

Stem Cell Therapy in Neurological Disorders

**4th
Edition**



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A NeuroGen Publication

Stem Cell Therapy In Neurological Disorders

Fourth Edition

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This book is basically a compilation of information / literature on the available on the topic, from various sources (which have been acknowledged duly). However, this is by no means an exhaustive resource, since the field is evolving at a very rapid pace. Every effort is made to ensure accuracy of material, but the publisher, printer and author will not be held responsible for any inadvertent error(s).

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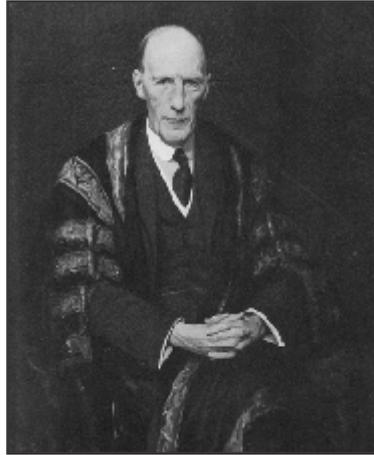
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This Book is Dedicated to all our Patients



A Prayer

From inability to let well alone; from too much zeal for the new and contempt for what is old; from putting knowledge before wisdom, science before art, and cleverness before common sense, from treating patients as cases, and from making the cure of the disease more grievous than the endurance of the same, Good Lord, deliver us.

- Sir Robert Hutchison

(British Medical Journal, 1953; 1: 671.)

“This is the true joy in life, the being used for a purpose recognized by yourself as a mighty one. The being a force of nature rather than a selfish feverish little clod of ailments and grievances complaining that the world will not devote itself to making you happy. I am of the opinion that my life belongs to the whole community and as long as I live its my privilege to do for it whatever I can. I want to be thoroughly used up when I die for the harder I work the more I live. I rejoice in life for its own sake. Life is no brief candle to me but a splendid torch that I have got hold of for the moment and I want to make it burn as brightly as possible before handing it over to future generations.”

- George Bernard Shaw

PREFACE

"Stem cell Therapy - An idea whose time has come"

There are times in human history when quantum leaps occur in our thinking and approach to the various issues that confront us as a race. The discovery of electricity, the combustion engine, the telephone, the microchip and the internet being amongst a few of these. In the world of medicine, such landmarks have been the discovery of microbes as the source of infections, the discovery of x-rays, vaccines and antibiotics etc. The last decade has seen the evolution of another such landmark. This is the field of regenerative medicine where healthy tissues could be used to replace damaged tissues, to help get relief from various so called incurable conditions.

Whilst this has opened up an entire new world of newer treatments for conditions for which there was earlier no hope, it has also unfortunately resulted in a storm of ethical debates that have more to do with religion, politics and personal beliefs than with science. So whereas on one hand there are millions of suffering patients who could possibly benefit from these treatments, there are also hundreds of people and organizations who are opposed to these on various grounds, from their not being enough evidence for use of them as a treatment form, to those that believe that use of cellular therapy is unacceptable on religious, political and ethical grounds. The unfortunate part of this ethical debate is that whilst the main objections and problems are regarding the use of embryonic stem cells, these have resulted in the lack of acceptance and misunderstanding of other non embryonic stem cells such as adult stem cells that have similar properties but are not of embryonic origin. Its time that the medical community, activists and patients recognized that stem cells are not one common entity but that stem cells come from different sources and the objections to the use of one source need not come in the way of the use of others.

Another important facet of the debate on the use of stem cells is based on the principles and practice of "evidence based medicine". Whereas there is no denying the fact that evidence based medicine is the bedrock on which more recent practices are based, it is also a fact that the principles of evidence based medicine, as we now practice are a creation and evolution of the past few decades. The notion of evidence based medicine did not exist from the 1800's to the 1970's, a period in which almost all of the modern aspects of medicine we now practice were discovered. In fact, it would not be an exaggeration to say that none of the discoveries and innovations of medicine in the 20th century would have happened if the present day yardsticks of evidence based medicine had been in place then. A realization that the systems we created to protect ourselves from the exploitation of commercial agencies is now hampering the very growth and development of medicine has led to us now turning to the concept of "practice based evidence". Clinical trials are expensive. Geron spent US\$ 56 million before it could embark on its historic embryonic stem cell study this year. Outside of the pharmaceutical and biotechnology companies these sort of resources are almost unavailable. It is time, therefore, that we relooked at "evidence based medicine" and turned to "practice based evidence" so that the individual practitioner of medicine could be a part of the newer developments and evaluation of the systems of medicine. Ninety percent of current

neurosurgical practice is not supported by prospective randomized double blind clinical trials. The same is true for many other surgical branches too. Progress in medicine has come when individual physicians pioneered newer form of therapy that they believed in. Day to day decisions made in clinical practice specially in intensive care setups and operating rooms are made empirically based on the treating physicians experiences and approach and the clinical circumstances at hand. Life is not a randomized trial and all decisions in medicine cannot be based on randomized clinical trials. Evidence generated from the individual physicians practice needs to be respected too. Thus "practice based evidence" needs to be looked at in a way similar to "evidence based medicine."

Nowhere is this more applicable than in the field of stem cell therapy. Despite the above, caution needs to be exercised in the practise of this therapy since neither the enthusiasm of the medical practitioner, nor the pressure from the patient community and emotional aspects of suffering are enough reasons to overlook the safety aspects of any new medical therapy. However, once safety is established it would further the cause of medicine as a whole, as well as the well being of the patient community, if more practitioners participated in these treatments. This would not only make more data available regarding safety and efficacy, but also by balancing out the supply demand imbalance, make such treatments more available and affordable.

There is a very thin line that separates "helping someone" and "taking advantage of someone's helplessness". It is important that we never cross this line. There are two sides to the ethical debate on basing our treatment options on evidence based medicine. [1] One side of the debate is " Is it ethical for doctors to offer to patients 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.

Another question that remains unanswered is when does a treatment that is "unproven or experimental" become a treatment that is "proven or established". How many publications documenting safety and efficacy will it take to make that shift ? Is a single publication enough, or are 10, 50 or 100 ok, or are multicentric international trials the only basis to make any treatment option an excepted form of treatment. Is it necessary to go on reinventing the wheel just to satisfy our intellectual considerations whilst millions of patients continue to suffer? Our own belief is, that based on the already published work and our own clinical experience, this form of treatment is no more experimental since the safety and efficacy of stem cell treatment in many of the neurological disorders has been established and documented in several published articles from several countries. However getting a consensus on these issues is not easy. The role of regulatory bodies in this field also needs to be relooked. 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 or slow down the evolution of newer forms of treatment. 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. That the regulations need to be modified as more evidence pours in from all over the world. That the regulations need to adapt and evolve as the research and clinical results are evolving. That individual doctors, medical institutions and medical associations need to be trusted and given the responsibility to both develop and implement these newer forms of therapy as well as monitor and prevent its misuse.

Stem cell therapy is a new paradigm in medicine since never before in the history of modern medicine have we had the capability to repair and replace damaged tissue. This is an opportunity of epic proportions. As we have a greater aging population worldwide which is likely to be affected by many of the degenerative processes that stem cells can help with, the possible benefits to humanity as a whole are unprecedented. This is too important a work to let social activists, politicians, bureaucrats and regulatory bodies hinder or hijack its progress. This is science and medicine at its very best (and maybe even its very worst) and decisions regarding its potential uses and benefits and precautions to prevent its misuse must remain in the hands of scientists and medical doctors. We need to take responsibility for what we are doing and for what is possible always keeping patient safety and benefits in mind. We need to take a stand on what we believe is the right thing to do. We must respect different points of view and at times agree to disagree. But we must keep moving ahead. 400 years ago when Galileo first observed that the planets including the earth moved around the sun, he was forced to recant or withdraw his observations under pressure from the church. Will we let history repeat itself in the 21st century? Will we let religious and political beliefs and various regulators stop or slow down a science that can possibly help millions of suffering people. The choice is ours.

This book attempts to put together information to help answer some of these difficult issues and questions. Whereas there exists a wealth of published information on the basic science work and animal experimental work to show the efficacy of stem cells in neurological disorders, in this book we focus on trials and clinical treatments done in human patients. The book has been created for those medical practitioners, who are keen to start using stem cell therapy for their patients with incurable neurological disorders, to understand some of the fundamental principles as well as practical aspects that are involved in this line of therapy as well as get informed about all the current clinical data from all over the world that is already published. Our own clinical experiences and techniques have also been incorporated. We believe that this therapy should be available conveniently in all the cities and towns at an affordable cost. This will not only make a big difference to the lives of millions of patients suffering from incurable neurological disorders, but will also further the cause of medicine and science. This book we hope is one small step in that direction. Yes we believe that "Stem cell therapy is an idea whose time has come."

Dr. Alok Sharma

Preface to the Second Edition

“Two sides of the Coin”

Its 3 years since we wrote the preface to the first edition of this book. Whilst on one hand there has been a huge increase in the number of scientific papers published since then and many patients have safely received stem cell therapy, on the other hand not much change has happened on the regulatory front in most countries. Exceptions to these have been Japan and some of the South American countries. We need to ask of ourselves that had the regulations been more accommodating of stem cell therapy as an accepted form of treatment then over these last few years :- How many lives could have been saved? How much patient suffering and disability would have been reduced? How much pressure would have eased on the hospitals, support services and families?

In no other field of medicine have regulations so much slowed down the development of the field as in Stem Cell Therapy. The genesis of this goes back to the ban President George Bush placed on the federal funding of embryonic stem cells lines developed after 2001. (This ban has subsequently been lifted by President Obama). Whereas regulatory bodies are just doing their job in having stringent standards to ensure patient safety, we believe there are two sides to this issue. The other side is that many patients are being deprived of treatments that could potentially save their lives or help reduce their suffering. In strictly adhering to the letter of the regulations are we compromising on the spirit of the regulations? Are the regulations now doing more harm than good by limiting the availability of treatments to patients ? It would not be an exaggeration to state that there are thousands of patients who are dying today or suffering from serious disability whose lives could be save or whose suffering could be reduced from available treatments had the regulations been more accommodating worldwide. Is sticking to strict regulation worth these lives lost or suffering incurred? These are difficult and uncomfortable questions to answer but its time regulatory bodies came to terms with these and then took a more humane approach.

To look at the other side we believe that regulatory bodies need to make the following distinctions in creating future guidelines. To explain this we quote from the International Society for Cellular Therapy (ICST) "White paper" published in 2010 in Cytotherapy [1] Distinction between Experimental therapies and medical innovation:- The White paper states:- "It is important to recognize the difference between clinical trials of experimental treatments and medical invocation. Medical innovation in cellular therapy may be viewed as ethical and legitimate use of non-approved cell therapy by qualified healthcare professionals in their practice of medicine . Patients not eligible for controlled clinical trials should be able to choose unproven but scientifically validated cell therapy medical innovations, if the researchers are competent and those seeking treatment are truthfully and ethically informed. There is a place for both paradigms in the cell therapy global community. “We wish to emphasize this last sentence that - there is place for both paradigms in the cell therapy global community.

[2] Distinctions Between Different types of centers doing this work:- The ICST White paper states centers doing this work should be defined and differentiated as follows:- "[a] approved/standard therapies (e.g hematopoietic stem cell transplant and other cellular therapies approved for marketing)[b] Controlled clinical trials [c] Valid compassionate use of unapproved therapies [d] Treatments not subject to independent scientific and ethical review" We wish to emphasize that is a need to have centers practicing - valid compassionate use of unapproved therapies. Therefore regulations should be different for each of these categories. According to us those falling in category [c] would be those who work in accordance with the Helsinki declaration of the World Medical Association which states "In the treatment of an individual patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

"Another Distinction that also needs to be made is between the 3 broadly different types of stem cells (embryonic, umbilical cord derived , adult) and between autologous and allogenic:- If one were to give an example from daily life then Embryonic stem cells could be compared to Alcohol, Umbilical cord stem cells to Cold drinks like Pepsi, Coke and Adult autologous stem cells to Homemade Fruit juice. Whereas alcohol is potentially dangerous and there should definitely be tight regulations so also embryonic stem cell work should be tightly regulated. Cold drinks may not be dangerous but can be harmful so there should be quality checks in place, so also for umbilical cord cells there should be quality checks in place and these types of cells should be treated like drugs / medicines and the same regulations and quality control systems should be in place for them. However there is no need for any strict regulations for home made orange juice and so autologous adult cells should be freed up from regulations and their availability in fact encouraged since they are completely safe and have shown clinical benefits in many conditions in various published scientific papers.

We also believe that the centers / practitioners working with the following principles should be looked upon in a more permissive manner :- [a] Those who strictly treat patients in accordance with the Helsinki Declaration. That means they do not treat patients where other more established treatment forms are available and the patients have not already taken them. [b] The medical practitioners practicing this are working within the general broad specialty of their qualifications and are dealing with diseases anatomically and physiologically that concern their broad specialty and that they have received specialized training in cell therapy or

done some basic research work in their fields.[c] Whilst doing this treatment they are also making this an object of their research and evaluating its safety and efficacy.[d] They are publishing the results and outcomes of their clinical work, including their negative results and complications if any.[e] They are taking special informed consent [f] There is a honesty and transparency to their work as shown by the fact that their clinical results are in the public domain and they present their results in national and international scientific conferences.[g] They have Institutional Committees that monitor the ethical, scientific and medical aspects of the work.[h] That quality standards are maintained that is they have GMP facilities, follow GCP standards &/or have other accreditations such as NABH/JCI/ISO etc.

With the above principles in place we shall be able to simultaneously ensure that patients with serious illnesses get the benefit of available stem cell treatments and an adequate check is kept on medical practices in this field to ensure the safety of patients. In the last Edition of this book we ended the preface with the statement "Stem cell therapy is an idea whose time has come". Looking at the large number of scientific publications in this field and looking at the number of patients opting for these treatment it looks like for the patients and some parts of the medical community this is true. However the regulatory authorities need to catch up with this. Regulations should not be decided by a handful of people sitting in offices based on their likes, dislikes , preferences and beliefs. They need to meet up and talk with patients both those who are suffering from the serious ailments as well as those who have taken stem cell therapy and benefitted from it. They also need to evaluate read all the available scientific literature available in this field. They need to see which direction the wind is blowing. They need to stop being rigid and be more flexible and open to accepting newer concepts. Whilst always ensuring that only safe and effective treatments are offered to patients there needs to be a human and caring side to regulations too. This will not only make a difference to the lives of millions of patients but result in the progress and advancement of the medical sciences too.

Dr. Alok Sharma

Preface to the Third Edition

The very fact that we have had to bring out a 3rd edition of this book within 6 years of writing the first edition is evidence of the fast moving pace of research and clinical applications of stem cell therapy. The last two years have seen a quantum increase in the number of publications highlighting the clinical efficacy of stem cell therapy in various disorders. The public opinion too is changing in a major way towards making stem cell therapy more available to the patient population. This has resulted in governments all over the world making serious efforts to draft new regulations for stem cell therapy. The lead in this was taken by Japan which has formulated an excellent set of regulations which simultaneously make the more low risk types of stem cell therapy more easily available and have stricter regulations for the high risk types of therapies. Korea is another country which has come up with a progressive set of regulations. In India the scenario has shifted with the Drug Controller General of India (DGCI) taking over the regulations from the Indian council of medical research (ICMR). A key change in the regulatory environment in the country has been the fact that unlike in the past, the present regulators (DGCI) are far more open to considering the views of stem cell therapists as well as patients. This progressive approach is likely to result in India coming out with a set of regulations which might be better than that of Japan and Korea. Thus the overall change in the public perception, medical opinion as well as regulatory bodies as well as the large evidence that is now available in published literature has resulted in a new found acceptance of this new form of therapy. When we wrote the first edition of this book we had no publications, when we wrote the 2nd edition of this book we had 28 publications and when we are now publishing our 3rd edition two year after the second we have 41 publications. This itself tells the whole story of rapid pace at which stem cell therapy is evolving. We believe that in another 5 years stem cell therapy will become a standard of care for many incurable neurological conditions.

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Preface to the Fourth Edition

When we first wrote this book in 2010, little did we realize that within 8 years we would be printing the 4th Edition. Whereas, lots has happened in the field since then. However, the availability of stem cell therapy is still limited. This has to do with the lack of availability of progressive regulations. When we published the 1st edition we had done 400 patients, by 4th edition we have treated 6500 patients. Yet, the service providers in this field remain very few. But, the last year has seen a major shift in the CDSCO and DCG(I) playing an aggressive role. Earlier, only ICMR was involved in making stem cell guidelines. But, since ICMR is all about research, therapy never got the importance it deserved in the guidelines. However, now that DCG(I) is taking over, they have done something remarkable. In the new Drugs and Cosmetics Rule, 2018, stem cells have been divided into processed and non-processed stem cells. Processed stem cells will be considered as a new drug and will have to undergo the regulatory pathway. While, non processed stem cells will be free from the same. This important distinction gives freedom to the doctors working in hospitals to offer minimally manipulated, non -processed cells as a form of therapy without needing regulatory approval from CDSCO.

Our own clinical experience shows us if children with Autism spectrum disorder (ASD) are treated early, they can get complete relief from the symptoms and declared free from ASD. In Cerebral palsy, dramatic improvement is seen in body's tightness and functionality. In Intellectual Disability, significant improvement is seen in cognitive functions. In Duchenne muscular dystrophy, children who would have otherwise died in early 20s are surviving beyond that. Patients paralysed due to spinal cord injury, brain stroke and head injury improve in their muscle strength and movements. We have published two landmark papers which are first of its kind.

1. Autologous bone marrow mononuclear cell therapy for autism – an open label proof of concept study published in Stem cell international in 2013
2. An open label proof of concept study of intrathecal Autologous Bone Marrow Mononuclear Cells transplantation in Intellectual Disability published in Stem cell research and therapy in 2017

Apart from these, we have published over 80 publications in peer reviewed journals, 4 chapters in international books and 14 books.

We believe that stem cell therapy for incurable neurological conditions is no more experimental and should be made available to those who need it the most.

Dr. Alok Sharma

Primum non nocere
(First do no harm)

The ethical basis of offering stem cell therapy as a treatment option is based on the Paragraph no. 37 of World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subject.

**WORLD MEDICAL ASSOCIATION
DECLARATION OF HELSINKI -
ETHICAL PRINCIPLES FOR
MEDICAL RESEARCH INVOLVING HUMAN
SUBJECTS**

"In the treatment of an individual patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available."

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Scientific Publication on Stem Cell Therapy in Neurological Disorders by the Authors

A) AUTISM:

1. Sharma A, et al., The baseline pattern and age related developmental metabolic changes in the brain of children with autism as measured on positron emission tomography/computed tomography scan. *World J Nucl Med* 2018;17
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SECTION A

Basics and Technical Aspects



*"I would go anywhere in the world
for a therapy that is safe and that
could accomplish the goal
of recovery"*

- Christopher Reeve

1

Introduction : Neuroregenerative and Neurorestorative Medicine

“The regenerative medicine revolution is upon us like iron and steel to the industrial revolution, like the microchip to the tech revolution. Stem cells will be the driving force of this next revolution” - Cade Hildreth

Regenerative medicine focuses on restoration, repair and replacement of damaged tissues by a safe and effective transplantation of living cells in solitude or in combination with specially designed materials. It has opened up new avenues of therapeutic strategies for multiple disorders with no definitive treatment or cure available, such as neurological disorders, diabetes, cardiovascular disorders, bone disorders, hematopoietic disorders, cancers, hepatic, renal and dermatological disorders. With its potential to heal and revolutionize health care, regenerative medicine has been called the “next evolution of medical treatments”, by the US Department of Health and Human Services.

The most powerful impact of regenerative medicine has been on treatment of neurological disorders. Since ages it was believed that brain and spinal cord cannot regenerate, but now the exploding field of stem cell research has defied this belief. The basic footprints of regenerative medicine can be traced back to the discovery of stem cells. Stem cells can be viewed as transformers. They have the ability to create any type of cell in the body. They are the ones who repair the daily wear and tear in the body tissues. They have unique potential to multiply manifolds and differentiate into any specialized tissue cells. The first origin of embryonic stem cells was criticized due to ethical issues and side effects of teratomas. However, this

triggered scientists and researchers to find numerous other types of stem cells e.g.: adult stem cells, umbilical cord stem cells and induced pluripotent stem cells (iPSCs) which evade moral issues. Adult stem cells modified the views of the world towards stem cell. These cells can be obtained from body tissues such as bone marrow, adipose tissue, olfactory ensheathing mucosa, endometrium, peripheral blood etc. They have been studied extensively and have shown relatively better safety profile.

Neuroregeneration is a modern concept which uses neuroplasticity, neurorestoration and neurogenesis to develop novel therapeutic strategies. Neurorestoration is a subdiscipline of neuroscience which studies neural regeneration, neural structural repair or replacement, neuroplasticity and neuromodulation. (*As defined by International Association of Neurorestoratology*) The four steps of neurorestoratology include restoration of neural structure, signal transmission, neurorehabilitation and neurofunction. Neuroprotection also plays a vital role in repair process of damaged nervous system. Stem cell's neuroregenerative capacity along with endogenous neuroplasticity can make ground breaking advancements in treatment of neurological disorders. Takahashi and Yamanaka, Nobel prize winners, reprogrammed adult mature cells back into pluripotent stem cells. These iPSCs are being projected to be used in personalized medicine for neurological disorders. Currently, attempts are being made to develop patient-specific iPSCs which are safe with genomic stability. They are being studied to be placed into biological scaffoldings which can be transplanted in diseased patients.

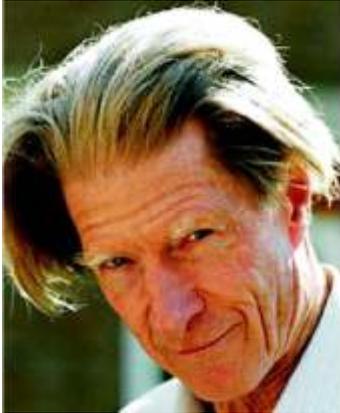
Stem cell therapy does not offer a cure as yet, but has definitely paved new ways to treat neurological disorders such as autism, cerebral palsy, spinal cord injury, brain stroke, muscular dystrophy, traumatic brain injury, motor neuron disease, Ataxia etc. In neurodegenerative disorders, stem cell therapy may positively alter (slowdown or arrest) the disease progression along with symptomatic improvements. In neurodevelopmental or traumatic disorders, stem cell therapy can augment the outcome of currently available standard treatments. The results of a particular cell therapy needs to be evaluated in the light of various factors like diagnosis, disease stage, severity, chronicity, age, gender etc. The way forward would be to study and compare the effects of various cell related aspects for e.g.: cell type, source, processing, dose, frequency, route of administration, etc

This book is focused on clinical application after stem cell therapy for incurable neurological and neuromuscular disorders. It has tried to summarize the vast information on pre clinical and clinical studies of various stem cells for neurological disorders. It exhibits the journey of stem cells from ambiguity to hope to reality.

"Stem cell research can revolutionize medicine, more than anything since antibiotics" - Ronald Reagan

Nobel Prize Winners in Stem Cell Research

2012



John B. Gurdon



Shinya Yamanaka



2007

1990



Sir Martin Evans



Dr. E. Thomas

2

Historical Review: Evolution of Stem Cell Therapy

“Stem cell research can revolutionize medicine, more than anything since antibiotics” - Ronald Reagan

The ability of animals to regenerate the lost parts is a dramatic but poorly understood aspect of biology. The sources of new cells for these regenerative phenomena have been sought for decades. Although humans cannot replace a missing finger or limb, we share some of the above abilities since our bodies are constantly regenerating blood, skin and other tissues.

In this Chapter we trace the history of stem cells from the early history almost a 100 years ago when the term was first coined to the recent development in the last 10 years where the stem cells are being researched and used for treatment of many diseases. This discovery raised the hope in the medical potential of regeneration as a possible treatment for a multitude of diseases that were considered incurable. For the first time in human history it became possible to regenerate damaged tissue with a new supply of healthy cells by drawing upon the unique property of stem cells to differentiate into specialized cells.

Introduction to the Concept of Stem Cells

The origins of stem cell research lie in a desire to understand how tissues are maintained in adult life, rather than how different cell types arise in the embryo. It was appreciated long ago that within a given tissue there is cellular heterogeneity: in some tissues, such as the blood, skin and intestinal epithelium, the differentiated

cells have a short lifespan and are unable to self-renew. This led to the concept that such tissues are maintained by stem cells, defined as cells with extensive renewal capacity and the ability to generate daughter cells that undergo further differentiation. Such cells generate only the differentiated lineages appropriate for the tissue in which they reside and are thus referred to as multi-potent or uni-potent.

Stem cells are defined as having the capacity to both self renew and give rise to differentiated cells. Given their proliferation and differentiation capacities, stem cells have great potential for the development of novel cell-based therapies. In addition, recent studies suggest that dysregulation of stem cell properties may be the cause of certain types of cancer.

