the patient's perspective respecting the fact that even small functional improvements can mean a lot to a particular patient.

(3) That there is an ethical ground for offering stem cell therapy as a treatment option based on the Helsinki declaration.

(4) That there is enough published clinical evidence about the efficacy and safety of adult stem cells in neurological disorders and based on this evidence there is no need to continue carrying out trials.

The unfortunate part about the raging ethical debate is that although the main objections are concerning the use of embryonic stem cells, these have resulted in the lack of acceptance and misunderstanding towards other non-embryonic stem cells. It is essential that the medical community, patients and activists realized that stem cells are not one common entity but originate from different sources and that the objections to the use of one source need not come in the way of the use of others. Hence, it is time that we re-evaluated the term "evidence based medicine" and turned to "practice based medicine".

There are two sides to the ethical debate for basing our treatment options on evidence based medicine. (1) One side of the debate is "Is it ethical for doctors to offer treatment options that have not become a standard of care as yet?" (2) The other side of the debate is "Is it ethical to deny patients suffering from disabling diseases, treatments options that are safe and available, whilst we wait many years for the results of multicentric international trial to prove that these treatments work?" Both these questions are answered differently by different people depending on what is at stake for them. The role of regulatory bodies in this field also needs to be re-evaluated. Whereas there is no denying the importance of regulation in all aspects of medical care and research, it is also important for the regulatory bodies all over the world to ensure that regulations do not hinder the evolution of newer treatment options. They also need to realize that in this field that is evolving at a breathtaking speed, regulations made several years ago may no longer be valid in the present and that the regulations need to be modified as more evidence pours in from all across the globe. However, getting a consensus on these issues is not easy.

Regulations for stem cell therapy in India

In India for all these years the regulatory authority for stem cell therapy and research has been the Indian Council of Medical Research (ICMR). They have formulated draft guidelines in the years 2003, 2007 and 2013. These are available at www.icmr.nic.in. Although, these cover many aspects of stem cell therapy and research, briefly speaking they had put the use of embryonic stem cells in the restrictive category and the use of

umbilical cord cells as well as adult stem cells in the permissive category. There is a requirement for the formation of an Institutional Committee for Stem cell research and therapy (ICSCRT). This has now been recently modified to an ICSCR. In any case these are guidelines and they as of now have not yet passed by the Parliament as a legally binding law or bill. In the recent past, the Drug Controller General of India (DCGI) has also taken up responsibility of this field and had put up the proposed new guidelines for public opinion on their website in February 2014. As is obvious from all the above is that there are a lot of grey areas. However, in the next few months/ years we should hopefully see something more definite and progressive happening on the regulatory front that will be in the interest of patient care.

Conclusion

In summary what this means in our view is that ethically for conditions for which there is no other treatment option available or all available treatment options have exhausted we can offer stem cell therapy as a treatment option on compassionate grounds. However it is important that all clinical results are documented and published. From a regulatory viewpoint if minimally manipulated autologous adult stem cells, such mononuclear cells, are being used (like we do at NeuroGen BSI) then approval is needed from the Institutional stem cell committee / Ethics Committee. Special informed consent is important before doing this treatment.

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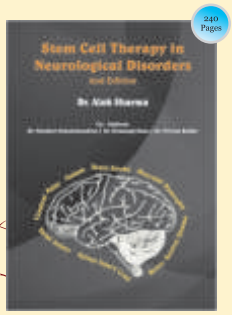

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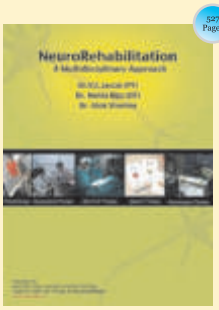

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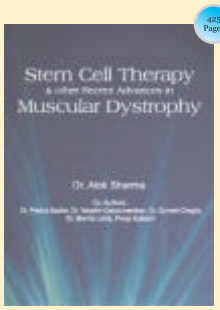

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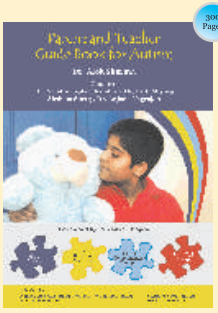

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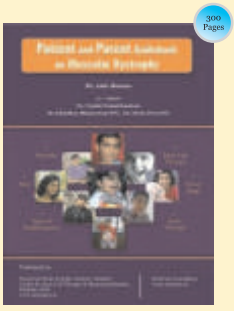

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