Historical Review And Evolution of Stem Cell Therapy

Due to these widespread basic and clinical implications, it is of interest to put modern stem cell research into historical context. This time line takes you through the roller coaster of ups and downs in the stem cell evolution .

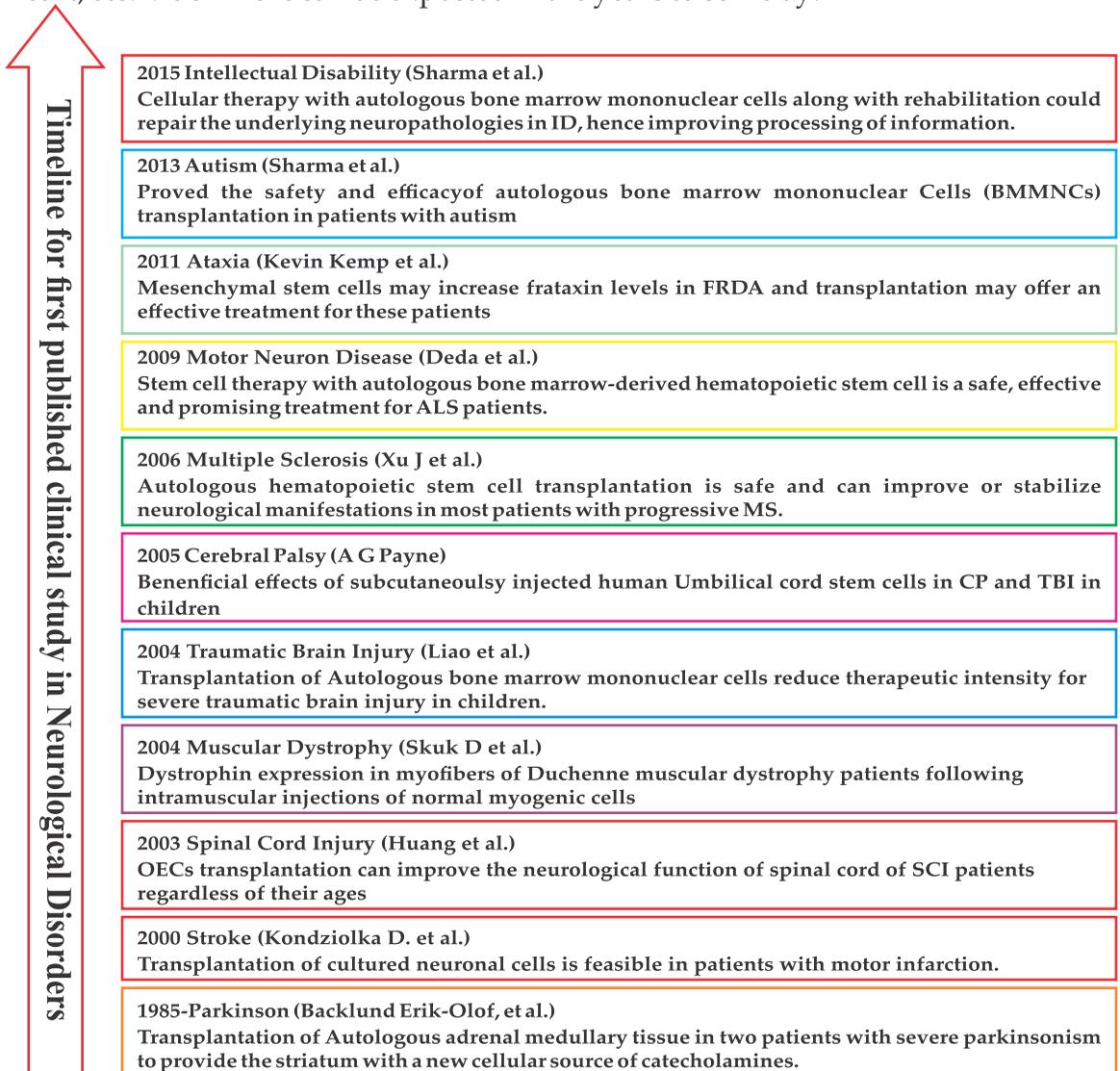
Year	Events
1868	Coining of the term Stammzelle by Ernst Haeckel, a German biologist
1892	Boveri proposed that cells along the germ line lineage between the fertilized egg and committed germ cells can be called stem cells
1896	Pappenheim used stem cell to describe a precursor cell capable of giving rise to both red and white blood cells.
Early 1900(s)	Physicians administered bone marrow cells by mouth to patients with anemia and leukemia
Early 1950(s)	Identity of cells that could regenerate into tissues was first revealed; experiments with bone marrow established the existence of stem cells
1956	Dr. E Donall Thomas, a bone marrow transplant specialist, administered donor adult stem cells to leukemia patients who went into complete remission
1958	Jean Dausset, a French medical researcher identified that the bone marrow transplant between identical twins guaranteed complete HLA compatibility between donor and recipient.
1963	Mc Culloch and Till started series of experiments involving administration of bone marrow cells into irradiated mice.
1964	Researchers isolated cells from teratocarcinoma and replicated in cell culture as stem cells. These were termed as Embryonic Carcinoma Cells(ECs)
1973	First unrelated bone marrow transplant was performed
1980(s)	James Thomson et al. at the University of Wisconsin extracted the first line of embryonic cells (ESCs) from mice
1981	Embryonic stem cells were derived from mouse embryo by 2 groups. The term Embryonic Stem Cells was coined; it was shown that these cells could be cultured in vitro.

1984	Congress passed National Organ Transplant Act which included the evaluation of unrelated marrow transplantation and feasibility of establishing a national donor registry.
1985	Yamamura detected the presence of stem cells in the pulp tissue
1988	Cord blood stem cells were used in treatment of blood cancer and Fanconi Anemia.
1990	Dr E Thomas and Joseph E Murray were awarded Nobel prize in Physiology for discoveries concerning cell and organ transplantation in treatment of human diseases.
1996	<i>Dolly - the sheep</i> . First mammal cloned from an adult somatic cell, using the process of nuclear transfer by Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute.
1998	Scientist isolated the first human embryonic stem cells
1999	Mc Donald J W et al. published a paper showing differentiation of transplanted embryonic stem cells into the injured rat spinal cord. The cells were witnessed to home at the site of injury and differentiate into astrocytes, oligodendrocytes and neurons.
2001	George W Bush announced his decision to allow federal funding of research only on existing human embryonic stem cells putting a halt on any further derivation of human stem cells and research.
2002,2004	CD34+ stem cells from bone marrow and umbilical cord blood were investigated and found to differentiate into different cell types.
2006	Generation of induced pluripotent stem cells (iPSCs) from the laboratory of Shinya Yamanaka which demonstrated the reprogramming of mouse somatic cells to pluripotency.
2007	Researchers in China reported that cell population derived from human fetal bone marrow had osteogenic, adipogenic, neural and erythroid lineage at a single cell level. Focus on induced Pluripotent stem cells(iPSCs) began
2009	President B Obama reversed the decision clearing the way again for stem cell research to progress in US
2014	Teams led by Dieter Egli of the New York Stem Cell Foundation and Young Gie Chung from CHA University in Seoul, South Korea, independently produced human embryonic stem cells from adult cells, using therapeutic cloning
2014	Masayo Takahashi started world's first trial of a therapy based on induced pluripotent stem cells, to treat a form of age-related blindness.

What is in store for the future?

The field of stem cell research and therapy has evolved and come a long way since 1868, when the term "stem cells" was coined. The discovery of embryonic stem cells opened up a new era in the use of stem cells. However stem cell research got embroiled in a controversy over the use of human embryonic stem cells for research. This led to scientists and clinicians looking at other sources of stem cells

such as from the umbilical cord or from the bone as alternative sources of stem cells. Various different kinds of stem cells are being explored for treating incurable disorders of organs other than hematopoietic, such as, the brain, muscles, liver, heart, etc. Much more can be expected in the years to come by.

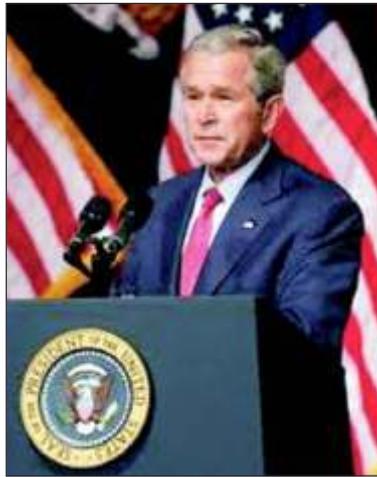


Stem cells have now entered the era of clinical studies. Numerous clinical studies using different types of cells and protocols are being conducted worldwide. The adult stem cells are now at the forefront of clinical studies due to their safety and feasibility. It is now believed that the future of healthcare and personalized medicine lies in stem cell therapy.

Interestingly the whole global ethical debate surrounding stem cell research is very concisely and clearly summed up in the speeches of the two presidents of the United States of America. These have been reproduced here as a depiction of two opposite sides of the same coin.

President George W. Bush's address on stem cell research

August 09, 2001



(Source: White House Press Office)

“All of us here today believe in the promise of modern medicine. We're hopeful about where science may take us. And we're also here because we believe in the principles of ethical medicine.

As we seek to improve human life, we must always preserve human dignity. And therefore, we must prevent human cloning by stopping it before it starts.

All of us here today believe in the promise of modern medicine. We're hopeful about where science may take us. And we're also here because we believe in the principles of ethical medicine.

As we seek to improve human life, we must always preserve human dignity. And therefore, we must prevent human cloning by stopping it before it starts.

Science has set before us decisions of immense consequence. We can pursue medical research with a clear sense of moral purpose or we can travel without an ethical compass into a world we could live to regret. Science now presses forward the issue of human cloning. How we answer the question of human cloning will place us on one path or the other.

Human cloning is the laboratory production of individuals who are genetically identical to another human being. Cloning is achieved by putting the genetic material from a donor into a woman's egg, which has had its nucleus removed. As a result, the new or cloned embryo is an identical copy of only the donor. Human cloning has moved from science fiction into science.

One biotech company has already begun producing embryonic human clones for research purposes. Chinese scientists have derived stem cells from cloned embryos created by

combining human DNA and rabbit eggs. Others have announced plans to produce cloned children, despite the fact that laboratory cloning of animals has led to spontaneous abortions and terrible, terrible abnormalities.

Human cloning is deeply troubling to me, and to most Americans. Life is a creation, not a commodity. Our children are gifts to be loved and protected, not products to be designed and manufactured. Allowing cloning would be taking a significant step toward a society in which human beings are grown for spare body parts, and children are engineered to custom specifications; and that's not acceptable.

In the current debate over human cloning, two terms are being used: reproductive cloning and research cloning. Reproductive cloning involves creating a cloned embryo and implanting it into a woman with the goal of creating a child. Fortunately, nearly every American agrees that this practice should be banned. Research cloning, on the other hand, involves the creation of cloned human embryos, which are then destroyed to derive stem cells.

I believe all human cloning is wrong, and both forms of cloning ought to be banned, for the following reasons. First, anything other than a total ban on human cloning would be unethical. Research cloning would contradict the most fundamental principle of medical ethics, that no human life should be exploited or extinguished for the benefit of another.

Yet a law permitting research cloning, while forbidding the birth of a cloned child, would require the destruction of nascent human life. Secondly, anything other than a total ban on human cloning would be virtually impossible to enforce. Cloned human embryos created for research would be widely available in laboratories and embryo farms. Once cloned embryos were available, implantation would take place. Even the tightest regulations and strict policing would not prevent or detect the birth of cloned babies.

Third, the benefits of research cloning are highly speculative. Advocates of research cloning argue that stem cells obtained from cloned embryos would be injected into a genetically identical individual without risk of tissue rejection. But there is evidence, based on animal studies, that cells derived from cloned embryos may indeed be rejected.

Yet even if research cloning was medically effective, every person who wanted to benefit would need an embryonic clone of his or her own, to provide the designer tissues. This would create a massive national market for eggs and egg donors, and exploitation of women's bodies that we cannot and must not allow.

I stand firm in my opposition to human cloning. And at the same time, we will pursue other promising and ethical ways to relieve suffering through biotechnology. This year for the first time, federal dollars will go towards supporting human embryonic stem cell research consistent with the ethical guidelines I announced last August.

The National Institutes of Health is also funding a broad range of animal and human adult stem cell research. Adult stem cells which do not require the destruction of human embryos and which yield tissues which can be transplanted without rejection are more versatile than originally thought.

We're making progress. We're learning more about them. And therapies developed from adult stem cells are already helping suffering people.

I support increasing the research budget of the NIH, and I ask Congress to join me in that support. And at the same time, I strongly support a comprehensive law against all human cloning. And I endorse the bill -- wholeheartedly endorse the bill -- sponsored by Senator Brownback and Senator Mary Landrieu.

This carefully drafted bill would ban all human cloning in the United States, including the cloning of embryos for research. It is nearly identical to the bipartisan legislation that last year passed the House of Representatives by more than a 100-vote margin. It has wide support across the political spectrum, liberals and conservatives support it, religious people and non-religious people support it. Those who are pro-choice and those who are pro-life support the bill.

This is a diverse coalition, united by a commitment to prevent the cloning and exploitation of human beings. It would be a mistake for the United States Senate to allow any kind of human cloning to come out of that chamber.

I'm an incurable optimist about the future of our country. I know we can achieve great things. We can make the world more peaceful; we can become a more compassionate nation. We can push the limits of medical science. I truly believe that we're going to bring hope and healing to countless lives across the country. And as we do, I will insist that we always maintain the highest of ethical standards.

Thank you all for coming. God bless."

President Obama Speech on Stem Cell Policy Change

March 9, 2009



(Source: White House Press Office)

"Today, with the Executive Order I am about to sign, we will bring the change that so many scientists and researchers; doctors and innovators; patients and loved ones have hoped for, and fought for, these past eight years: we will lift the ban on federal funding for promising embryonic stem cell research. We will vigorously support scientists who pursue this research. And we will aim for America to lead the world in the discoveries it one day may yield.

At this moment, the full promise of stem cell research remains unknown, and it should not be overstated. But scientists believe these tiny cells may have the potential to help us understand, and possibly cure, some of our most devastating diseases and conditions. To regenerate a severed spinal cord and lift someone from a wheelchair. To spur insulin production and spare a child from a lifetime of needles. To treat Parkinson's, cancer, heart disease and others that affect millions of Americans and the people who love them.

But that potential will not reveal itself on its own. Medical miracles do not happen simply by accident. They result from painstaking and costly research - from years of lonely trial and error, much of which never bears fruit - and from a government willing to support that work. From life-saving vaccines, to pioneering cancer treatments, to the sequencing of the human genome - that is the story of scientific progress in America. When government fails to make these investments, opportunities are missed. Promising avenues go unexplored. Some of our best scientists leave for other countries that will sponsor their work. And those countries may surge ahead of ours in the advances that transform our lives.

But in recent years, when it comes to stem cell research, rather than furthering discovery, our government has forced what I believe is a false choice between sound science and moral values. In this case, I believe the two are not inconsistent. As a person of faith, I believe we are

called to care for each other and work to ease human suffering. I believe we have been given the capacity and will to pursue this research - and the humanity and conscience to do so responsibly.

It is a difficult and delicate balance. Many thoughtful and decent people are conflicted about, or strongly oppose, this research. I understand their concerns, and we must respect their point of view.

But after much discussion, debate and reflection, the proper course has become clear. The majority of Americans - from across the political spectrum, and of all backgrounds and beliefs - have come to a consensus that we should pursue this research. That the potential it offers is great, and with proper guidelines and strict oversight, the perils can be avoided.

That is a conclusion with which I agree. That is why I am signing this Executive Order, and why I hope Congress will act on a bi-partisan basis to provide further support for this research. We are joined today by many leaders who have reached across the aisle to champion this cause, and I commend them for that work.

Ultimately, I cannot guarantee that we will find the treatments and cures we seek. No President can promise that. But I can promise that we will seek them - actively, responsibly, and with the urgency required to make up for lost ground. Not just by opening up this new frontier of research today, but by supporting promising research of all kinds, including groundbreaking work to convert ordinary human cells into ones that resemble embryonic stem cells.

I can also promise that we will never undertake this research lightly. We will support it only when it is both scientifically worthy and responsibly conducted. We will develop strict guidelines, which we will rigorously enforce, because we cannot ever tolerate misuse or abuse. And we will ensure that our government never opens the door to the use of cloning for human reproduction. It is dangerous, profoundly wrong, and has no place in our society, or any society.

This Order is an important step in advancing the cause of science in America. But let's be clear: promoting science isn't just about providing resources - it is also about protecting free and open inquiry. It is about letting scientists like those here today do their jobs, free from manipulation or coercion, and listening to what they tell us, even when it's inconvenient - especially when it's inconvenient. It is about ensuring that scientific data is never distorted or concealed to serve a political agenda - and that we make scientific decisions based on facts, not ideology.

By doing this, we will ensure America's continued global leadership in scientific discoveries and technological breakthroughs. That is essential not only for our economic prosperity, but for the progress of all humanity.

That is why today, I am also signing a Presidential Memorandum directing the head of the White House Office of Science and Technology Policy to develop a strategy for restoring scientific integrity to government decision making. To ensure that in this new

Administration, we base our public policies on the soundest science; that we appoint scientific advisors based on their credentials and experience, not their politics or ideology; and that we are open and honest with the American people about the science behind our decisions. That is how we will harness the power of science to achieve our goals - to preserve our environment and protect our national security; to create the jobs of the future, and live longer, healthier lives.

As we restore our commitment to science, and resume funding for promising stem cell research, we owe a debt of gratitude to so many tireless advocates, some of whom are with us today, many of whom are not. Today, we honor all those whose names we don't know, who organized, and raised awareness, and kept on fighting - even when it was too late for them, or for the people they love. And we honor those we know, who used their influence to help others and bring attention to this cause - people like Christopher and Dana Reeve, who we wish could be here to see this moment.

One of Christopher's friends recalled that he hung a sign on the wall of the exercise room where he did his grueling regimen of physical therapy. It read: "For everyone who thought I couldn't do it. For everyone who thought I shouldn't do it. For everyone who said, 'It's impossible.' See you at the finish line."

Christopher once told a reporter who was interviewing him: "If you came back here in ten years, I expect that I'd walk to the door to greet you."

Christopher did not get that chance. But if we pursue this research, maybe one day - maybe not in our lifetime, or even in our children's lifetime - but maybe one day, others like him might.

There is no finish line in the work of science. The race is always with us - the urgent work of giving substance to hope and answering those many bedside prayers, of seeking a day when words like "terminal" and "incurable" are finally retired from our vocabulary.

Today, using every resource at our disposal, with renewed determination to lead the world in the discoveries of this new century, we rededicate ourselves to this work.

Thank you, God bless you, and may God bless America."

Dear Mr. President,
My name is Gavin Nore. I am a 15 year old young man from Fort Dodge, Iowa. I first met you when I was eight years old. Back in 2007, you gave a speech about your campaign. Once you were done, people were allowed to ask you questions. I got the chance to meet you and I asked, "Would you continue stem^{cell} research?" You told me, you would continue the research. When I turned 14, I was diagnosed with Hodgkins Lymphoma on February 14, 2013. I beat the battle. During the summer of 2013, I was cancer free. Then, in August of last year, I was re-diagnosed. I had to have a stem cell transplant. I beat the battle once again. I would like to thank you very much for continuing the research. If the research hadn't continued, I wouldn't be here today. Once again, thank you very much Mr. President!

Sincerely,
Gavin Nore

A letter written to US President Obama by a young boy suffering from Hodgkins Lymphoma, thanking him for lifting the ban on stem cell research.

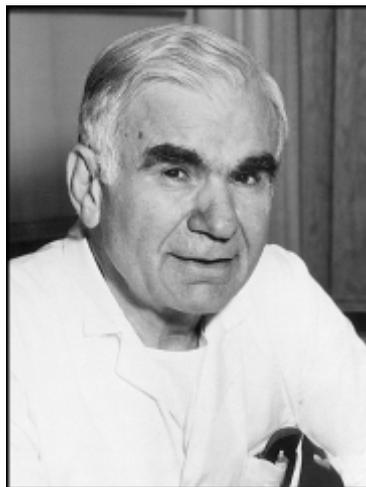
Courtesy: letterstopresidentobama.tumblr.com

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"Our enduring hope is invested in Biological research"



M. Gazi Yasargil
(Neurosurgeon of The Millenium)

3

Basics of Stem Cells: Types and Sources

“We are not made of drugs, we are made of cells” – Cade Hildreth

The field of stem cell therapy has advanced with time to such an extent that it has percolated in every branch of medicine. The understanding of stem cells has been increasing exponentially with sophisticated biotechnology and laboratory experiments. This basic research is now translating into clinical studies in an attempt to ameliorate various disorders. Thus understanding the basics of these stem cells has become imperative for the medical community. Here we make an effort to simplify the complex scientific information regarding stem cells.

The human body is intricate, with respect to its structure and function. It is made up of diverse cell types, each with a different cytoskeleton, genetic make-up, different cellular processes and functions. Despite of this intricacy, the origin of each of these cells is from a pool of stem cells in the early embryo. During early development as well as later in life, these stem cells give rise to the specialized or differentiated cells that make up the human body. Over the past 2 decades scientists have been constantly decoding the processes by which unspecialized stem cells become the different types of specialized stem cells. Stem cells can regenerate themselves or produce specialized cell types. This property of differentiation and trans-differentiation makes them unique for constructing medical treatment that can replace lost or damaged cells. In this chapter we will look at some of the fundamental basic properties of Stem cells.

What Are Stem Cells?

A stem cell is defined by two distinct properties of self renewal and differentiation into various cell types. These cells can divide indefinitely, producing a population of identical cells. Stem cells can, on cue, undergo differentiation by asymmetric division to produce two different cell lines. One is identical to the parent and continues to contribute to the original stem cell line. The other cell contains a different set of genetic instructions and is characterized by a reduced proliferative capacity and more restricted developmental potential than its parent. Eventually a stem cell becomes known as a "progenitor" or "precursor" cell, committed to producing one or a few terminally differentiated cells such as neurons, muscle cells etc. (1)

Potency of Stem Cells:

There exists a hierarchy in the stem cell compartment, depending on their 'potency' or fate restriction:

- 1) **Totipotent stem cells** give rise to embryonic as well as the extra embryonic tissue. The physiological totipotent stem cell is a fertilized oocyte (zygote) or first blastomere which comprises of the 8 cell stage and the artificial counterpart is a clone obtained by somatic cell nuclear transfer (SCNT) to an enucleated oocyte.
- 2) **Pluripotent stem cells** have the capacity to give rise to cells of all the three germ layers of the embryo which is endoderm, mesoderm and the ectoderm.
- 3) **Multipotent stem cells** give rise to cells of one of the germ cell layers only, either ecto-, meso- or endoderm. Sources range from 8 day old embryo to adult bone marrow.
- 4) **Monopotent/Unipotent stem cells** are tissue-committed stem cells that give rise to cells of one lineage, e.g., hematopoietic stem cells, epidermal stem cells, intestinal epithelium stem cells, neural stem cells, liver stem cells or skeletal muscle stem cells. (2)

Classification of Stem Cells

Stem cells are broadly divided into embryonic origin and adult origin. We describe them into the following groups for the better understanding with respect to clinical application

- A. Embryonic Stem Cells
- B. Fetal Stem Cells
- C. Umbilical Cord Stem Cells
- D. Adult Stem Cells
- E. Induced Pluripotent Stem Cells

A. Embryonic Stem cells:

Embryonic stem cells are pluripotent in nature which are derived from the inner cell mass (ICM) of 5 to 7 day blastocyst, obtained from IVF clinics. (3) The ICM ultimately gives rise to the three germ layers and subsequently the whole embryo. The potential of the embryonic stem cell to form the "germ layers" & its capacity to self renew indefinitely as well as its ability to form any cell type of the body, has led to opening up of this field widely but has thrown up debates regarding ethics and legalities.

Recently, hES cell lines have now been cultivated both on human feeder cells to avoid xenogenic (8) and in the absence of feeder cells under serum-free conditions (9) as had been previously done for mES cells. These technological advances suggest that new hES cell lines free from potential retroviral infections will be prepared and that these cells, might be suitable for eventual therapeutic applications in future.

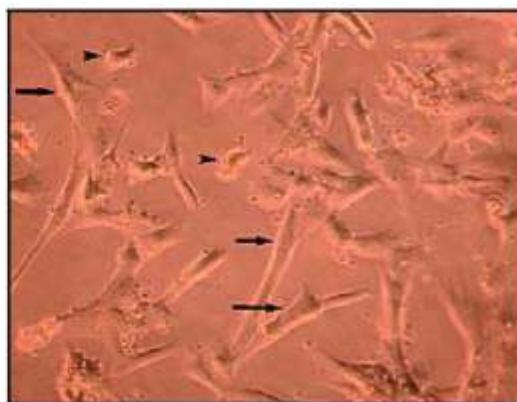
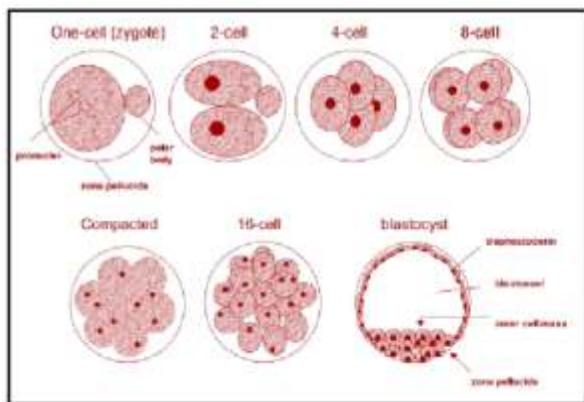
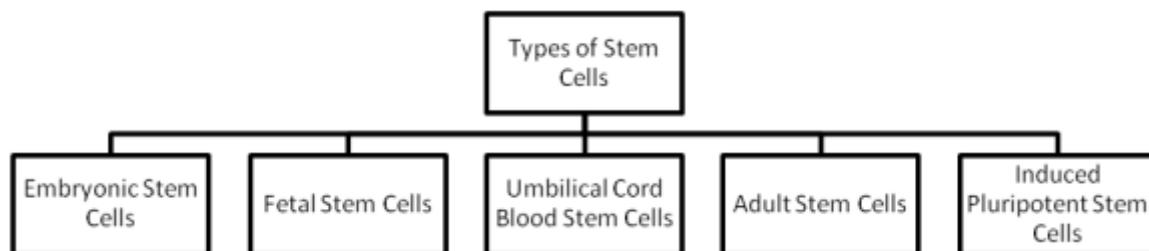


Figure 1: Development of a zygote to a blastocyst (from where embryonic stem cells are derived)

Figure 2 : Mesenchymal stem cells

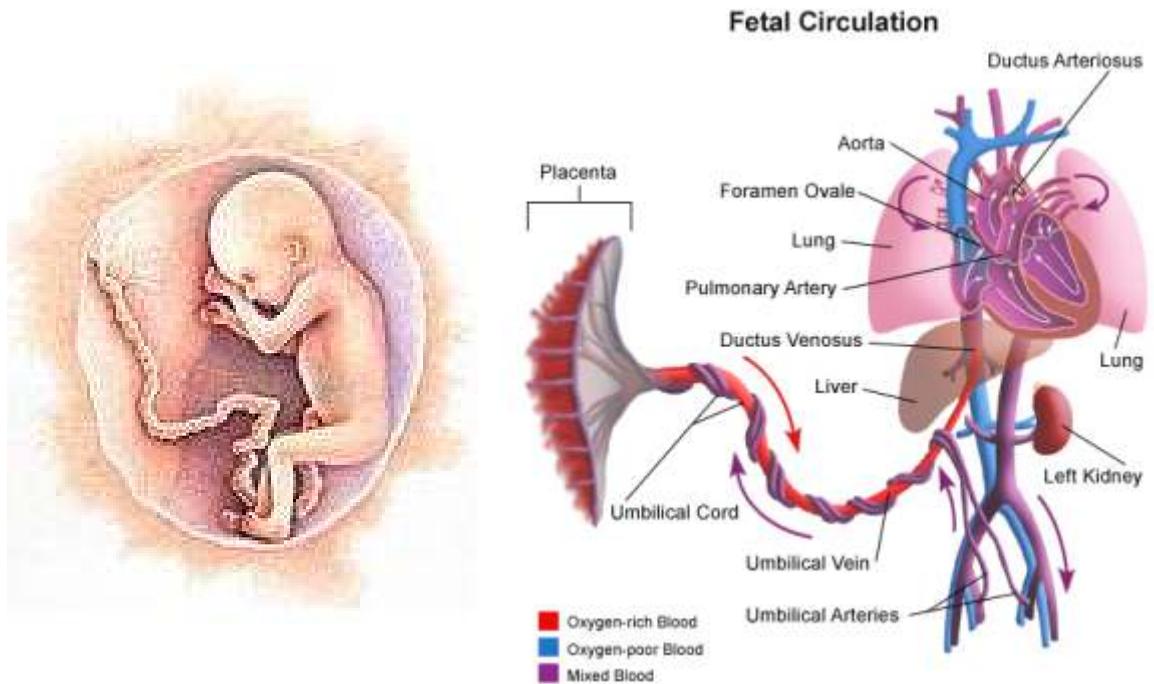


Figure 3 : The umbilical cord and placenta : a rich source of stem cells

B. Fetal Stem Cells:

Fetal Stem Cells (FSCs) are relatively a new addition into the community of different sources of stem cells, exhibiting unique and fascinating features (15). FSCs can not only be isolated from the fetal blood and hemopoietic organs in early pregnancy, but also from a variety of somatic organs as well as amniotic fluid and placenta throughout gestation (16). They can also be extracted from extra-embryonic sources (17). Fetal blood is a rich source of hemopoietic stem cells (HSCs). Mesenchymal stem cells, endothelial stem cells, epithelial stem cells and neural stem cells are other types of stem cells obtained from fetal blood (18). These cells exhibit rapid proliferative rate as compared to those present in cord blood or adult bone marrow. As these cells share similar growth kinetics and express pluripotency markers, it provides us with a strong notion that these cells may be biologically closer to embryonic stem cells. These cells represent as intermediates between embryonic stem cells and adult stem cells, with respect to proliferation rates and plasticity features.

C. Umbilical Cord Stem Cells

Umbilical cord blood stem cells can be obtained from the umbilical cord immediately after birth. Like bone marrow, umbilical cord blood is another rich source of hematopoietic stem cells. The blood remaining in the umbilical vein following birth contains a rich source of hematopoietic stem and progenitor cells,

has been used successfully as an alternative allogeneic donor source to treat a variety of genetic, hematologic, immunologic, and oncologic disorders. Fresh cord blood is also a promising source of non-hematopoietic stem cells. Among others, it contains endothelial cells, MSCs and unrestricted somatic stem cells (USSC). These hematopoietic stem cells are less mature than those stem cells found in the bone marrow of adults or children.

There have been reports that matrix cells (Wharton's jelly) from the umbilical cord also contain potentially useful stem cells. Wharton's jelly has been a source for isolation of mesenchymal stem cells. These cells express typical stem cell markers such as c-kit and high telomerase activity and can be induced to differentiate in vitro into neurons.

The advantages of using cord blood as a source of stem cells are:

1. It is a non-invasive source and can be obtained from the umbilical cord immediately after birth.
2. Available in vast abundance; thousands of babies are born each day and the umbilical cord and placenta are discarded as waste.
3. Despite its high content of immune cells, it does not produce strong graft-versus-host disease
4. Therefore, cord blood grafts do not need to be as rigorously matched to a recipient as allogenic bone marrow grafts.
5. Higher proliferative capacity

However, there are a few disadvantages (20):

1. Slow engraftment
2. Limited cell dose- small volume of unit, additional cell dose unavailable
3. Autologous donation- limited benefit owing to hereditary disorders
4. Storage issues - unknown length of long term storage, Cost related to long term storage,
5. Quality control

Hence, cord blood has recently emerged as an alternative source of hematopoietic stem cells for treatment of leukemia and other blood disorders.

All over the world, innumerable cord blood banks have been established for storage of umbilical cord stem cells. These are generally either pure public banks or private banks. There are certain banks which offer both types of banking (mixed type). Umbilical cord stem cells banks also differ in the type of biological material

that they store. Some banks only store the cord blood (from the umbilical vein) which predominantly carries the haematopoietic stem cells. Increasingly, banks have started storing pieces of the placenta and cord, which are a rich source of mesenchymal stem cells.

D. Adult Stem Cells

Adult stem cells are pluripotent, self renewing and have the ability to differentiate into the mature cell of its resident environment and also, may have transdifferentiating abilities. The primary role of these adult stem cells is initiation of repair process in the organ following an injury/damage. There is practical difficulty to obtain these cells due to the following reasons:

- 1) Inaccessibility and small numbers (e.g. neural stem cells)
- 2) Lack of markers for characterization and isolation of the "stem cell population" from various organs (21).

These cells are a preferred choice of cells as their use does not involve any ethical, moral or legal issues as compared to the use of embryonic stem cells. However, the debate over their pluripotency is ongoing and the concept of adult stem cell plasticity has been extremely dynamic.

Adult stem cells have been identified in many organs and tissues, including bone marrow, CNS, nose, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis.

Bone Marrow Derived Cells

Bone marrow is the most accessible and most studied source of adult stem cells. Different types of stem cells have been found to be present in the bone marrow, which differ in their potential to differentiate and form cells from one or more germ layers.

Initially, the bone marrow was thought to contain only haematopoietic stem cells. However, increasing evidence suggests presence of heterogeneous population of cells with varying plasticity.

Potential Pluripotent Stem Cells candidates identified in bone marrow are:

1) Mononuclear Cells:

Bone marrow mononuclear cells are a heterogeneous population that includes hematopoietic lineage cells such as lymphocytes, monocytes, stem cells and progenitor cells as well as mesenchymal stromal cells, along with endothelial progenitor cells (EPCs) and very small embryonic like (VSELs) stem cells. Mononuclear cells are isolated from human adult bone marrow, peripheral blood and umbilical cord. This mixture of cells has shown promising therapeutic

potential in various neurological conditions (53).

2) Mesenchymal Stem Cells (Multipotent Mesenchymal Stromal Cells):

Human mesenchymal stem cells (MSCs) are thought to be multipotent cells that have the potential to differentiate into multiple lineages including bone, cartilage, muscle, tendon, ligament fat and a variety of other connective tissues. Bone marrow-derived cells seem to retain a remarkable plasticity, since they have much wider differentiation potential than thought previously. Marrow cells have been reported to contribute to angiogenesis, somatic muscle development, liver regeneration, and the formation of central nervous system cell types. It is likely that MSC may be contaminated by other populations of primitive non-hematopoietic stem cells. This possibility should be considered whenever a "transdifferentiation" of MSC into cells from other germ layers is demonstrated. Because various inconsistencies have come to light in the field of MSC research, the International Society for Cellular Therapy recently recommended avoiding the name of MSC stem cells and changing it to multipotent mesenchymal stromal cells instead. (22)

3) Multipotent Adult Progenitor Cells (MAPC):

MAPC are isolated from BM as well from various adult organs as a population of CD45⁻GPA⁻A⁻ adherent cells and they display a similar fibroblastic morphology to MSC. Interestingly MAPC are the only population of BM derived stem cells that have been reported to contribute to all three germ layers after injection into a developing blastocyst, indicating their pluripotency. (23) The contribution of MAPC to blastocyst development, however, requires confirmation by other, independent laboratories.

4) Marrow-isolated adult multilineage inducible (MIAMI) cells:

This population of cells were isolated from human adult BM by culturing BM MNC in low oxygen tension conditions on fibronectin. MIAMI cells were isolated from the BM of people ranging from 3- to 72-years old. Colonies derived from MIAMI cells expressed several markers for cells from all three germ layers, suggesting that, at least as determined by in vitro assays, they are endowed with pluripotency. However, these cells have not been tested so far for their ability to complete blastocyst development. The potential relationship of these cells to MSC and MAPC is not clear, although it is possible that these are overlapping populations of cells identified by slightly different isolation/expansion strategies.

5) Multipotent Adult Stem Cells (MACS):

These cells express pluripotent-state-specific transcription factors (Oct-4, Nanog and Rex1) and were cloned from human liver, heart and BM-isolated mononuclear cells. MACS display a high telomerase activity and exhibit a wide range of

differentiation potential. Again the potential relationship of these cells to MSC,MAPC and MIAMI described above is not clear, although it is possible that these are overlapping populations of cells identified by slightly different isolation/expansion strategies.

6) Very Small Embryonic Like (VSEL) Stem Cells:

Recently, a homogenous population of rare (~0.01% of BM MNC) Sca-1+ lin- CD45- cells was identified in murine BM. They express (as determined by RQ-PCR and immunohistochemistry) markers of pluripotent stem cells such as SSEA-1, Oct-4, Nanog and Rex-1 and Rif-1 telomerase protein (24) Direct electron microscopical analysis revealed that VSEL (2-4 μm in diameter) display several features typical for embryonic stem cells such as i) a large nucleus surrounded by a narrow rim of cytoplasm, and ii) open-type chromatin (euchromatin). Interestingly, these cells despite their small size possess diploid DNA and contain numerous mitochondria. VSEL, however, do not express MHC-1 and HLA-DR antigens and are CD90-CD105-CD29.

2. Central Nervous system (CNS)

Adult CNS is a potential niche for isolation of neural stem cells (NSCs) Adult-derived neural progenitor and stem cells have been transplanted in animal models, and shown functional engraftment, supporting their potential use for therapy. (29) Stem cell niches have now been identified in adult mammalian forebrain, a) in the subventricular zone (SVZ), subgranular zone (SGZ) and b) dental gyrus of the hippocampus. The most active NSC compartment is found in SVZ. Two main cell types are found in the SVZ: migratory, proliferating neuroblasts and astrocytes.

3. Nose:

it contains cells with considerable regeneration potential, including neural cells, progenitor/ stem cells, and olfactory ensheathing cells. OECs can promote axonal regeneration by producing insulating myelin sheaths around growing and damaged axons, secreting growth factors, and generating structural and matrix macromolecules that lay the tracks for axonal elongation. (33, 34)

4. Skin:

The skin harbors a distinct population of stem cells such as melanoblasts and epidermal Stem cells. These cells can generate both neural and mesodermal cell types and that most of the neural cells generated by them have characteristics of peripheral neurons and Schwann cells (35)

5. Adipose tissue :

The adipose tissue is a highly complex tissue and consists of mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident

monocytes/macrophages and lymphocytes. Hence, this tissue compartment provides a rich source of pluripotent adipose tissue-derived stromal cells. It has been demonstrated that AT contains stem cells similar to BM-MSCs, which are termed processed lipoaspirate (PLA) cells. Exhibiting a neuronal-like morphology and expressing several proteins consistent with the neuronal phenotype. (36, 37)

6. Peripheral Nervous system (PNS):

Schwann cells are the supporting cells of the PNS. Like oligodendrocyte, Schwann cells wrap themselves around nerve axons, but the difference is that a single Schwann cell makes up a single segment of an axon's myelin sheath. Schwann cells originating from dorsal and ventral roots are one of the cellular components that migrate to the site of tissue damage after spinal cord injury. The remyelinating capability of Schwann cells has been demonstrated in a number of studies and the functioning status of this myelin in conduction of neural impulses has confirmed. (38, 39).

7. Eye stem cells

Stem cells have been identified in the adult mouse eye. Single pigmented ciliary margin cells were shown to clonally proliferate in vitro to form sphere colonies of cells that can differentiate into retinal-specific cell types, including rod photoreceptors, bipolar neurons and Muller glia. The adult retinal stem cells were localized to the pigmentary ciliary margin and not to the central and peripheral retinal pigmented epithelium. (40)

8. Dental Stem Cells:

Different types of dental stem cells have been isolated from mature and immature teeth, dental pulp, exfoliated deciduous teeth, periodontal ligament, apical papilla and dental follicle. Dental stem cells are rich source of mesenchymal stem cells and neural cells. They are multipotent stem cells which are being widely explored for its potential in treatment of neurodegenerative and ischemic diseases (54).

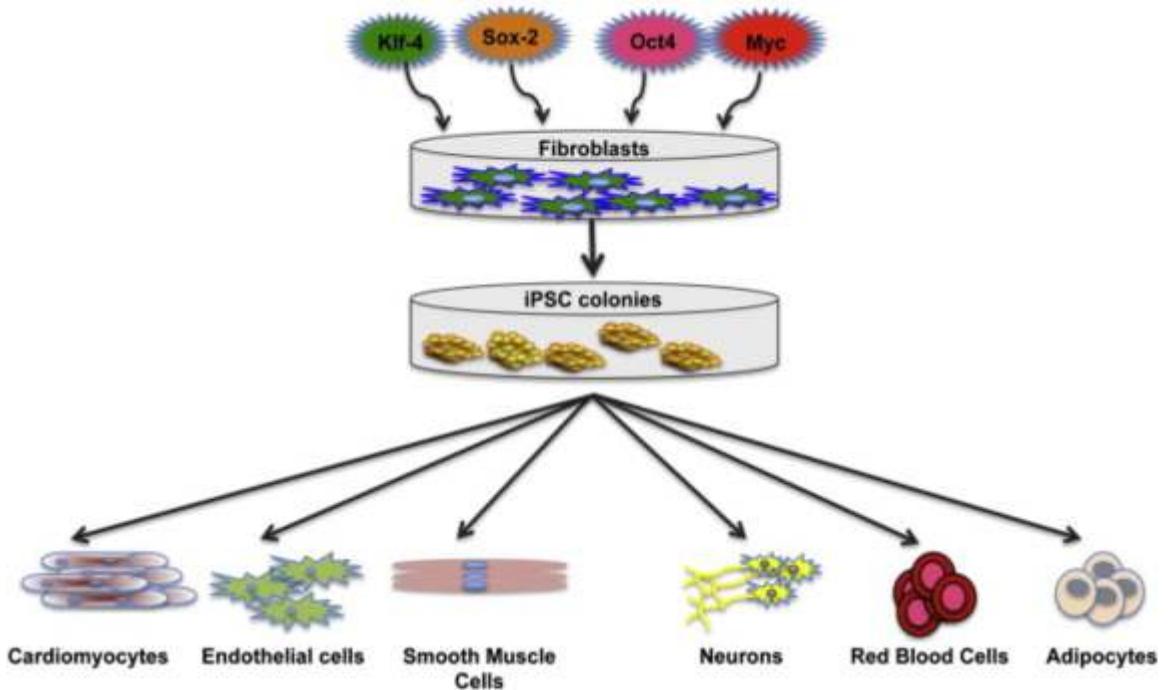
9. Muscular Stem Cells:

The progenitor/stem cells are also found in skeletal muscles which are also known as satellite cells and side progenitor (SP) cells. These stem cells are involved in repair of regular wear and tear of muscle fibers. These cells help to regenerate the damaged muscles.

E. Induced Pluripotent Stem Cells:

One of the emerging areas in laboratory investigations of stem cells is the attempt to induce differentiated somatic stem cells into pluripotent stem cells by inducing certain factors which will initiate cellular reprogramming (48, 49). The induced pluripotent human stem cells have normal karyotypes, express telomerase activity,

express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers (50). These iPSCs sidesteps the ethical issues that have limited the use of embryonic stem cells, as they can be generated without the use of oocytes or cell from the preimplantation embryo (51). These cells can be autologous, thereby surmounting the problem of immune reaction. Thus, development of IPS cell technology can add to the sources of autologous cells for transplantation therapy (52).



Courtesy: Nsair, Ali, and W. Robb MacLellan. "Induced pluripotent stem cells for regenerative cardiovascular therapies and biomedical discovery." Advanced drug delivery reviews 63.4 (2011): 324-330.

The progression of Adult Stem Cells to Induced Pluripotent Stem Cells (iPSCs) is already a dynamic area of research in stem cell therapy. However, recent work has exhibited strong evidence that the adult somatic cells can be reprogrammed into mature neurons, without the in-between transition into iPSCs (41-43). There are recent reports which provide us sufficient evidence that transcription-mediated reprogramming of human fibroblasts into subtype specific neurons can be achieved without undergoing the proliferative progenitor stage (44-46). In one of the studies, the authors reported that the fibroblasts were reprogrammed into motor neurons, by forced expression of select transcription factors (47).

Conclusion

Stem cells have received much attention for their potential use in cell based therapies for various human diseases. Understanding different types of stem cells

and their niches is essential for future clinical applications. All the above mentioned stem cells have distinct differential potentials which need to be explored and manipulated to optimize their therapeutic potential. Until now, adult bone marrow stem cells have been the most studied type of cells.

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School yourself to demureness and patience and learn to inure yourself to drudgery in science. Perfect as the wing is of the bird, it would never raise the bird up without resting on air. Facts are the air of the scientist. Without them your theories are vain efforts. By learning, experimentation and observation try not to stay on the surface of facts. Do not become an archivists of facts. Try to penetrate to the secret of their occurrence and persistently search for the laws that govern them"

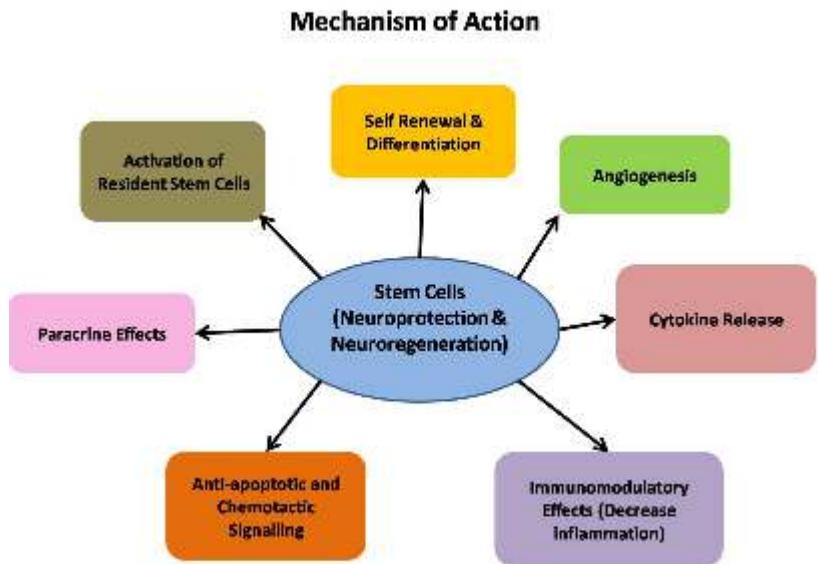
- Ivan Pavlov

4

Mechanism of Action

*“If power is defined as the ability to do anything and create anything, then the stem cell is the most powerful *known* life source.”*

The naturally occurring stem cells in the organs constantly repair the daily wear and tear of tissues through multitudes of mechanisms. In various disease models, the mechanism of action of stem cells has been studied in great depths. The pathways through which they act have also been studied in vitro. The micro cellular environment



plays a crucial role in deciding the fate of stem cells. Stem cells carry out the repair process by neuromodulation, neuroprotection, axon sprouting, neural circuit reconstruction, neurogenesis, neuroregeneration, neurorepair, neuroreplacement and muscle regeneration. Mechanism of stem cells is divided into two categories- Direct cell replacement and indirect repair via paracrine mechanisms.

1. Direct Cell Replacement:

Homing of the stem cells

Stem cells are known to migrate to injured/ damaged sites on transplantation. This property is attributed to the expression of growth factors, chemokine and extracellular matrix receptors on the surface of cells. Evidence also exists that both endogenous and exogenous stem cells are able to 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. (1) This homing mechanism may also depend on expression of stromal cell-derived factor 1 (SDF-1), monocyte chemoattractant protein-3 (MCP-3), stem cell factor (SCF), and / or IL-8.

SDF-1 represents the major chemokine for initiating stem cell migration.⁷⁸ The majority of cytokines that mediate stem cell migration do so via modulation either of SDF-1 or of its receptor, CXCR4. Thus the SDF-1/CXCR4 axis is central for stem cell mobilization. Post damage/ injury, injured tissue releases SDF-1 which stimulates the mobilization and homing of cells. These cells get recruited to the damaged/ injured tissue and carry out further repair. (2)

These cells self renew and differentiate into host cells and replace the damaged or dead cells. Their ability to differentiate into various other cell types is known as stem cell plasticity.

Stem Cell Plasticity

Stem cell plasticity refers to the capacity of the tissue derived stem cells to give rise to cell types, other than that of the resident tissues. One of the mechanisms explaining stem cell plasticity is "cell fusion". (3) An undifferentiated stem cell fuses directly with a more differentiated cell resulting in a single cell expressing properties of either cells. However, many reports have excluded this mechanism as experimental studies have not shown any evidence of cell fusion. Another mechanism justifying plasticity of stem cells is "transdifferentiation" or "dedifferentiation" (4) wherein 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. Also, there is a possibility of true pluripotent stem cells existing in various tissues of the body making it the most appealing explanation for the plasticity of stem cells.

Adult stem cells like bone marrow cells have been reported to generate a wide spectrum of different cell types, including hepatocytes, endothelial, myocardial, neuronal, and glial cells. In 1998, Ferrari et al. reported that mouse bone-marrow-

derived cells give rise to skeletal muscle cells when transplanted into damaged mouse muscle. (5) Hematopoietic cells can differentiate into cardiac myocytes and endothelial cells, functional hepatocytes and epithelial cells of the liver, gut, lung, and skin. (6-12) 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. (13-15) 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, which suggest that even cell types are plastic in their developmental potential.

A critical observation of adult stem cell plasticity is that in order for plasticity to occur, cell injury is necessary(16), thus micro-environmental exposure to the products of injured cells may play a key role in determining the differentiated expression of marrow stem cells. (17)

2. The Paracrine Effect

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 neuroprotection, 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. (18)

Neuroprotection

Stem cells secrete a vast array of neuroprotective growth factors including BDNF, nerve growth factor (NGF), neurotrophin-3 (NT-3), glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor-2, and insulin-like growth factor type 1. These growth factors activate a number of signalling pathways and help in survival of cells. Brain-derived neurotrophic factor (BDNF) is one of the most important growth factors. It has shown to enhance differentiation, survival of neurons and maintain neuronal functions. (19)

Increased Angiogenesis

Stem cells produce local signaling molecules like vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (FGF2) that may improve perfusion and enhance angiogenesis to chronically ischemic tissue.(20,21)

Chen et al. have recently shown that treatment with bone marrow stromal cells enhances angiogenesis by increasing endogenous levels of VEGF and VEGFR2.(22) They previously demonstrated that administration of recombinant human VEGF165 to rats 48 h 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. (23)

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 and 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. Thus, VEGF, HGF, and FGF2 may be important paracrine signaling molecules in stem cell-mediated angiogenesis, protection and survival. (24)

Immunomodulation

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. (25)

Anti-Apoptosis

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 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. (26)

Beneficial Remodeling of the Extracellular Matrix

Stem cell transplantation alters extracellular matrix, resulting in post-infarct remodeling, strengthening of the infarct scar, and prevention of deterioration in organ function. MSCs improved this function by increasing 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

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. (27)

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 is the phenomenon by which new myelin sheaths are generated around axons in the adult central nervous system. 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.

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, 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 promote differentiation of endogenous cells. Interactions with viable axons and supportive astrocytic responses are required for endogenous immature cells to fulfill their potential remyelinating capacity.(28,29)

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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*“We are what we repeatedly do.
Excellence is therefore not an act but a habit”*

-Aristotle

5

Laboratory Aspects of Stem Cell Therapy

“This field [stem cell research] isn’t growing, its EXPLODING.” – Barth Green

Stem cell harvesting is preliminary and important part of the whole process of stem cell therapy. There are various methods of procuring, culturing, differentiating and preserving. All these have specific heterogeneous protocols which are followed by different scientists. As these cells are introduced into humans for clinical application stringent aseptic precautions are mandatory. Safety of the cells has to be ensured before transplantation. The cells' viability also needs to be ascertained for correlation to efficacy. The type of stem cells also needs to be confirmed by cell markers. For all these processes Good Clinical Laboratory Practices should be followed.

Various sources of stem cells have already been discussed in the previous chapters. Currently, stem cells are being procured for therapeutic application primarily from haematopoietic sources such as the bone marrow, peripheral blood and umbilical cord and other tissues such as adipose tissue, dental pulp, muscles, etc, due to easy accessibility and absence of ethical issues. Certain aspects of harvesting and mobilization of these cells is being discussed in this chapter.

Basic methodology

The cells procured from any source are a heterogeneous population of various progenitor cells. The cells of interest for clinical application need to be separated

from this mixture. Then either they are purified and cultured before use or introduced without culturing.

Separation of the cells can be performed based on the following properties

1. Density (and size): Density Gradient centrifugation
2. Adhesion: Adhesion to surfaces, Rosette formation
3. Surface markers: Panning, Dynal beads, magnetic activated cell sorting (MACS), Fluorescence-activated cell sorting (FACS)
4. Lab-on-a-chip methods- Microfluidics-New Label free separation methods still in experimental stage.

However, the choice of cell separation protocol depends upon the cell source, the characteristics of the desired cell type and its required purity.

There are a multiple methodologies available for both maintaining stem cells in an undifferentiated state and for differentiating them into different lineages and cell types. The culture conditions and types of media used for stem cell culture depend on the type of stem cell to be cultured. This is an extensive subject of discussion which is out of scope of this chapter. Therefore, we have focused only on separation of commonly used cells.

Bone marrow derived stem cell separation

Open Method

Bone marrow (100-150 mL) is aspirated from the anterior or posterior superior iliac spine and is collected in heparin containing tubes/ balanced salt solution such as Hank's balanced salt solution (HBSS) at a ratio of 1:1. Sample is centrifuged at 400g for 30 to 40 min at 18°C to 20°C through a density gradient method using Ficoll-Paque Premium, 1.073 g/ml; GE Healthcare. The mononuclear cell layer is recovered from the gradient interface and washed with salt solution. The cells are centrifuged at 400 to 500 g for 10 to 15 min at 18°C to 20°C and resuspended in 6 to 8 ml balanced salt solution and again centrifuged at 400 to 500 × g (or 60 to 100 × g for removal of platelets) for 10 min at 18°C to 20°C.

Closed Method

Commercial platforms for harvesting bone marrow concentrates are being engineered to facilitate harvesting in a closed system. One such system is Harvest's BMAC™ (Bone Marrow Aspirate Concentrate) System (Harvest Technologies Corporation, www.harvesttech.com)

A total of 240 mL of marrow aspirate was processed using the point of care SmartPREP System (Harvest Technologies, Plymouth, MA) to yield 40 mL of treating volume.

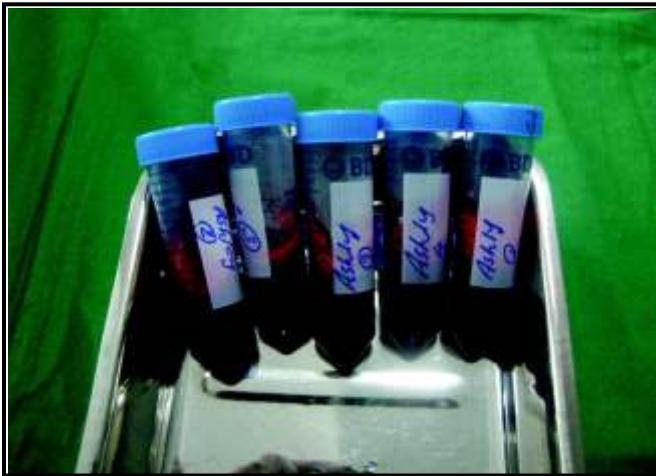


Figure 1: Aspirated bone marrow in tubes. Each tube contains about 20 ml bone marrow mixed with heparin.

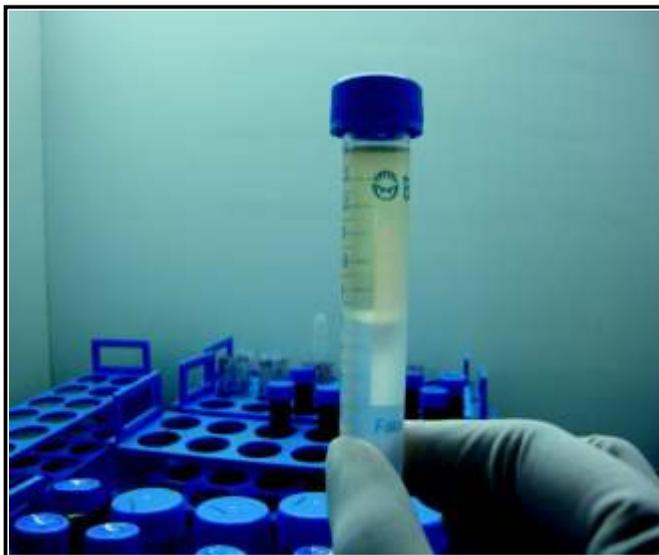


Figure 2: Buffy coat containing separated fraction of mononuclear concentrate (arrow indicating)



Figure 3 : Purified concentrate of mononuclear cells in solution (heterogenous mixture of stem cells - mainly hematopoietic)

Stem cell isolation from Peripheral blood

Mobilization and harvesting of peripheral blood stem cells for Autologous haematopoietic stem cell transplant (AHSCT):

One of the methods 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, administration of cytokines such as granulocyte colony-stimulating factor (G-CSF) with or without chemotherapy is necessary to mobilize HSCs and subsequently collect HSCs from the blood through apheresis.

Hematopoietic Stem cell selection

Most mononuclear cells collected by peripheral blood apheresis/ leukaphereses are immune cells such as lymphocytes and monocytes and not HSCs. Not much is known about the phenotype or biology of peripheral blood stem cells, however, CD34 or CD133 are predominant cell markers of hematopoietic stem cells (HSC) and hematopoietic progenitor cells. The isolation of these cells is carried out either via positive selection, i.e., isolation according to the marker molecules they express or negative selection which is depletion of all other cells according to the markers they express. Positive selection is considered to be superior to negative selection methodology.

The selection or purification of hematopoietic cells is usually performed using combination of cell sorting technologies such as MACS, flow cytometry and FACS. In general, a minimum number of 2×10^6 CD34 cells per kilogram of recipient weight with the viability count of 98% will ensure engraftment. (1)

Isolation of Adipocyte cells

Adipose derived stem cells are usually isolated from lipoaspirate waste from liposuction. They are comprised of three distinct layers: 1) an upper layer of oil due to the lysis of mature adipocytes, 2) a middle layer of adipose tissue, and 3) a bottom, liquid infranatant containing saline and blood cells.

Standard protocol involves isolation of the cells from above 2 layers however, recently, a rapid protocol was developed which isolates ASCs from the saline and blood cell layer.

Standard Protocol for ASC isolation:

The standard protocol, uses enzymatic digestion and differential centrifugation.(2)

The lipoaspirate is washed using PBS until the adipose layer has a yellow/ gold color. The next step includes enzymatic digestion of the sample using collagenase. Followed by isolation using control medium (CM), containing DMEM, fetal bovine

serum and antibiotics. CM is used for collagenase inactivation. The isolated ASCs are also further cultured in CM.

The standard protocol is time consuming hence, a rapid protocol was established to obtain viable population of ASCs in a short period of time.

Rapid protocol for ASC isolation

This protocol includes 5 simple steps. (3) First, the blood/saline phase is isolated and cells pelleted (10 minutes). Second, the resulting pellet is gently re-suspended in NH₄Cl for red blood cell lysis (2–5 minutes). Third, the cells are pelleted again (10 minutes). Fourth, the cell pellet is gently re-suspended in DMEM with 40–50% fetal bovine serum (FBS), followed by plating the cells (2–5 minutes) and incubation overnight. Finally, the non-adherent cells and debris are washed away with phosphate-buffered saline (PBS), and the ASC cultures are grown. This isolation method not only requires less 30 mins to complete but uses only standard tissue culture materials and equipment without the need for enzymatic digestion, Percoll gradients, or extensive washing.

Umbilical Cord blood processing

Currently, cord blood cells are derived using two techniques viz. manual and automated.

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. This method was the only method available for a long period of time. However, there are two potential problems with manual processing. Firstly, manual methods led to loss of mononuclear cells and could recover only 40%-80% of cells and secondly, there is increased risk of bacterial contamination. However, automated processing avoids bacterial contamination by using a completely closed system and, allows up to 99% recovery of necessary cells for transplantation. In addition, the possibility of human error is reduced. Unfortunately, these advancements make automated processing expensive for use. (4)

Endometrial cell processing and expansion

Harvesting

Before the collection procedure a "collection tube" is prepared in a class 100 Biological Safety Cabinet located in a Class 10,000 Clean Room. To prepare the collection tube, 0.2 ml amphotericin B (Sigma-Aldrich, St Louis,MO), 0.2 ml penicillin/streptomycin (Sigma) and 0.1 ml EDTA-Na₂ (Sigma) are added to a 50 ml conical tube containing 30 ml of GMP-grade phosphate buffered saline (PBS). Collection of 5 ml of menstrual blood is performed according to a modification of the published procedure. Collection is performed by the donor. A sterile Diva cup

inserted into the vagina and left in place for 30-60 minutes. After removal, the contents of the Diva cup are to be decanted into the collection tube. The collection tube is then taken to the clean room where it is centrifuged at 600 g for 10 minutes. The collection tube is then transported to the Biological Safety Cabinet where the supernatant is removed, and the tube is topped up to 50 ml with PBS in the Biological Safety Cabinet and cells are washed by centrifugation at 600 g for 10 minutes at room temperature. The cell pellet is to be washed 3 times with 50 ml of PBS, and mononuclear cells are collected by Ficoll- Paque (Fisher Scientific, Portsmouth NH) density gradient. Mononuclear cells are washed 3 times in PBS and resuspended in 5 ml complete DMEM-low glucose medium (GibcoBRL, Grand Island, NY) supplemented with 10% Fetal Bovine Serum selected lots having endotoxin level ≤ 10 EU/ml, and hemoglobin level ≤ 25 mg/dl clinical grade ciprofloxacin (5 mg/mL, Bayer A.G., Germany) and 4 mM L-glutamine (cDMEM).

The resulting cells are mononuclear cells substantially free of erythrocytes and polymorphonuclear leukocytes as assessed by visual morphology microscopically. Viability of the cells is assessed using a Guava EasyCyte Mini flow cytometer, Viacount reagents, Cytosoft Software version 4.2.1, Guava Technologies, inc. Hayward, CA (Guava flow cytometer).

Expansion

Cells are plated in a T-75 flask containing 15 ml of cDMEM, cultured for 24 hours at 37°C at 5% CO₂ in a fully humidified atmosphere. This allows the ERC precursors to adhere. Non-adherent cells are washed off using cDMEM by gentle rinsing of the flask. Adherent cells are subsequently detached by washing the cells with PBS and addition of 0.05% trypsin containing EDTA (Gibco, Grand Island, NY, USA) for 2 minutes at 37°C at 5% CO₂ in a fully humidified atmosphere. Cells are centrifuged, washed and plated in T-175 flask in 30 ml of cDMEM. This results in approximately 10,000 ERC per initiating T-175 flask. The flask is then cultured for 5 days which yields approximately 1 million cells in the T-175 flask (Passage 1). Subsequently cells are passaged at approximately 200,000 cells in a T-175 flask. At passage 3-4, approximately 100-200 million cells are harvested. (5)

Induced pluripotent cell processing

Induced pluripotent cells (iPSCs) are generated by reprogramming somatic cells to embryonic-like state cells. The somatic cells are introduced with a defined and limited set of factors and are cultured under embryonic stem cell like conditions. (6) For the first time, Yamanaka et al carried out a retroviral mediated introduction of four transcription factors - octamer-binding transcription factor-3/4 (OCT3/4), SRY-related high-mobility-group (HMG)-box protein-2 (SOX2), MYC and Kruppel-like factor-4 (KLF4) in mouse fibroblast to produce iPSCs. (6,7) These iPSC cell lines exhibit similar morphology and growth properties as ES cells and express

ES cell-specific genes. Transplantation of iPS cells into immunodeficient mice resulted in the formation of germ-cell-tumor (teratoma)-containing tissues from all three germ layers, confirming the pluripotent potential of iPS cells. Since then, the same protocol has been used for other types of mouse cells and human somatic cells. Once, the factors are introduced, cells are cultured where they form colonies resembling pluripotent cells. These cells are then isolated based on the morphology, surface markers, etc. Generation of iPSCs takes around 1-2 weeks for mouse cells and 3-4 weeks for human cells. Recently, the iPSCs are being generated virus and vector free to avoid viral induced tumor formation. The growth factors and cytokines used for differentiation of iPSCs should be extensively tested to ensure high biological activity, high purity, freeze-thaw stability, and structural homogeneity.(8) They should also allow optimal growth, expansion, and storage of differentiated cells. The major steps in obtaining iPSCs are reprogramming, culturing, engineering, differentiation and cell analysis. It is essential to validate their pluripotency and differentiation capacity into the desired cell lineage. (9)

Isolation of Neural Stem Cells

Neural stem cells reside within specific niches of the adult brain. These regions are located in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) in the hippocampus. (10)

Their isolation procedures follow common steps including tissue dissection, digestion and cell enrichment. The origin of the tissue influences the type of isolated cells as well as their proliferation and differentiation capacity. (11) Firstly, brain tissue is dissected. NSCs are removed from the rest of the tissue by enzymatic digestion which is a critical step as it influences the survival rate of the cells. At times, enzymatic digestion alone is not sufficient hence a mechanical method is used after or during the enzymatic digestion such as trituration to break up the digested pieces into a single cell suspension. Lastly, the NSCs are enriched using different procedures such as methods based on adherent properties of cells, Differential gradient centrifugation, FACS, immunopanning, etc. These cells are maintained and propagated either using methods such as free-floating cell clusters (neurospheres) or as adherent cultures forming a monolayer on the plate surface. The neurosphere assay has been the most extended method to demonstrate the presence of NSCs in culture and it is still used with different modifications. However, these cells are yet to be translated for therapeutic use.

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"The best research questions come from the patient's bedside"



Prof. Harvey Cushing
Neurosurgeon of the Millenium

6

Surgical Aspects of Stem Cell Therapy: Routes of Administration

The stem cell therapy process using autologous bone marrow derived stem cells consists broadly of 3 stages. (1) Procurement of the stem cells from the Bone marrow via a Bone marrow aspiration in the Operating theatre, (2) Separation, harvesting, enriching &/or expansion and differentiation in the laboratory and finally (3) Transplantation or delivery of the cells to the desired location. The laboratory aspects have already been dealt with in the previous chapter therefore in this chapter the procurement and transplantation aspects will be discussed.

Procurement of Stem cells - 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 of the iliac crests (posterior or anterior). The posterior superior iliac spine is easily accessible and identifiable, however to access this, the patient has to be turned 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 patient, the landmarks may be obliterated due to fat distribution. Sampling is not normally discordant between the anterior or posterior iliac spines.

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 is 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 intradermally administering 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 make 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 done. A total of 100-120 ml is aspirated in adults and 80-100 ml in children. This is collected in heparinized tubes which need to be appropriately labeled. The bone marrow collected is transported to the laboratory in a special transporter under sterile conditions.(1)

Transplantation of Stem Cells in neurological disorders

The other surgical aspect in the process of stem cell therapy is the delivery of the cells which may either be done systemically (through intravenous or intraarterial routes) or locally (intrathecal or direct implantation into the spinal cord or brain). Different centers are following different routes to transplant the cells and as of now there are no comparative studies that could tell us which is the preferred method. However keeping in mind the existence of the Blood Brain barrier, local delivery would seem to be a more logical option.

Intrathecal delivery

The patient is positioned in the lateral decubitus position, in the curled up "foetal ball" position. Occasionally, the patient is made to sit, leaning over a table-top. Both these maneuvers help open up the spinous processes. The back is painted and draped and local anaesthetic is injected into the L4-5 or L3-4 space. An 18G Touhy needle is inserted into the sub-arachnoid space. After ascertaining free flow of CSF, an epidural catheter 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 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. General anesthesia is given to children.



Figure 1: Bone marrow J needle



Figure 2: Bone marrow aspiration



Figure 3: Epidural set (18 G) for intrathecal Inj.



Figure 4: Intrathecal Injection step 1



Figure 5: Intrathecal Injection step 2



Figure 6: Intrathecal Injection step 3



Figure 7: Intrathecal Injection step 4



Figure 8: Intrathecal Injection - delivery of stem cells



Figure 9 & 10 : Intraspinal transplantation of stem cells in a case of thoracic spinal cord injury.



Figure 11: Intra-arterial direct injection of stem cells into the carotid artery following carotid endarterectomy



Figure 12: STA-MCA bypass



Figure 13: Leksell Stereotactic Frame for direct stem cell implantation into the brain.

A spinal needle instead of a catheter is preferred in patients with cardiac problems, where excessive intravenous infusion is to be avoided, in patients on anti-coagulant or anti-platelet drugs so as to avoid bleeding into the sub-arachnoid space, in case where the spine is scoliotic which happens often in patients with muscular dystrophy and in some previously operated cases of lumbar spine surgery.

Sometimes in patients with severe spinal deformities such as scoliosis it is very difficult to get the needle intrathecally and at times assistance has to be taken of the C arm to exactly locate the point and direction of needle placement.

Callera et al (2007) demonstrated for the first time that autologous bone marrow CD 34+ cells labelled with magnetic nanoparticles delivered into the spinal cord via lumbar puncture (LP) technique migrates into the injured site in patients with spinal cord injury. They conducted the trial on 16 patients with chronic SCI. 10 of them were injected intrathecally with labelled autologous CD 34+ cells and the others received an injection containing magnetic beads without stem cells. Magnetic resonance images were obtained before and 20 and 35 days after the transplantation. Magnetically labelled CD 34+ cells were visible at the lesion site as hypointense signals in five patients, which were not visible in the control group.(2)

Intraspinal transplantation

Direct implantation into the spinal cord may be done in one of many 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. The dura is incised, sparing the arachnoid, which is subsequently opened separately with a microscissors. The dorsal surface of the contusion site is located under high-power microscopic magnification. After exposure of sufficient surface in the contusion site, 300µL aliquots of cell paste (total volume, 1.8 mL) are injected into six separate points surrounding the margin of the contusion site. To avoid direct cord injury, 2×10^8 cells are delivered at a rate of 30 µL/min, using a 27-gauge needle attached to a 1-mL syringe. The depth of the injection site is 5 mm from the dorsal surface. To prevent cell leakage through the injection track, the injection needle is left in position for 5 min after completing the injection, after which the dura and arachnoid are closed. The muscle and skin are closed in layers.(3)
- b) Though a minilaminectomy and exposure of the spinal cord. The dura is opened and a 27 gauge scalp vein is used by cutting one of the wings. The other wing is held by a hemostat and inserted at a 45 degree angle into the Dorsal root entry zone. It is inserted 3mm deep into the spinal cord. Two injections are made on either side above the injury site and two injections are made below the injury site. In China, surgeons are injecting 35 µL of stem cells. In his planned trials, Wise Young is intending to inject an escalating dose of 4 µL, 8 µL and 16 µL.

- c) In their ongoing trials, Geron and Neuralstem are using stereotactic systems specifically designed for intraspinal injections. They have the advantage of precision as well as being less invasive. Geron is using a stereotactic frame with a straight needle and injecting 25 μ L.

Intra-arterial injection

Following revascularization surgery such as Carotid endarterectomy or Superficial Temporal artery to Middle Cerebral artery bypass, stem cells could be injected directly intraarterially immediately after the completion of the revascularization procedure. The advantage of this approach is that the stem cells would go directly to the ischemic brain and also that since the artery is already exposed no separate procedure needs to be done for the stem cell injection. The other method of direct 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.

Stereotactic implantation into the brain

Cell transplantation for neurological conditions started with Stereotactic implantation of fetal cells for Parkinson's disease.(4) However after a randomized trial done by Freed et al showed that the clinical outcomes were not significantly different from non transplanted patients this has now been given up.(5) There are many stereo tactic systems available all over the world however the two most popular ones are the Leksell Stereotactic system and the CRW Stereotactic system. The Leksell system involves fixing the frame on the patients head and then getting a MRI done with the frame on. 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. The patient is now shifted to the operating room where a small burr hole is drilled into the skull and then through this the cells to be transplanted and inserted at the desired location using the X,Y and Z coordinates. The entire procedure is done under local anesthesia.

Intramuscular injection In certain disorders, especially

Muscular dystrophy, cells are also transplanted into the muscle. The points at which these have to be injected are termed as the "motor points"(described in detail in chapter 7).At these motor points, the area is cleaned with povidone iodine.The cells diluted in CSF are injected with the 26G needle going into the muscle at an angle(approx. 45 degrees).The piston/plunger of the syringe is slightly withdrawn to verify the the needle is not inside a blood vessel. The cells are then injected, the needle removed and the site immediately sealed with a benzoin seal.

Intravenous injection

Intravenous injectin (IV) is the most widely used route of administration for stem cells. It is safe, minimally invasive and has no ethical issues involved. In spite of these advantages, it is not the most efficient mode of transplantation. Studies have shown that on IV administration, majority of the cells get trapped in organs other than the target organ. They are also more susceptible to the host immune system.

Anaesthesia considerations

Muscular Dystrophy

Pre-operative evaluation: Heart is affected to varying degrees, depending on the stage of the disease and the type of mutation. The myocardium is replaced by connective tissue or fat, which leads to delayed cardiomyopathy. There may also be tachycardia, T-wave anomalies, ventricular arrhythmias etc. This necessitates a good pre-operative cardiac assessment with an ECG and an echocardiogram, with a 24 hr Holter monitoring in the presence of arrhythmias. Pulmonary insufficiency is another cause of concern, due to abdominal muscle weakness, scoliosis, and other factors such as altered chest wall and lung mechanics. Pulmonary function tests are recommended, though always not feasible. An arterial blood gas study gives a fair idea of respiratory reserve.

Intra-operative and anaesthetic considerations: increased sensitivity to anaesthetic agents, with hypersomnolence, increased chances of respiratory problems due to hypotonia, chronic aspiration, and central and peripheral hypoventilation. hypotension due to decreased cardiac reserve, difficulty in lumbar puncture due to scoliosis, delayed gastric emptying due to hypomotility of the GI tract, predisposing to regurgitation and possible aspiration.

Multiple Sclerosis

Cardiac and respiratory systems are generally spared, as this condition primarily attacks the nervous system.

Anaesthesia considerations: corticosteroid supplementation during the peri-operative period is advised. Symptoms of MS are known to exacerbate post-operatively, esp. in the presence of infection and fever. But on the whole, general anaesthesia is relatively safe.

Cerebral Palsy

Pre-operative Evaluation: these children are usually on anti-convulsants and other drugs to reduce spasticity. They are prone to respiratory tract infections, and also have increased salivation.

Anaesthesia Considerations: Increased chances of GE reflux. Increased chances of aspiration, both from the regurgitant contents and pooled salivary secretions. Skeletal and muscle spasticity resulting in contractures and joint deformities, which

can hamper positioning, increased sensitivity to anaesthetic drugs, resulting in slow emergence.

Spinal Cord Injury

Intra-operative and anaesthesia considerations: Impaired alveolar ventilation, especially in cervical cord injury, with impaired ability to cough and clear secretions, cardiovascular instability manifesting as autonomic hyperreflexia, chronic pulmonary and genitourinary infections, altered thermoregulation, decubitus ulcers, osteoporosis and skeletal muscle atrophy due to prolonged immobilization, increased predisposition to deep venous thrombosis, difficulty in positioning, difficulty in lumbar puncture if surgery and instrumentation has been done on the lumbar spine.

*"Whatever you can do or dream you can do, begin it.
Boldness has genius, power and magic in it. Begin it now"*

- Goethe

7

Novel Concepts and Technique of Motor Points for Intra-Muscular Stem Cell Transplantation

There's always something new to learn.

Motor point is the point at which the innervating nerve enters the muscle or, in case of deeply placed muscle, the point where the muscle emerges from the under covers of the more superficial ones. It also represents the area of the skin above the muscle where maximal visible contraction is obtained with the lowest possible intensity of electrical stimulation.

Motor points are usually situated at the junction of the upper & middle one thirds of the fleshy belly of the muscles, although there are exceptions e.g.: The motor point of vastus medialis, whose nerve enters the lower part of the muscle, is situated a short distance above the knee joint. Deeply placed muscles may be stimulated most satisfactorily where they emerge from beneath the more superficial ones, e.g.: extensor hallucis longus in the lower one third of the lower leg. Motor point is the point on the skin where an innervated muscle is most accessible to percutaneous electrical stimulation at the lowest intensity. This point on the skin generally lies over the neuro vascular hilus of the muscle & the muscles band or zone of innervations. Muscle fibres do not always extend the whole length of a muscle & myoneural junctions are not uniformly spread out all over the muscle. They are concentrated in a confined area-the zone or band of innervations where there is greatest concentration of motor endplates & where the other large diameter nerve

fibres may be reached with less concurrent painful stimulation of the smaller diameter cutaneous fibres.

The exact location of motor point varies slightly from patient to patient but the relative position follows a fairly fixed pattern. Some motor points are superficial & are easily found, while others belonging to deep muscles are more difficult to locate.

Concept of motor point stimulation

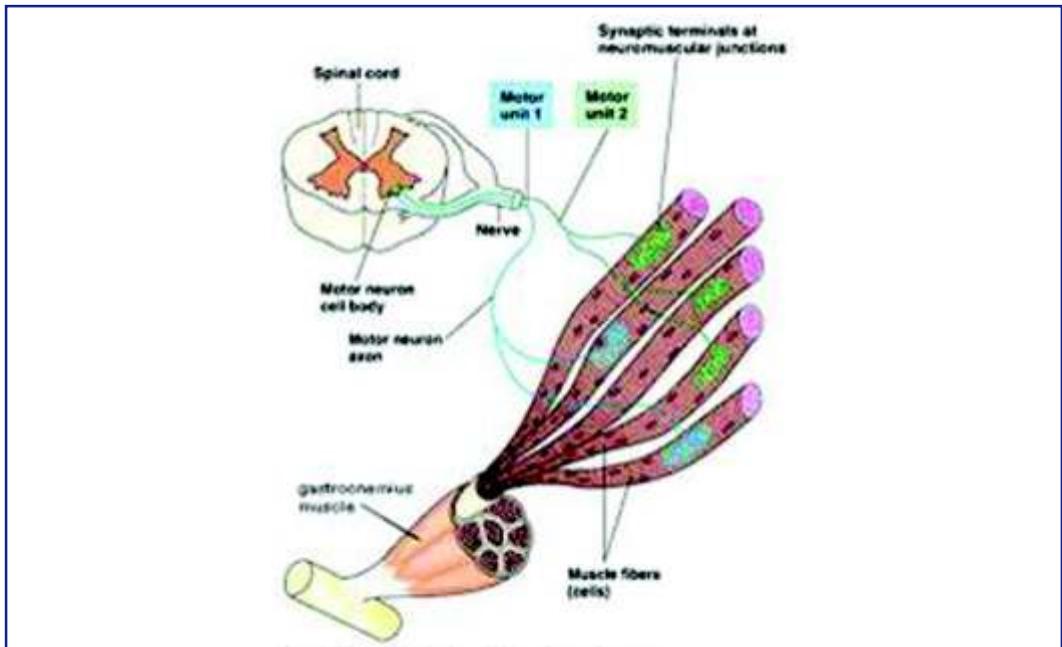


Figure 1: A Neuromuscular Junction

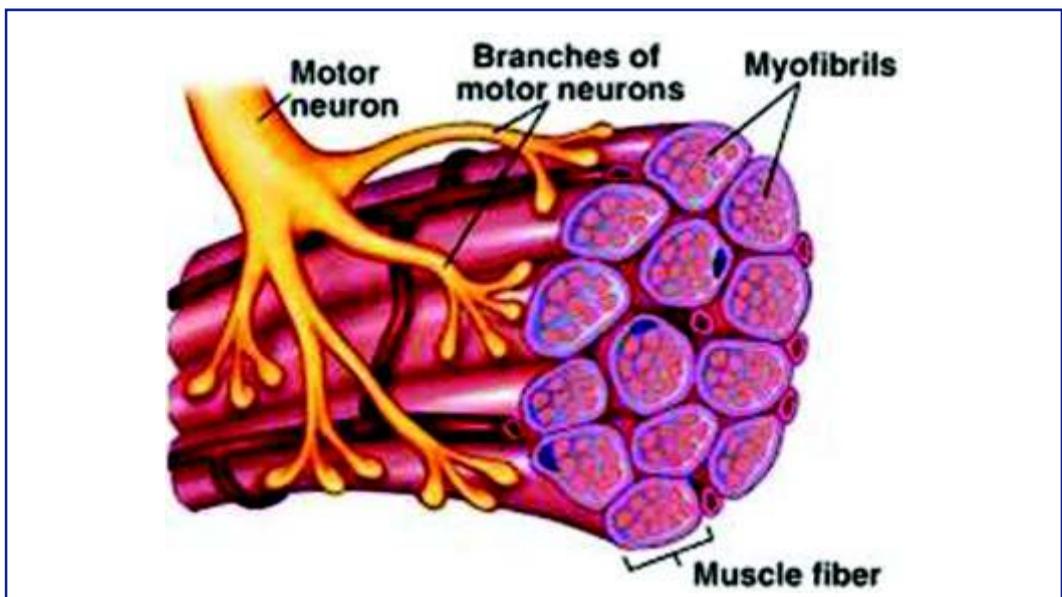


Figure 2 : The Motor Unit

When a nerve is stimulated at a nerve cell or an end organ, there is only one direction in which it can travel along the axon, but if it is initiated at some point on the nerve fibre it is transmitted simultaneously in both directions from the point of stimulation.

When a sensory nerve is stimulated the downward travelling impulse has no effect, but the upward travelling impulse is appreciated when it reaches conscious levels of the brain. The sensory stimulation experienced varies with the duration of the impulse. Impulses of long duration produce an uncomfortable stabbing sensation, while impulses of 1 ms & less produce only a mild prickling sensation.

When a motor nerve is stimulated, the upward -travelling impulse is unable to pass the first synapse, as it is travelling in the wrong direction, but the downward travelling impulse passes to the muscles supplied by the nerve, causing them to contract.

When a stimulus is applied to a motor nerve trunk, impulses pass to all the muscles that the nerve supplies below the point at which it is stimulated, causing them to contract.

When a current is applied directly over an innervated muscle, the nerve fibres in the muscle are stimulated in the same way. The maximum response is thus obtained from stimulation at the motor point.

Preparation of the patient

The area to be plotted is exposed & the patient is supported comfortably in good light. The skin has high electrical resistance as the superficial layers being dry, contain few ions. The resistance is reduced by washing with soap & water to remove the natural oils & moistening with saline immediately before the electrodes are applied. Breaks in the skin cause a marked reduction in resistance which naturally results in concentration of the current & consequent discomfort to the patient. To avoid this broken skin is protected by a petroleum jelly covered with a small piece of non absorbent cotton wool to protect the pad. The indifferent electrode should be large to reduce the current density under it to a minimum. This prevents excessive skin stimulation & also reduces the likelihood of unwanted muscle contractions, as it may not be possible to avoid covering the motor points of some muscles.

Preparation of apparatus

Faradic type of current

A low frequency electronic stimulator with automatic surge is commonly used. A faradic current is a short -duration interrupted direct current with a pulse duration of 0.1 - 1 ms & a frequency of 50 - 100 Hz. Strength of contraction depends on the number of motor units activated which in turn depends on the intensity of the

current applied & the rate of change of current. To delay fatigue of muscle due to repeated contractions, current is commonly surged to allow for muscle relaxation.

Stimulation of Motor points

This method has the advantage that each muscle performs its own individual action & that the optimum contraction of each can be obtained, by stimulating the motor



Figure 3 : Electrical stimulator used for stimulation and plotting of motor points.



Figure 4 : Preparation of the patient for motor point plotting



Figure 5 : Plotting of motor point (sternomastoid muscle)



Figure 6 : Marking of sternomastoid muscle motor point.



Figure 7 : Plotted motor points of tibialis anterior and peronei muscle



Figure 8 : Injection of stem cells in tibialis anterior muscle motor point.

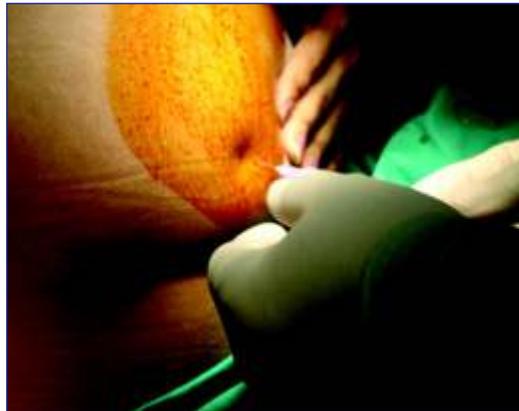


Figure 9 : Injection of stem cells in the glutei muscle motor point.



Figure 10 : Injection of stem cell injection in the adductor pollicis muscle motor point.



Figure 11 : Injection of stem cells in the lumbrical muscle motor points

point. The indifferent electrode is applied & secured in a suitable area. The stimulating electrode is placed over the motor point of the muscle to be stimulated. Firm contact ensures a minimum of discomfort. The operator's hand may be kept in contact with the patient's skin so that she / he can feel the contractions produced.

Selection of the Individual muscles for Stem cell transplant

The physiotherapist selects the weak muscles for stem cell injection on the basis of manual muscle testing & patient's complain of weakness & difficulty in ADL. Post stem cell injection these muscles need specific training & individual muscle strengthening program so that the patient can gain efficiency & independency in ADL. Apart from injecting stem cells intrathecally, injecting them in the motor points of the muscles facilitates further specific implantation of the stem cells in isolated individual muscles.

A) Major muscles of UL that are generally considered:

- a) Deltoid: Anterior, middle & posterior fibres.
- b) Biceps brachii.
- c) Triceps: long, lateral & medial heads.
- d) Thenar muscles: Opponens pollicis, abductor pollicis brevis & flexor pollicis brevis.
- e) Hypothenar muscles: abductor, flexor & opponens digiti minimi.

B) Major muscles of LL that are generally considered:

- a) Quadriceps: vastus medialis, vastus lateralis, rectus femoris.
- b) Hamstrings: Biceps femoris, semimembranosus & semitendinosus.
- c) Glutei.
- d) Dorsiflexors: Tibialis anterior, Peronei longus & brevis, EHL.

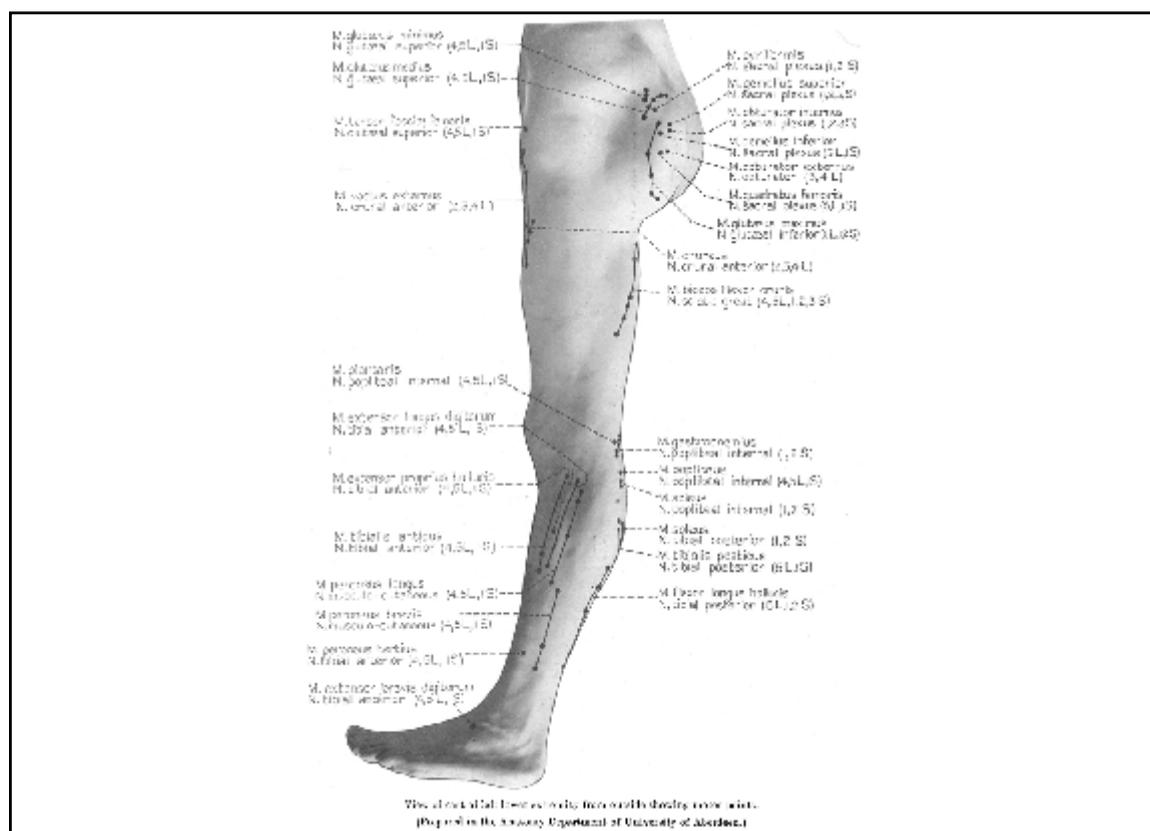
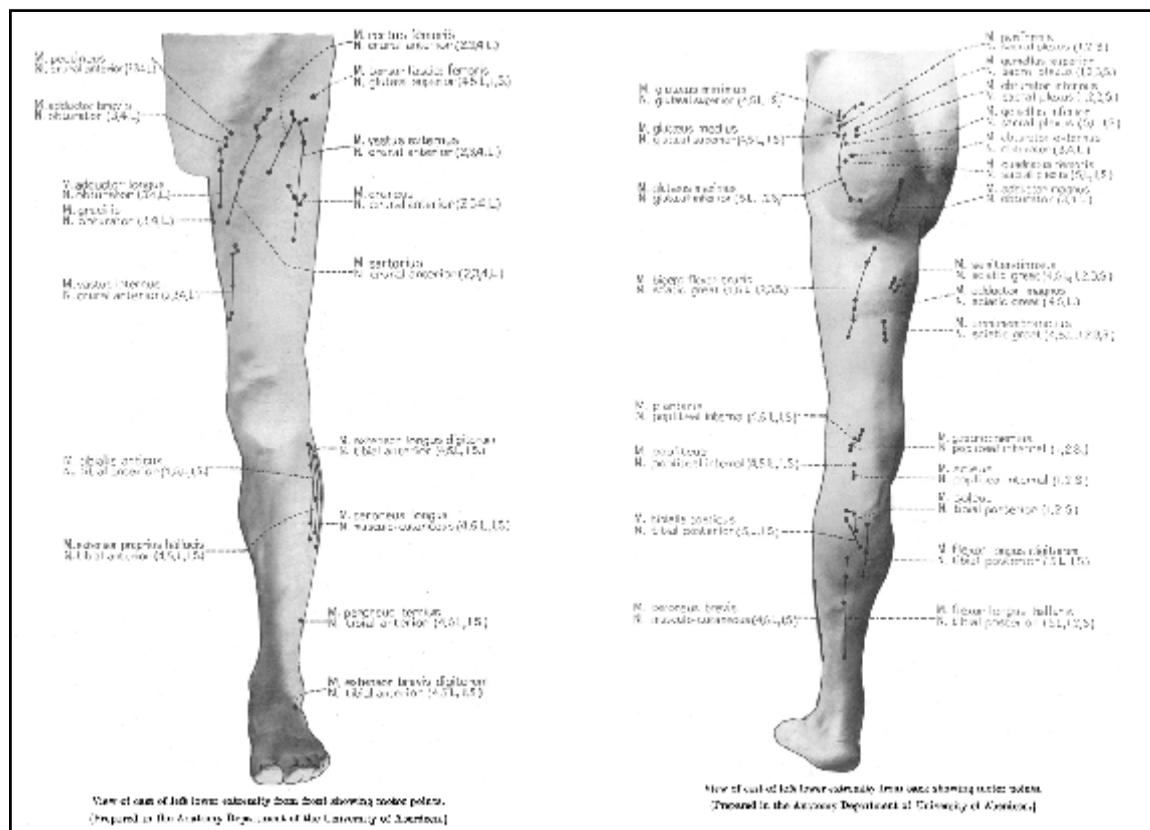
C) In trunk:

Abdomen & back extensors are considered, & in neck muscles sternocleidomastoid.

D) Facial Muscles:

In case of facial muscle weakness in conditions like Motor Neuron Disease & a few muscular dystrophies, facial muscles motor points are also selected for intramuscular injections e.g.: orbicularis oris, orbicularis oculi, Buccinator, rhizorius, frontalis, mentalis, etc.

Intramuscular stem cells injection in motor points within the muscle is very specific



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SECTION B

Clinical Application of Stem Cells

*“Things don't change.
You change your way of looking at them”*

- Carlos Castaneda

8

Role of Stem Cells in Autism Spectrum Disorders

Stem cells have the potential to restore the underlying abnormal neural network of brain in Autism Spectrum Disorders.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which begins in early childhood and is characterized by persistent deficits in social interaction and communication, along with presence of restricted, repetitive patterns of behavior, interests, or activities. (1)

According to DSM V, ASD is a spectrum of disorders which includes Autism, Asperger syndrome and pervasive developmental disorder – not otherwise specified (PDD-NOS). (2) Individuals diagnosed with ASD have a range of symptoms which are usually showcased in the early developmental period and last throughout the lifetime. Severity of the disorder depends on the symptoms and levels of impairment or disability.

The prevalence of ASD has increased radically over few decades for reasons not yet known. It is seen three to four times more in boys than girls. Autism, similar to other neurodevelopmental disorders, is incurable and requires lifelong management. It impacts the health, economic wellbeing, social integration and quality of life of individuals with the disorder, and also on their families and potentially the rest of the society. Hence, a great deal of research is being conducted all over the world to understand the etiology of autism and subsequently find a cure for it.

Newer treatments like stem cell therapy have shown immense potential as a

treatment strategy for ASD. This chapter mainly focuses on detailed understanding of how stem cell therapy works in ASD. It has been supported with adequate scientific data in the form of worldwide review of clinical studies and their results.

Pathophysiology of Autism

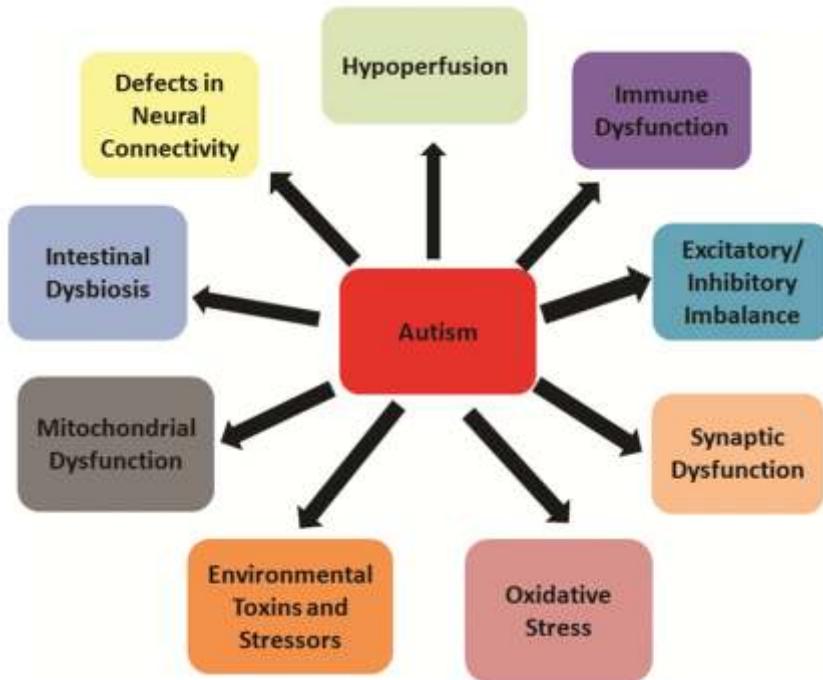


Figure 1: Pathophysiology of ASD

The etiology of ASD is complex and a more definitive understanding of the pathophysiology is required to develop a cure for ASD. It is likely to be multifactorial encompassing both genetic predisposition and environmental factors (3) Research has shown structural and functional abnormalities in the brain of ASD patients. To understand the brain pathology of autism, we conducted a study using PET CT scan brain which demonstrated an atypical neurodevelopmental trajectory of brain maturation in autism. We found that unlike the healthy controls, as the age increases, there is a linear decrease in brain metabolism of autism children. Hypometabolism was observed in amygdala, hippocampus, parahippocampal gyrus, cerebellum, mesial temporal lobe, thalamus, superior and middle temporal pole, and hypermetabolism in calcarine fissure and Heschl's gyrus in autism. The trajectory of the brain metabolism in autism varies across different parts of the brain with the frontal and temporal being affected more as compared to the parietal and occipital cortices. These abnormalities explain the developmental, cognitive, and behavioral deficits observed in these children. (4) Autism has also been strongly associated with underconnectivity of long pathways and increased connectivity in short pathways. This causes an imbalance in the connectivity of the brain of autism. (5)

Unmet medical needs

It is difficult to identify autism-specific biomarkers as ASD is considered to be the final common pathway of multiple etiological and neuropathological mechanisms. (6) Hence, the diagnosis relies on the recognition of an array of behavioral symptoms that vary from case to case and overlap with other childhood neuropsychiatric disorders.

Also, due to heterogenous nature, developing a standard cure for ASD is difficult. Currently, the treatment options available for autism are rehabilitation, behavioral, nutritional and medical intervention. These do not address the core pathophysiology of autism but only manage the symptoms and associated medical conditions. The medicines alleviate the symptoms temporarily but, do not cure them permanently. Hence, establishing a strategy to target the underlying abnormal neuronal connectivity which will have a longlasting effect is the need of the hour.

Stem cell therapy in autism

As autism is a complex neurodevelopmental disorder, different studies have tried understanding its basic pathophysiology. It is assumed that neural hypoperfusion and immune dysregulation are the two core underlying pathologies associated with autism. (7) In the past decade stem cell therapy has emerged as one of the most potential treatment strategies ASD. (7-9) It has the therapeutic potential to repair the damaged neural tissue at molecular, structural and functional level. Hypoperfusion results in hypoxia. Reversal of hypoxia may lead to self-repair and neural proliferation. The angiogenic potential of stem cells facilitates reperfusion and restores the lost connections. (10) It also regulates the immune system, balances inflammation further exhibiting beneficial clinical effects in patients with ASD. These cells also secrete several biomolecules with anti-inflammatory properties through paracrine effect. This tries to maintain equilibrium in the immune system alterations and activate endogenous repair mechanisms in autism. (11) Thus, stem cells are capable of suppressing the pathological immune responses as well as stimulating neovascularisation. Cell therapy may also prove useful for the treatment of T cell defect associated with autism. (12) Cells may also play a role in stimulating the restoration and/or generation of functional synaptic pathways. (13) Additional neuroprotection may be offered via molecular mechanisms by inhibiting toxic processes such as neural apoptosis, microglial activation, astrocyte proliferation, and production of oxidative stress molecules.(14) Overall, stem cells carry out functional restoration of specialized neural systems by neuroprotection, neural circuit reconstruction, neural plasticity, neurogenesis and immunomodulation.

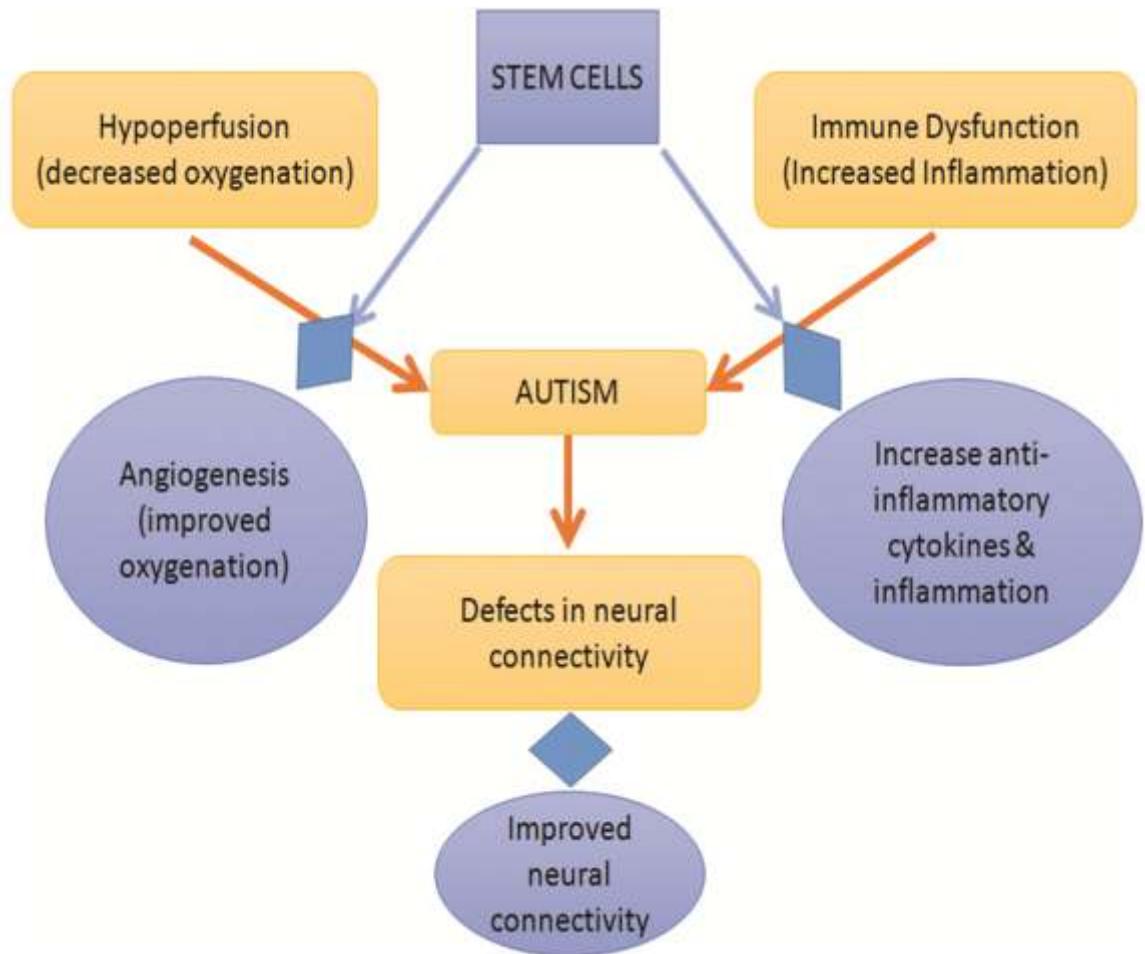


Figure 2: Stem Cell Therapy in Autism

Pre-clinical Studies

Some neurodevelopmental disorders like autism are strictly limited to humans. Autism is usually diagnosed only by the behavioral parameters. Hence, it is difficult to replicate autism in animal models with all the clinical characteristics. This also makes it challenging to study the effect of any intervention on these animal models. BTBR inbred mouse strain is a commonly used animal model for autism as it demonstrates robust behavioral deficits. In a recent study conducted by H Segal.Gavish et al, transplantation of MSCs in BTBR mice resulted in a reduction of stereotypical behaviors, a decrease in cognitive rigidity and an improvement in social behavior. Tissue analysis revealed elevated BDNF protein levels in the hippocampus accompanied by increased hippocampal neurogenesis in the MSC-transplanted mice compared with sham treated mice. (15)

Author	Type of cells used	Route of administration	Sample size	How many patients improved	Demonstrated safety
Sharma et al (16)	autologous bone marrow mononuclear cells	Intrathecal	32	29	yes
Yong-Tao Lv, et al (17)	human cord blood mononuclear cells (CBMNCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs)	Intravenous and intrathecal	37	18	yes
Bradstreet JJ, et al (18)	fetal stem cells	subcutaneous	45	35	yes
Dawson G et al (19)	Autologous Cord Blood cells	intravenous	25	13	yes
Shroff G (20)	Embryonic Stem cells	Multiple routes	3	3	yes

17 studies have been published including 5 clinical studies and 12 case reports demonstrating the effect of stem cell therapy in ASD. Different types of cells were used namely autologous bone marrow mononuclear cells, fetal stem cells and umbilical cord blood cells.

Sharma et al (16) published the first clinical study which was an open label proof of concept study in 32 patients of autism. They administered autologous bone marrow mononuclear cells intrathecally. The results of their trial demonstrated the safety and efficacy of stem cell therapy for autism. The results of this study have been described in detail below. The next clinical study was published by Yong-Tao Lv et al where they studied use of human cord blood MNCs and MSCs in 37 patients. (17) They used CARS, CGI and ABC scales to assess the therapeutic efficacy in this study. A positive outcome was obtained in the treatment group. In 2014, Bradstreet et al published their study using fetal stem cells. (18) 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. In 2017, Dawson et al published their Phase 1 safety and tolerability study wherein they administered a single intravenous infusion of autologous cord blood cells in 25 children with ASD. (19) The infusions were safe,

with no serious adverse events and occasional allergic reactions and irritability reported. Improvements in ASD symptoms were observed on caregiver-completed measures (Vineland Adaptive Behavior Scales Second Edition (VABS-II, see figure 1) and Pervasive Developmental Disorder Behavior Inventory (PDDBI)), clinician assessment (CGI-I, figure 1), and computerized eye tracking assessments. Positive changes, including increased social communication skills and receptive/expressive language and decreased repetitive behavior and sensory sensitivities were observed six months post infusion and maintained at 12 months. A Phase 2 randomized study is underway to evaluate the efficacy of autologous or allogeneic CB therapy versus placebo in children with ASD. In 2017, a case series from India was published by Shroff G wherein 3 cases of ASD were administered with embryonic stem cells. (20) The patients showed improvements in eye coordination, writing, balancing, cognition, and speech and showed reduced hypersensitivity to noises and smells. These patients also showed improvement in SPECT scans.

Our Published data

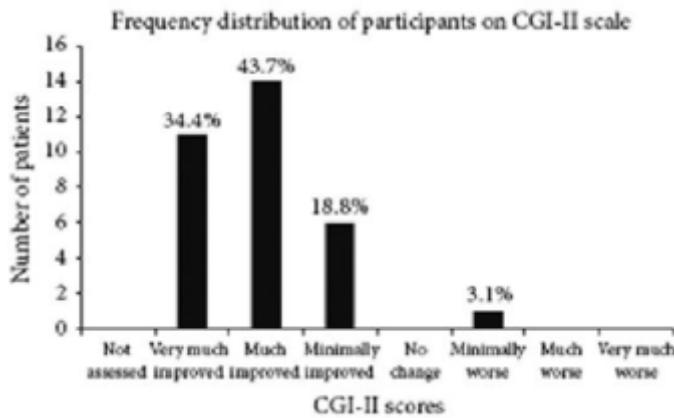


Figure 3: Frequency distribution of participants on CGI-II scale

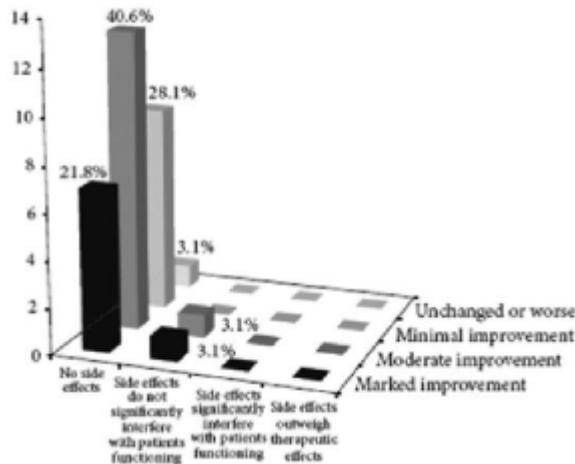


Figure 4: Frequency distribution of participants on CGI-III scale

An open label proof of concept study (Sharma et al) of autologous bone marrow mononuclear cells (BMMNCs) intrathecal transplantation in 32 patients with autism followed by multidisciplinary therapies was performed. All patients were followed up for 26 months (Mean 12.7) Outcome measures used were ISAA, CGI and FIM/ Wee-FIM scales. Positron Emission Tomography computed Tomography (PET-CT) scan recorded objective changes. 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. In the domain of Social relationships and reciprocity 29 out of 32 (90.6%) patients showed improvement. Improved emotional responsiveness was observed in 18 out of 32 (56%) patients. Under the Speech-language and communication domain there was an improvement observed in 25 patients out of 32 (78%). Behavior patterns of 21 out of 32 patients (66%) improved. Hyperactivity or restlessness (71%) and engaging in stereotype and repetitive motor mannerisms (65%) decreased significantly. Sensory aspects improved in 14 out of 32 patients (44%). Cognitively they showed improved consistency in attention and concentration and response time. 71% patients showed better attention and concentration, 45% patients showed reduction in the delay in responding. The difference between pre and post scores was statistically significant ($p < 0.001$) on Wilcoxon Matched-Pairs Signed Rank Test. On CGI-II 96% of patients showed global improvement. The efficacy was measured on CGI-III efficacy index. Functional neuroimaging in the form of PET - CT scan in eight patients, documented changes in brain metabolism which correlated with clinical improvements. Few adverse events including seizures in three patients were controlled with medications. The encouraging results of this leading clinical study provide future directions for application of cellular therapy in autism.

Scale	Median score before cellular therapy	Median score after the cellular therapy	Test statistics	Statistical significance
CGI-I	4.5	3	$Z = -3.509$	$P < 0.001^*$
ISAA scale	115.5	97	$Z = -4.670$	$P < 0.001^*$

*Statistically significant (level of significance at $P < 0.05$).

Table 1: Change in the scores of CGI and ISAA before and after intervention.

ISAA scale domain	Median score before cellular transplantation	Median score after cellular transplantation	Test statistics of Wilcoxon signed rank test for matched pairs	Statistical significance
Social relationship and reciprocity	35.5	13	-4.118	$P < 0.001^*$
Emotional responsiveness	23	20	-3.153	$P = 0.002^*$
Speech, language, and communication	13	11	-3.989	$P < 0.001^*$
Behavior patterns	29	10	-3.126	$P = 0.002^*$
Sensory aspects	21	17	-2.409	$P = 0.016^*$
Cognitive component	11	8	-3.508	$P < 0.001^*$

*Statistically significant (level of significance at $P < 0.05$).

Table 2: Change in the ISAA scores of individual domains measured before and after intervention.

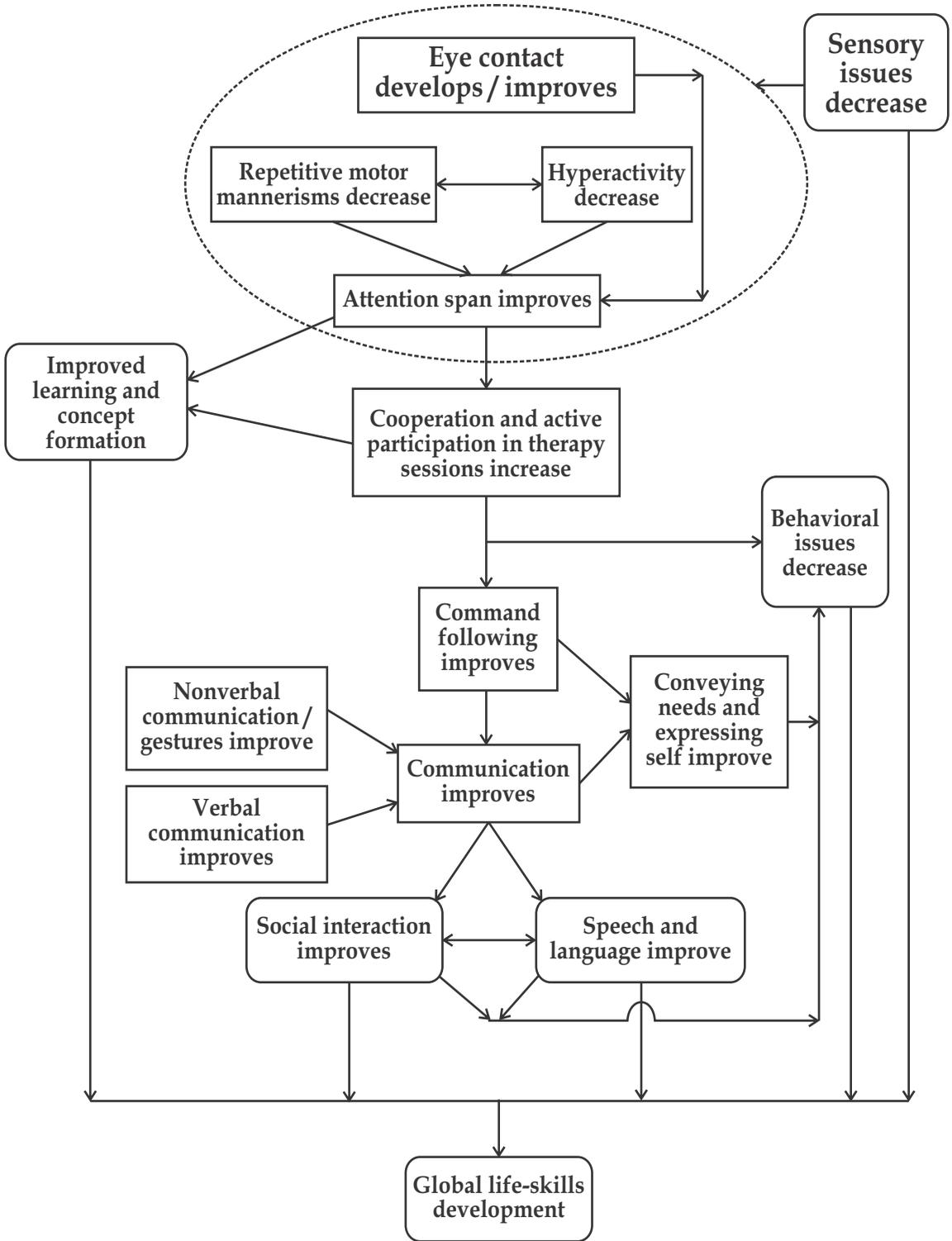


Figure 5: Schematic representation of clinical improvements after cellular therapy. This figure shows proposed theoretical outline of observed changes after cellular therapy.

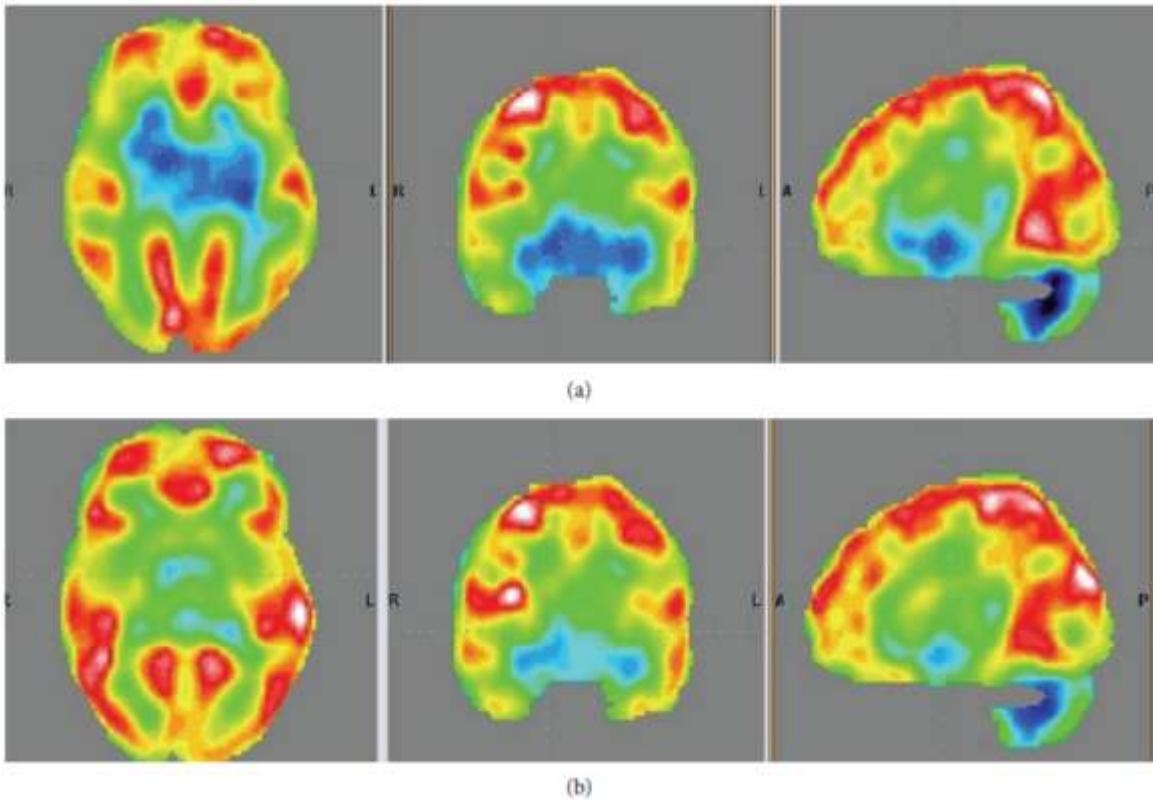


Figure 6: Findings in PET-CT scan before and after cellular therapy. (a) PET-CT scan before intervention showing reduced FDG uptake in the areas of frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus, and mesial temporal lobe. (b) PET-CT scan six months after intervention comparison shows increased FDG uptake in the areas of frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus, and mesial temporal lobe.

We have also published 12 case reports in various national and international peer reviewed journals demonstrating the effect of stem cell therapy in autism cases. (21-32) All these cases were different with respect to age, severity of autism and symptoms presented. One of the patient also had comorbid ID. These cases underwent autologous bone marrow mononuclear cell transplantation along with personalized neurorehabilitation program which included applied behavior analysis, psychological intervention, speech therapy, occupational therapy, art based therapy, etc. These patients were followed up regularly to record changes in clinical symptoms and outcome measures such as CARS, CGI, ISAA and FIM were used to quantify the changes. On follow up, improvements were observed in common symptoms such as speech, awareness, logical thinking, attention and concentration, eye contact, social interaction, emotional responses, command following, learning abilities, response time, sitting tolerance and ADLs. Hyperactivity, aggressive behaviour, Stereotypical and self-stimulatory behaviour were also reduced. They all showed positive changes on outcome measures. The

scores of the patient categorized as severe autism on CARS improved to no autism. Which indicates the patient required assistance but was out of the spectrum after the intervention. There was improvement in IQ as well of the patient with comorbid ID.

In 9 cases out of 12, PET CT scan was used as a monitoring tool to study the metabolic changes occurring after stem cell therapy. These scans showed improved brain metabolism in the areas affected before stem cell therapy. There was a balancing effect observed in the metabolism. Areas which were hypermetabolic or hypometabolic before treatment turned normal after treatment.

Unpublished

We have treated 193 cases of autism were treated with autologous BMMNCs intrathecal administration. The data of these cases was analyzed. Symptoms such as social interaction, eye contact, hyperactivity, aggressive behaviour, self-stimulatory behaviour, speech, attention, stereotypical behaviour and communication were analysed. On follow up we found that, 89.12% of patients with autism showed improvements while 10.88% did not show any change after intervention. 25.90% showed mild improvements, 29.53% showed moderate improvements and 33.67% patients showed significant improvements. No major adverse events were recorded. Children showed improvements on objective scales like CGI – II and III, ISAA and CARS. PET-CT scans also revealed improvements which correlated well with the clinical improvements.

Improvements in Autism After Stem Cell Therapy (N=193)

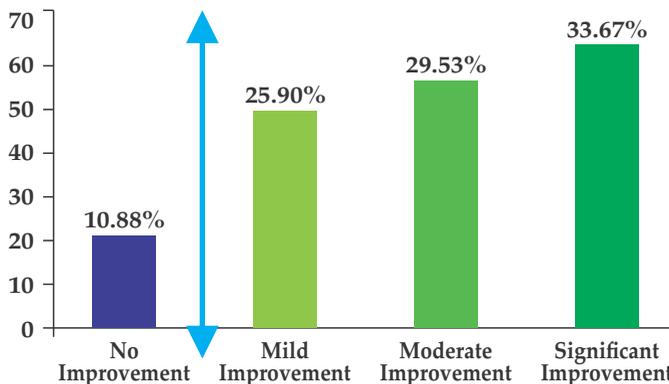


Figure 7: Graph representing improvements in Autism after Stem cell Therapy

PET-CT as a monitoring tool for effects of stem cell therapy in Autism

PET-CT is an imaging technique which utilizes 18-FDG, a dye that is analogous to glucose. This dye is entrapped in the brain cells, which can then be measured on the CT scan giving a diagrammatic representation of the function of cells. Uptake of the FDG is given as standard uptake value (SUV). The SUV of the patient undergoing

PET-CT evaluation is then compared with the SUV of control population and standard deviation (SD) is computed. If the SUV value of the patient is beyond 2SDs then it is considered as abnormal brain metabolism. Function of the brain cells is directly proportional to the glucose uptake and metabolism. Thus, hypofunctioning areas will depict reduced FDG uptake and hypometabolism (SD values of -2 and below, represented by shades of blue and black); while hyperfunctioning areas will depict increased FDG uptake and hypermetabolism (SD values of +2 and above, represented by shades of yellow and red). An increased FDG uptake in hypofunctioning areas or decreased FDG uptake in hyperfunctioning areas may be implicated as improvement in brain function depending upon the correlation with clinical improvement.

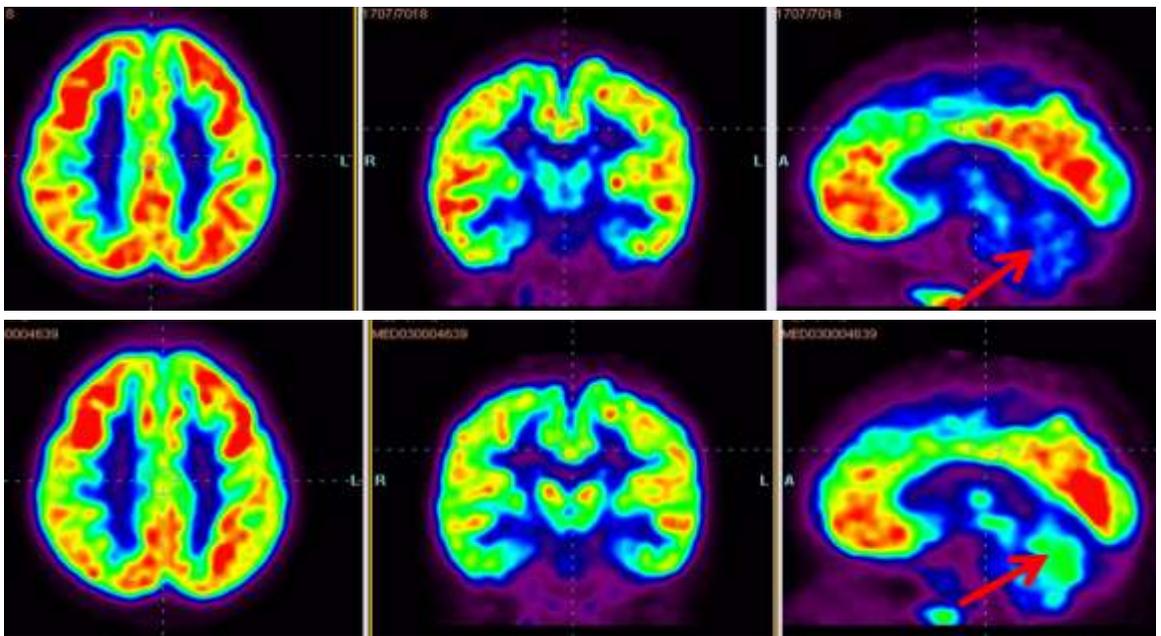


Figure 8: Comparative PET CT scans of an autism patient performed before and 6 months after stem cell therapy. Significant improvement was observed in bilateral medial temporal cortex, thalamus and cerebellum

Case Report

A 12 years old male child was diagnosed with Autism Spectrum Disorder based on his clinical presentation. On assessment we observed that his attention and concentration was affected as he got easily distracted easily. His imitation skills were poor and inconsistent, he followed basic commands but he required 2 to 3 repetitions. His problem solving and awareness was affected. There was presence of laughing and crying without any reason. His sitting tolerance was poor i.e. less than 5 seconds according to the parents, and his social interaction and social norms were also affected. There was presence of motor mannerisms like hand flapping and rocking behavior. He conveyed most of his basic needs non-verbally. His

speech was affected and he could make only sounds. He was dependent on his caretakers for day to day activities. On FIM, he scored 62. On ISAA he scores 123 which indicated moderate autism and on CARS he scored 46.5 which indicated severe autism. This patient underwent 4 transplantations in a period of 3 years.

Improvements after 1st Transplantation. Follow up period-9 months

- He could establish eye contact and maintain it for a long time.
- There was reduced vestibular, proprioceptive seeking. Oral seeking had also reduced (reduced rocking).
- Could follow 1 step commands better now as compared to before.
- Hand flapping and spitting has reduced.
- His sitting tolerance had improved. He could now sit at one place for doing assignments like colouring.
- Could perform activities like candle blowing, balloon blowing.
- There was improvement in day to day activities such as brushing – he does it in a better way but still requires assistance for cleaning. Dressing speed had improved. He could eat with spoon which was difficult 6 months ago.
- He could make his bed with assistance.
- FIM improved from 62 to 87
- ISAA improved from 123-108

Improvements after 2nd Transplantation. Follow up period-12 months

- Attention and concentration had improved
- Command following had further improved
- He could copy simple actions e.g. clapping hands
- Speech had improved
- He is aware of danger
- Screaming and hitting had reduced.
- Hyperactivity has reduced by 50%
- Spitting behavior had completely stopped
- He now woke up less frequently in the night
- He could now match colors and identify fruits like banana
- He could brush teeth independently with very little help.
- FIM further improved from 87 to 95
- ISAA remained same (108)

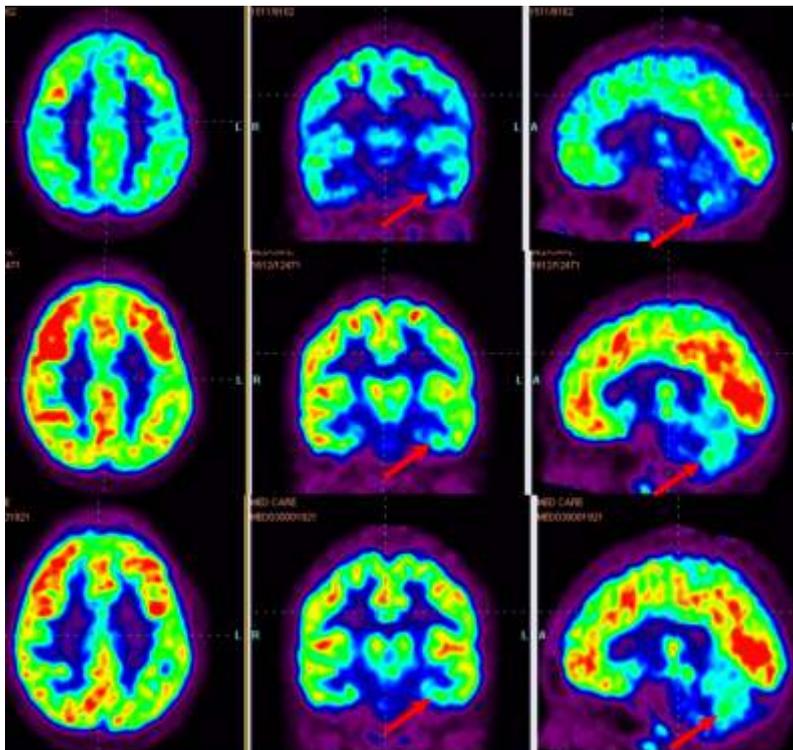
Improvements after 3rd Transplantation. Follow up period-12 months

- Eye contact had further improved. He could focus on one object for more than 2 minutes
- Attention span also improved further, as he could sit for more than 2 hours for performing tasks

- He had now started participating in social activities like playing football with friends.
- He had developed awareness with respect to his body
- He could understand complex commands now
- He started understanding use of different objects
- He could now speak 2-3 words
- His understanding was better as he now opened the door for his dad when he went for work
- Aggressive spells had further reduced
- At school he performed activities of making necklaces, 2 necklaces at a stretch (1.5 hrs)
- He was able to perform ADL like brushing, bathing, dressing, bed making tasks faster as compared to before.
- FIM remained same (95)
- ISAA improved from 108-104

On comparing the PET CT scans performed before and after stem cell therapy there was significant improvement observed in brain metabolism.

- In 2015, the first PET scan performed before stem cell therapy revealed reduced metabolism in bilateral thalamus, cerebellum, and medial temporal cortex
- In 2016, PET scan performed 9 months after stem cell therapy showed improved metabolism in thalamus, medial temporal cortex and cerebellum
- In 2018, the PET scan showed further improved metabolism in medial temporal cortex and left cerebellum.



Over three years, this patient had overall improved in behavior, cognition, communication and attention and concentration. He was more independent than before and also his quality of life had considerably improved. All these improvements correlated with improved brain metabolism observed on PET CT scan.

Adverse Events:

Stem cell therapy for ASD is a safe procedure. However, adverse event monitoring is important to achieve optimal outcome of the intervention. Adverse events are categorized into minor and major adverse events. Minor adverse events include procedure related events such as spinal headache, nausea, diarrhea, vomiting, pain or bleeding at the site of aspiration /injection, fever amongst others. These are treated using medications. Anesthetic complications and allergic reactions may also occur depending on the procedure. Major adverse events include episodes of seizures occurring after intervention. These can be managed prophylactically. Pre-existing epileptogenic focus in EEG also predicts the occurrence of seizures. Evidence suggests that antiepileptic prophylactic regimen decreases the incidence of seizures along with limiting the onset of new ones. (33)

Conclusion

Based on published literature and our clinical experience we consider that Stem cell therapy is a safe and effective treatment option for ASD. However, it is not a cure, but it can be used in combination with current rehabilitation and medical intervention to augment their clinical outcome. Stem cell therapy can repair the underlying abnormal neuronal connectivity, improve information processing and hence, give a long lasting symptomatic relief. It helps in improving the quality of life of the patients and making them functionally independent which may provide them mainstream opportunities. To optimize the benefits of stem cell therapy, it is important to explore the most beneficial types of cells, route of administration, quantity of cells to be injected, frequency of injections, etc. Neuroimaging techniques like PET - CT scan and functional MRI (fMRI) scan give more lucid information about neural connectivity in the brain of autism and hence need to be studied in detail and standardized.(34) It is also observed that the earlier the children undergo stem cell therapy, the better is the outcome. As, younger the age greater is the brain plasticity, therefore, these children respond better to the intervention.

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Security is mostly a superstition. It does not exist in nature nor do children of men as a whole experience it. Avoiding danger is no safer in the long run than outright exposure. Life is either a daring adventure or nothing"

- Hellen Keller

9

Stem Cell Transplantation for Cerebral Palsy

Neurodevelopment in Cerebral Palsy can be accelerated by trophic, paracrine and neuroregenerative properties of stem cells.

Cerebral Palsy is defined as “a disorder of movement and posture due to a defect or lesion of the immature brain.” (1) It is known to affect 2/1000 live-born children. CP may result from a variety of prenatal, perinatal or post natal factors. Evidence suggests that the cause of CP in 70-80% of cases is prenatal. (2) The symptoms of CP may 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.

The conventional treatments available currently for CP are physical and behavioral therapy, Hyperbaric oxygen therapy (HBOT), (3-5) Botulinum A toxin injection, (6) surgical treatments, assistive devices, and medical management of associated conditions play a supportive role. As a variety of symptoms in CP need to be addressed, a comprehensive multidisciplinary approach needs to be established.

Unmet medical needs

The incidence of CP is increasing at an alarming rate throughout the world. Recent

medical advances have improved medical care, but still many challenges remain in the management of these disorders. CP remains an incurable disorder. 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 underlying damaged brain. There are no definitive treatment options to accelerate the development of cerebral palsy patients.

Role of stem cell therapy in Cerebral palsy

Hypoxic ischemia is the most common risk factor of CP prenatally and during delivery. Periventricular leukomalacia (PVL), a diffused damage of cerebral white matter, could be one of the major underlying neuropathologies of CP. (7) With PVL, the area of damaged brain tissue can affect the nerve cells that control motor movements. Along with astrogliosis and microglial infiltration, there is loss of pre-myelinating oligodendrocytes (pre-OLs). (8) A discrepancy in neuronal functions is triggered, as the loss of pre-OLs lead to disruption in the production of mature OLs which further leads to disturbance in myelination. (9,10) In CP, microglial activation also instigates the secretion of tumor necrosis factor alpha (TNF- α), interferon gamma (INF- γ), Interleukin -1 beta (IL-1 β), superoxide radicals, nitrogen species, glutamates, adenosine which

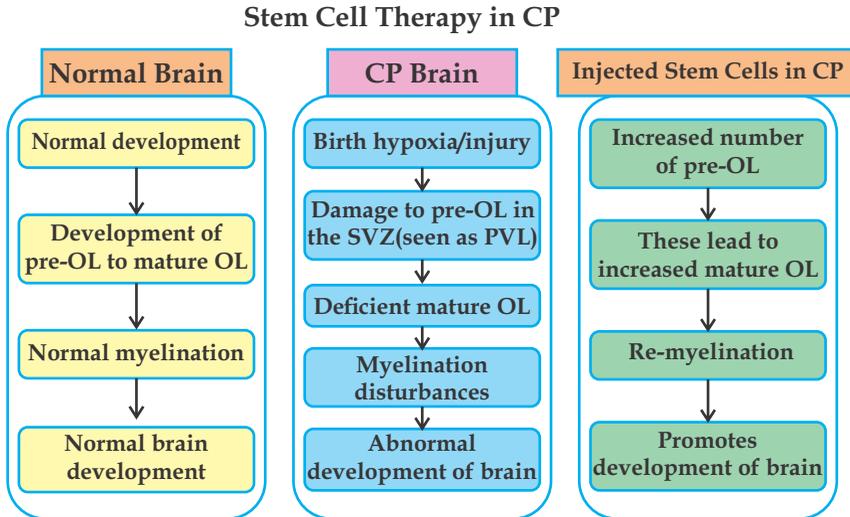


Figure 1: Stem cell therapy in cerebral palsy

exerts a toxic effect on neurons and oligodendrocytes.(11) Stem cell therapy regulates these cellular mechanisms by migrating and homing to the damaged areas and initiating the repair process. They reduce the levels TNF- α ,IL-1 β , IL-6 and increase levels of IL-10 and exert an anti-inflammatory effect (12); therefore, enhancing the endogenous brain repair. Stem cells also restore the damaged myelin by replacing lost OLs and pre-OLs.

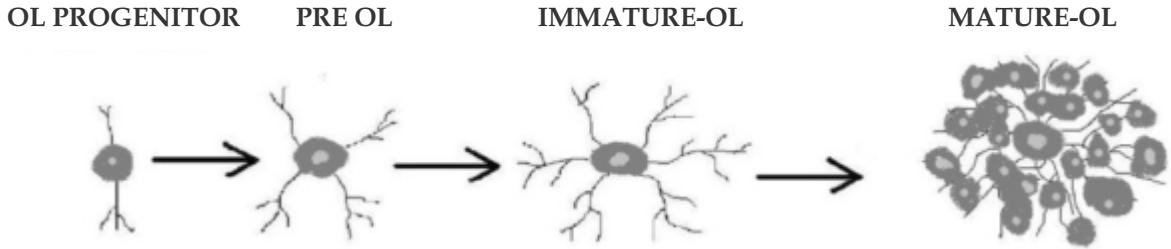


Fig. 2 Phases of Oligodendrocyte development

Animal studies

Various preclinical studies have demonstrated the potential of stem cell therapy in cerebral palsy. Administration of these cells in animal models have led to survival, homing and differentiation into neurons, oligodendrocytes, astrocytes, etc. (13-15) Woodbury et al. demonstrated the differentiation of bone marrow cells into neurons in adult rats. (16) Similarly, studies have shown that umbilical cord blood stem cells proliferate into neural cells via Sonic hedgehog (Shh) signaling pathway and also improve sensorimotor deficits along with other neurological functions. (17-23) Park et al and Titomanlio et al reported differentiation of neural stem cells (NSCs) into neurons and oligodendrocytes which triggered reduction in lesion size along with improvement in memory performance. (24,25) Other cells such as multipotent progenitor cells (MPCs) and oligodendrocyte precursor cells were also found to be efficacious in rat models. (26)

Human studies

30 studies which include 17 clinical studies and 13 case reports have been published to demonstrate the effects of various types of stem cells in cerebral palsy. These studies used different cells such bone marrow derived stem cells, umbilical cord blood cells, Olfactory Ensheathing cells, embryonic cells and neural cells.

Many studies used bone marrow derived stem cells such as bone marrow mononuclear cells, mesenchymal cells, enriched bone marrow progenitor cells and total nucleated cells. (27-36) These cells were administered either intravenously or intrathecally All the authors reported these cells to be safe and efficacious. Neurological and functional improvements were observed in all these studies. Umbilical cord blood cells have also shown a positive outcome after intravenous and intrathecal route of transplantation. (37-49) No major adverse events were recorded in any study. Shroff et al, administered human embryonic stem cells intravenously in 91 patients. 63 patients showed improvement however, seizures were recorded as a side effect of the intervention. (50) Seledtsov et al carried out a controlled study injecting a cell suspension from immature nervous and haematopoietic tissues. Their findings suggested that cell therapy was an effective, safe and immunologically justified method of therapy for patients with cerebral palsy. (51) Chen et al published the results of a randomized controlled study of

intracranial administration of olfactory ensheathing cells. (52) They reported these cells to be safe and efficacious. Similarly, Luan et al and Chen et al, administered neural stem cells and neural stem cell like cells intracranially and intrathecally in a large number of cases in China and reported it to be an effective treatment for CP. (53-55) Whereas, Rah et al, demonstrated the neuroregenerative potential of intravenous G-CSF and autologous peripheral blood stem cells in CP, through a randomized, double-blind, cross-over study. (56)

Our results

Published data

A study on 40 cases was carried out to evaluate the benefits of stem cell therapy in cerebral palsy. (27) These cases were administered autologous bone marrow mononuclear cells intrathecally. On follow up after 6 months, overall 95% patients showed improvements. The total population was further divided into diplegic, quadriplegic and miscellaneous group of cerebral palsy. On follow up patients showed improvements in various symptoms. (Figure3-5) On statistical analysis, a significant association was established between the symptomatic improvements and stem cell therapy in diplegic and quadriplegic cerebral palsy. PET-CT scan done in 6 patients showed metabolic improvements in areas of the brain correlating to clinical improvements.

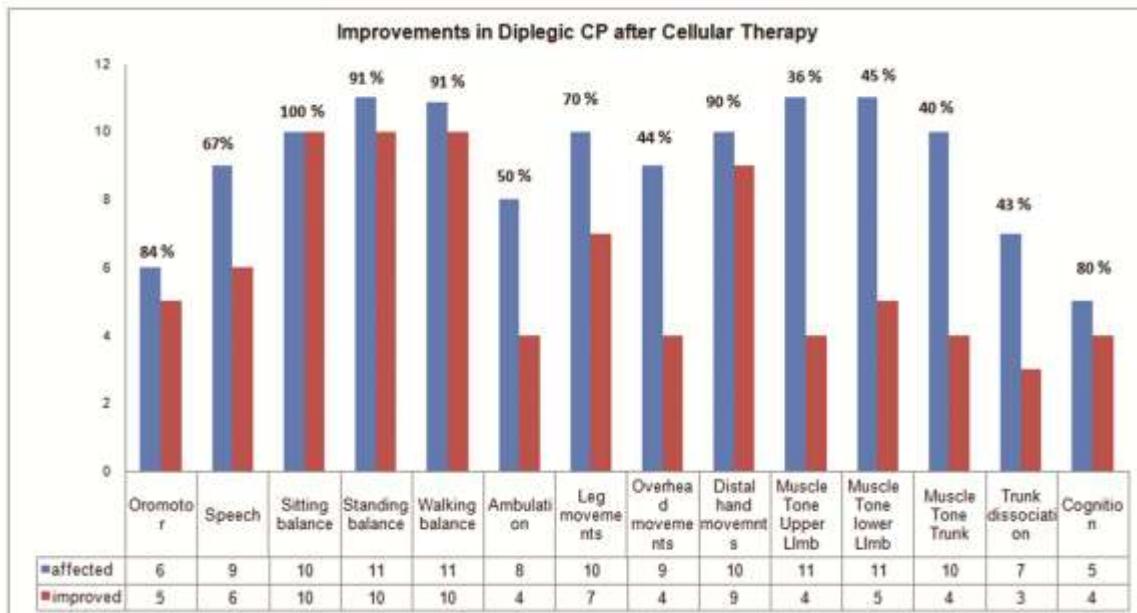


Figure 3: Graph demonstrating improvements in Diplegic CP after stem cell therapy

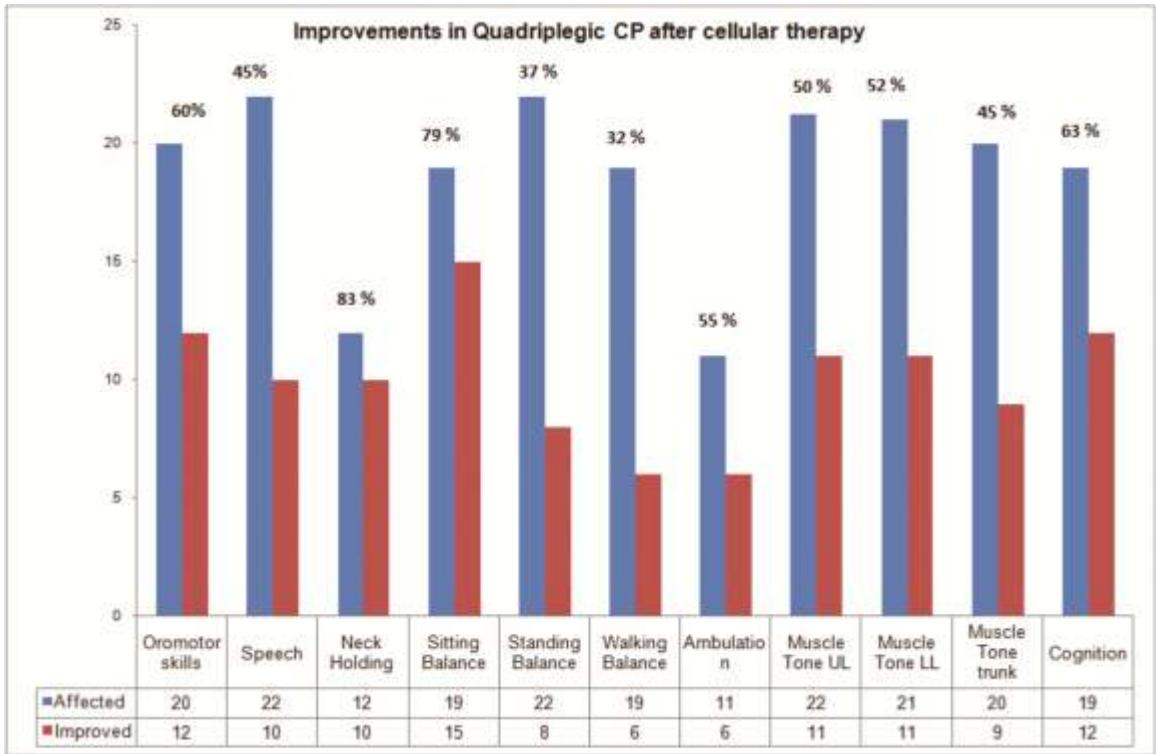


Figure 4: Graph demonstrating improvements in Quadriplegic CP after stem cell therapy

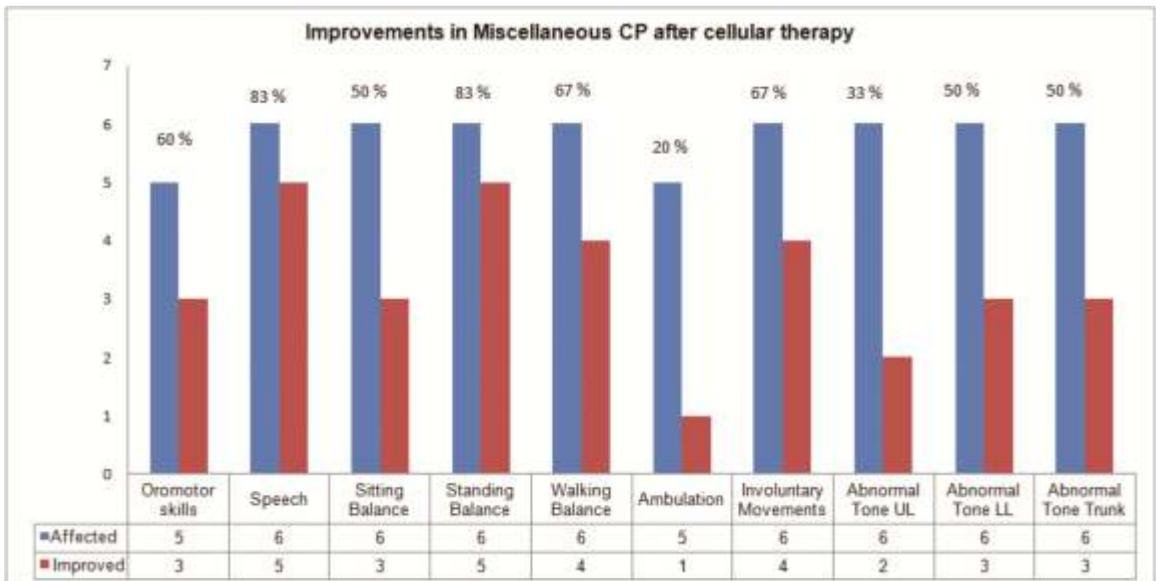


Figure 5: Graph demonstrating improvements in miscellaneous types of CP after stem cell therapy

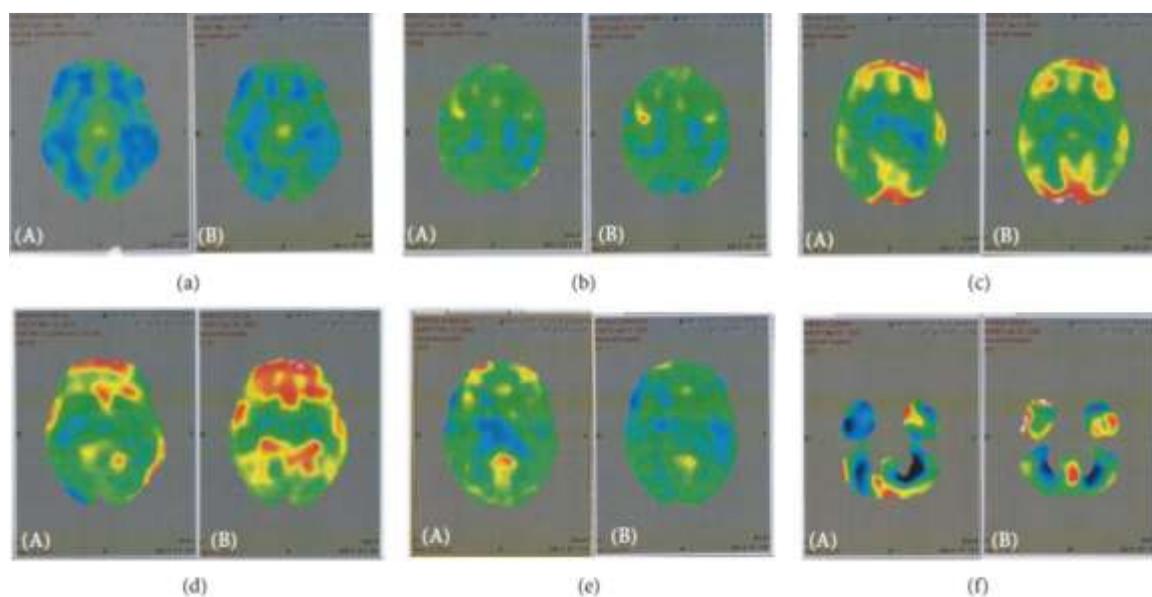


Figure 6: *Improvements in PET-CT scan images of (A) pre and (B) Post intervention showing increased metabolic activity in various areas. Blue areas indicate hypometabolism, green areas indicate normal metabolism and yellow areas indicate slightly high metabolism and red areas indicate high metabolism.*

Case reports

We have published 8 case reports in various national and international peer reviewed journals. (57-64) These cases were unique with respect to their age, severity of CP, type of CP, comorbidities such as autism and ID and their outcomes. Outcome measures such as GMFCS, GMFM, FIM were used to quantify the outcome. PET CT scan brain was also used to monitor the metabolic changes occurring in the brain after intervention. In all these cases, common symptomatic improvements such as improved trunk strength, limb control, hand functions, walking stability, balance, posture, spasticity and coordination was observed. Transfers such as bed, sitting and getting up from the floor had become easier. Their FIM scores improved indicating improvement in the ability to perform day to day tasks. The case with comorbid ID showed improved IQ scores suggestive of improved cognitive functions. These cases improved neurologically as well as functionally. Their PET CT scans showed improved metabolism of brain areas which were affected before the intervention. The FDG uptake of previously hypometabolic areas had increased after stem cell therapy.

Unpublished data

Improvements in Cerebral Palsy After Stem Cell Therapy (N=267)

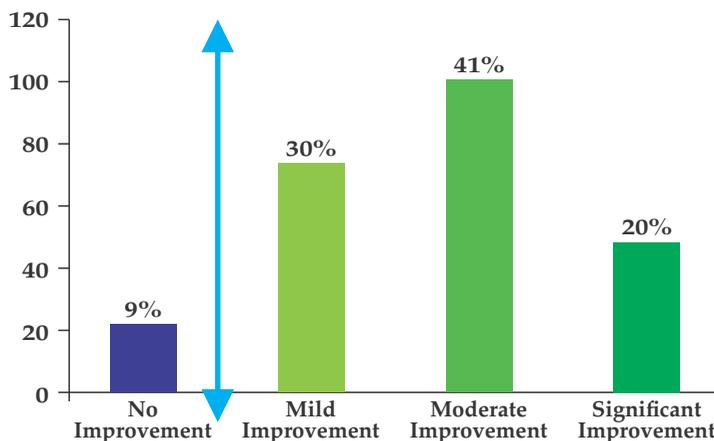


Figure 7: Graph demonstrating improvements after stem cell therapy in cerebral palsy.

We analysed the effect of stem cell therapy in 267 patients diagnosed with cerebral palsy. Changes in common symptoms like oromotor/speech, balance, trunk activity, upper limb activity, lower limb activity, muscle tone, ambulation and Activities of Daily Living were recorded on follow up. The improvements were graded as no change, mild improvements, moderate improvements and significant improvements. Analysis revealed that out of 267 patients, Overall 91.01% patients showed symptomatic improvements. 8.98% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 29.96% of patients, moderate in 41.19% of patients, whereas, 19.85% of patients showed significant improvements. We observed that patients who continued regular exercise program at home under supervision of professional therapists showed significant improvements. Patients who did not follow regular rehabilitation showed mild improvements. Therefore, in our experience stem cell therapy should be followed by regular rehabilitation under supervision of a therapist.

PET CT Brain in CP

As discussed in previous chapter, PET CT records brain metabolism by using fluorodeoxyglucose uptake. The glucose metabolism in the brain directly correlates with neuronal activity. Hence, PET CT scan is effectively used to monitor the effects of stem cell therapy on brain function in CP patients. We have observed that after stem cell therapy, areas which were hypometabolic before stem cell therapy showed improved metabolism after intervention. Improvement in these areas resulted in improved functions.

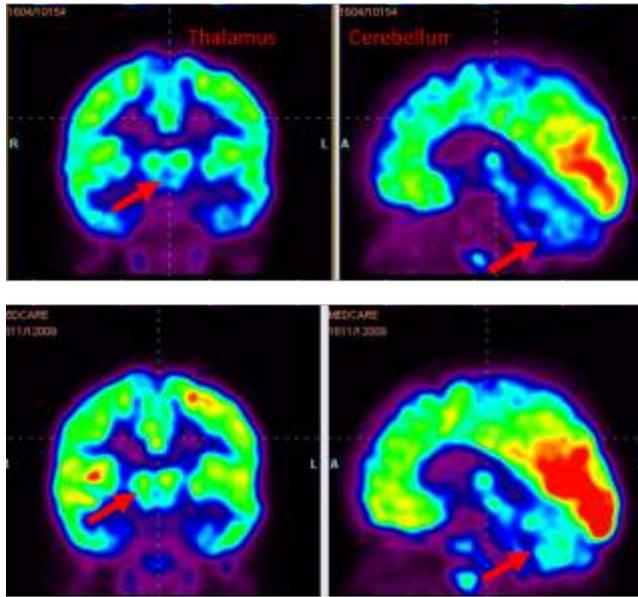


Figure 8: Comparative PET CT scan showing improved metabolism in CP. Before stem cell therapy PET CT showed reduced metabolism in left motor cortex, bilateral medial temporal cortex, bilateral Basal ganglia, Bilateral cerebellum. After stem cell therapy, improvement was seen in left motor cortex, significant improvement in bilateral basal ganglia, Thalamus and bilateral cerebellum

Case report

A 2 years old boy was diagnosed with CP at the age of 5 months. He had an emergency low section caesarean section birth. He did not cry immediately after birth. There was respiratory distress hence, he was intubated and put on ventilator for 4 days and 6 days in NICU. His MRI brain suggested periventricular leukomalacia with atrophic corpus callosum. He also had history of seizures at 6 months and since then he has been on medication. On observation, he was dependent for all ADLs. Responded very poorly to commands. There was no social smile. Visual tracking was poor. Oromotor functions were affected as there was no speech, drooling was present and he could only eat semisolid food. There was presence of macrocephaly. On examination, right upper and lower extremities were hypertonic while, left upper and lower extremities were normotonic. Reflexes were affected in right biceps, triceps and knee. However, they were normal in left biceps, triceps and knee. Voluntary Control was poor in left upper extremity and lower extremity, fair in right upper extremity and lower extremity.

On follow up at 9 months after transplantation following improvements were observed:

- Higher mental functions were improved, specifically cognition and

orientation. He could understand emotions better and sometimes responded to name call as well.

- Sitting and standing posture had improved. He sat and stood more erectly.
- Weightbearing on both upper and lower extremities had improved. He had started doing quadruped and crawling on his own. He could also perform sit to stand with support on his own and perform weight shifts on legs.
- Reflexive development had matured as he had developed equilibrium and anticipatory postural reactions.
- Voluntary control had improved in both upper and lower extremities. Earlier, he could not perform crawling and quadruped. Now, he could crawl and even perform sit to stand, and kneel standing.
- Trunk-pelvis, pelvis femoral, and interlimb dissociations had improved from poor to fair.
- Reach outs in all postures except quadruped had improved. He tried to grasp toys in sitting, kneeling, and standing.
- Balance was better. Earlier, he would not crawl and stand. Now, he started to stand with support. He could stand for 10-15 minutes at a stretch. Also, he started walking with tripod cycle.
- Transitions were improved. He could perform supine to sit, sit to stand, crawl and walks with support.
- Left hamstring and TA tightness had reduced and right hamstring and TA tightness was not there.
- Speech had improved as vocalization had started.
- Awareness and understanding of relationships was better as he could now recognize his father.
- Social interaction and play behavior had improved. He also developed social smile
- He had developed visual tracking too.
- ADLs were age appropriate;
- FIM Score had improved from 58 to 61.
- GMFM Score had improved from 6.54 to 32.28.
- GMFCS had improved from level V to level IV.

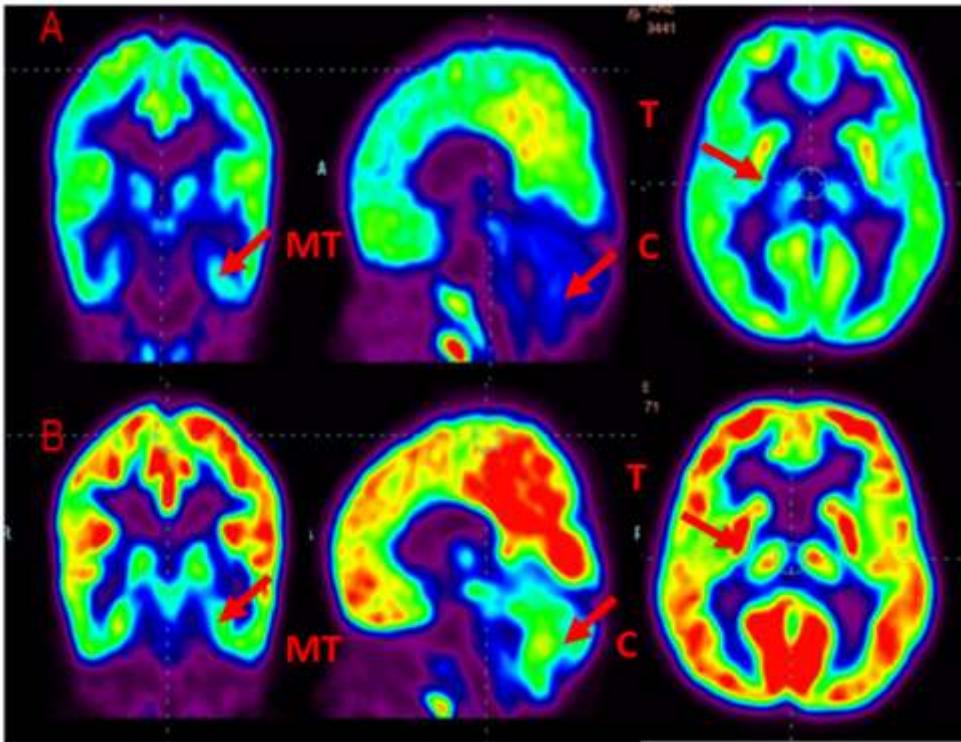


Figure 9: Comparative PET CT scan showing improved brain metabolism. Before stem cell therapy, PET CT scan showed severely reduced metabolism in the bilateral thalamus, medial temporal cortex and cerebellum. Post stem cell therapy, it showed improved metabolism in the bilateral thalamus, medial temporal cortex and cerebellum

Conclusion-

Various countries all over the world are performing clinical trials to study the benefit of stem cell therapy in cerebral palsy. However, for it to become a standard treatment it is important to optimize certain factors such as type of cells to be used, source of cells, number of cells to be administered, time and frequency of transplantation, etc. Umbilical cord blood cells and bone marrow derived cells are the most widely studied cells for their therapeutic potential. Use of umbilical cord blood cells has been approved for hematological conditions; however, protocols for their intrathecal transplantation in neurological disorders are still being established. Safety of these cells still needs to be established. Currently, there are safe and effective protocols available for autologous BMMNCs, which can be used to repair the underlying neurological damage in CP. PET CT scan brain is being studied as a monitoring tool to track changes occurring in the brain at a cellular level. Stem cell therapy may not be a cure for CP. But, it can be used as an adjunctive treatment modality with the current standardized medical and rehabilitation intervention to accelerate the development of children with cerebral palsy.

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"What is at stake, in the present moment, is not the future. What is at stake now is the stand you and I take for the future - whether our day to day lives could be lived in the context of a reality which we cannot now even imagine. Our work has never been about altering things within our realities, within the realm of possibilities. It is about being able to create the realm of possibilities itself, to bring forth that which heretofore was unimaginable"

- Werner Erhard

10

Role of Stem Cells in Muscular Dystrophy

“If a treatment (for muscular dystrophy) does not replenish the stem cell compartment, it will be likely fail; it would be like pushing the gas pedal to the floor when there is no reserve.”- Jason Pomerantz, MD

Muscular dystrophy (MD) is a heterogeneous group of genetic disorders that weaken the muscles of the body. It is characterized by progressive weakness and wasting of these muscles (1). Each type of muscular dystrophy is associated with a distinct genetic mutation. Mutation is seen in different components of dystrophin-glycoprotein complex (DGC) which links the extracellular matrix in muscle to the intracellular cytoskeleton resulting in destabilization of the muscle membrane, increased muscle fragility and degeneration, and muscle wasting (2, 3). The nature of the gene mutation and location of the chromosome determines the characteristics of the muscular dystrophy and their inheritance. Pre-clinical studies have shown that muscle weakness in muscular dystrophy is caused not only due to muscle fiber fragility but also due to hampered muscle regeneration caused by intrinsic satellite cell dysfunction (4). Although the disease progression is due to dystrophin and other muscle proteins' deficiency, it is ultimately a stem cell disease (5).

The types of MD vary according to severity, age of onset, and selective involvement of muscle groups. The most common types are Duchenne, Becker, limb girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal and Emery-Dreifuss (6). Of these, DMD is the most severe form of muscular dystrophy. All forms of MD are characterized by a progressive muscle weakness with some types

affecting cardiac muscle. Abnormal gait (waddling gait) with frequent falls, difficulty in rising from the floor and climbing stairs, pseudohypertrophy of calves, positive Gowers' sign and scoliosis or kyphosis are a few common symptoms presented by the affected population of MD (7). With decreasing muscle strength in those that are affected, function is compromised, interfering with the activities of daily living and ambulation. Once wheelchair-bound, contractures and spinal deformities further worsen. On an average, for every 10 degrees of thoracic scoliosis, there is a 4% decrease in forced vital capacity. This along with cardiac involvement may result in death (8).

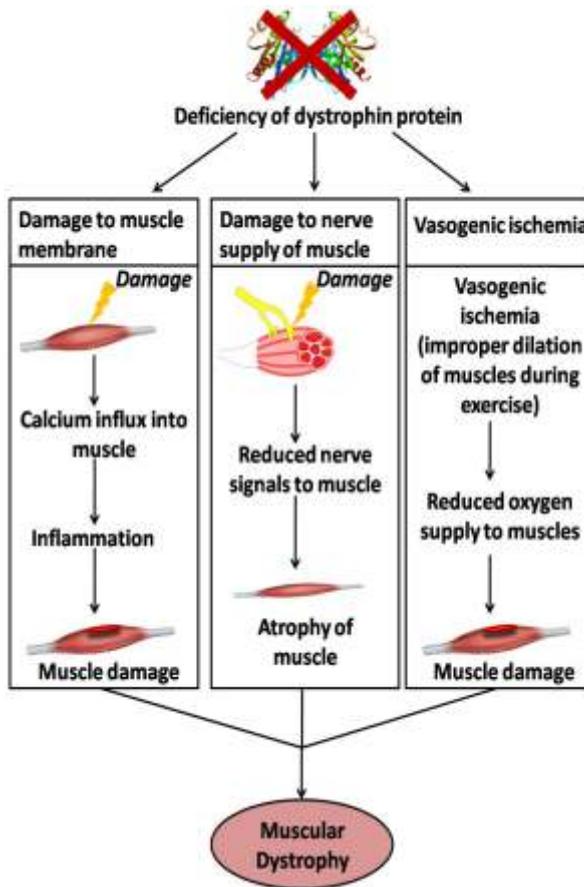


Figure 1: Pathophysiology of the most common muscular dystrophy type; Duchenne muscular dystrophy (DMD).

Despite extensive studies being carried out in this field, there is currently no effective treatment for the same (9). The conventional treatments include medical intervention such as corticosteroids, physical and occupational therapy, assistive devices, etc. Corticosteroids help by reducing muscle inflammation and improvements in muscle function (10). Steroids also delay the onset of cardiomyopathy (11).

Unmet Medical Needs

The treatments available currently for muscular dystrophy alleviate few symptoms of the disorder but do not act at the cellular level. They fail to carry out the repair and regeneration of the damaged muscles. Also, no treatment repairs the underlying mutation of the genes which cause MD. Gene therapy is being explored and though appealing as a treatment option in muscular dystrophy. But due to immune responses and safety issues, it has not been established as a treatment option. No standard therapeutic modality has been successful to halt the progression of the disease or increase the survival. Botulinum toxin Type A maybe indicated for improvement in joint range and minimizing contractures, however these effects are temporary. Exon skipping which makes use of synthetic antisense oligonucleotide sequences results in restoration of the reading frame and partial production of functional dystrophin (12). Recently, eteplirsen, an antisense oligonucleotide, has received approval, but is limited to treatment of patients who have a mutation in the dystrophin gene that is amenable to exon 51 skipping (13). Also, in the study 6MWT was used as an outcome measure. While the study demonstrated improvement in the 6 MWT distance and a slower rate of loss of ambulation, two of twelve patients still lost ambulation. The drug thus does not provide a cure.

Role of stem cell therapy in MD

Wallace et al postulated the underlying pathogenic mechanism of muscular dystrophy to be an imbalance between muscle damage or degeneration and muscle repair through stem-cell mediated regeneration (14). Continuous cycles of degeneration and regeneration of muscle fibers exhausts the muscle stem cell pool, leading to muscle being replaced by adipose and fibrotic tissue. Stem cell therapy holds promise as a treatment for muscular dystrophy by providing cells that can both deliver functional muscle proteins and replenish the stem cell pool (15).

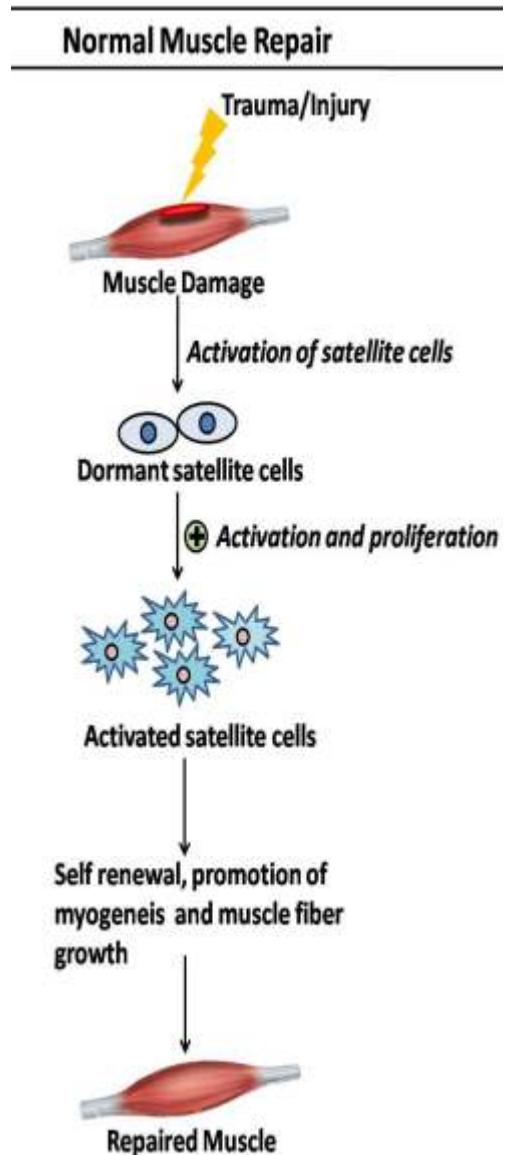


Figure 2: Normal process of muscle repair.

The mechanisms by which stem cells may function and reverse the effects of cell death include differentiation, cell fusion, and secretion of cytokines or paracrine effects (16-18). These cells have the capacity to mobilize and exert their reparative effects at the site of injury. They are known to enhance angiogenesis and contribute to neovascularization by producing signaling molecules such as vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF2) (19). Along with increase in angiogenesis, they also promote tissue remodeling, prevent apoptosis, decrease inflammation, release growth factors and activate the satellite cells (20). In animal studies, these cells have shown to produce the deficient proteins and make new muscle cells which fuse with the host fibers. Satellite cells, the adult skeletal muscle progenitor cells, are commonly considered to be the main cell type involved in skeletal muscle regeneration. Further, stem cell derived exosomes which are small membrane vesicles and are responsible for inter cellular communication; promote muscle regeneration by enhancing myogenesis and angiogenesis.

Although, MD is primarily a muscle disease, dystrophin-glycoprotein complex (DGC) is also a component of neurons and glia in the brain. Therefore, part of the cell fraction is injected intrathecally (21). Neuromuscular junction is also impaired in MD due to synaptic abnormalities. Injecting the cells at the motor points ensures repair of both, the muscles and the nerves.

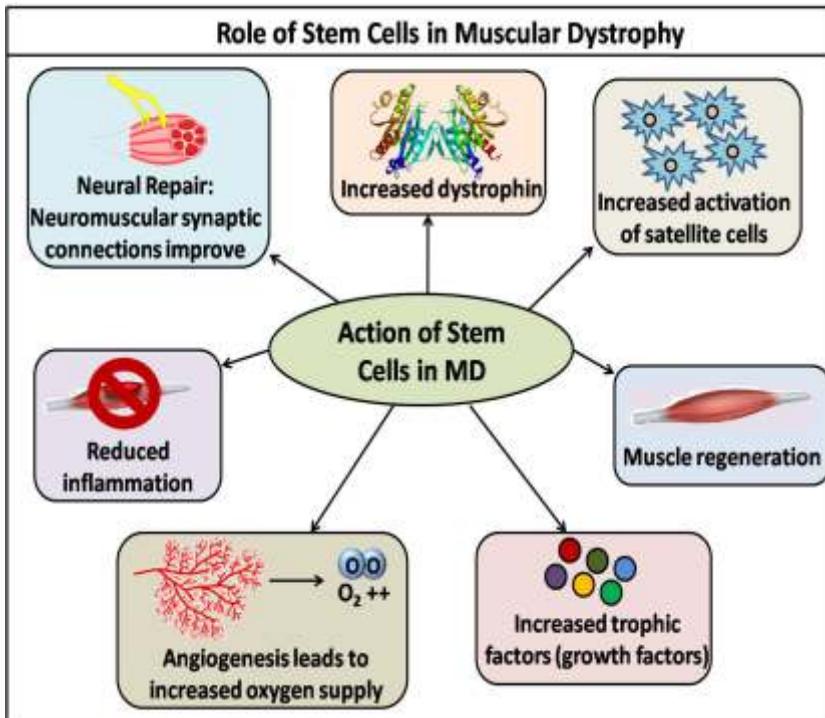


Figure 3: Role of stem cells seen in DMD (in other forms of muscular dystrophy, there is an increase in the deficient protein based on the type).

Precautionary measures before stem cell therapy in MD

Before stem cell therapy, biochemical and hematological blood tests, chest X-ray, 2-D Echo are performed. Patients with respiratory distress/ respiratory tract infections, or with acute infections such as, fever, hemoglobin less than 8 g/dl, bleeding tendencies, left ventricular ejection fraction less than 30% are at high risk for developing complications after stem cell therapy. High risk precautions should be taken in these cases before stem cell transplantation. Anaesthesia risk should also be evaluated for all MD patients before cell therapy.

It is recommended that following stem cell therapy, patients perform voluntary/ assisted active exercises, low-intensity exercises such as swimming avoiding overexertion. Eccentric exercises such as running or walking downhill should be avoided (22). Those suffering from MD are at risk of developing osteoporosis with a consequent susceptibility to fractures. Prolonged exposure to corticosteroids further negatively impacts bone density. It is therefore important that patients receive a daily vitamin D supplement. Regular screening of bone density, X-ray especially of the spine, pelvis and hips should be done. In those patients with cardiac involvement and in patients with DMD and BMD, complete cardiac evaluation is recommended. The prophylactic use of ACE inhibitors is recommended before the establishment of cardiomyopathy (23). Additionally, evaluation and treatment of respiratory abnormalities should be done.

Musculoskeletal Magnetic Resonance Imaging (MSK MRI) is a useful, non invasive tool to track disease progression and can serve as an objective measure for assessing efficacy of a treatment. Diffusion Tensor Imaging (DTI) measures the anisotropy of water diffusion. DTI may be applied to skeletal muscle as it defines structural details of a muscle. Since, muscle injury from muscular dystrophy affects the integrity of muscle microstructure; changes are noted in the DTI parameters. The fractional anisotropy (FA) is the most commonly used tensor value. The microstructural changes are thus reflected by changes in FA values (24). Progression of the disease process is marked by an increase in FA values. Conversely, decrease in FA values is a positive outcome to a treatment measure.

Preclinical studies

Preclinical studies in mouse models of various muscular dystrophies have demonstrated that myoblasts on transplantation into dystrophic muscle could repair damaged myofibres. However, myoblast transplantation did not show effective results due to rapid death of most injected myoblasts and the failure of injected myoblasts to migrate from the injection site (25). Other cells such as adult-derived stem cells, including bone marrow-derived stem cells, blood and muscle-derived CD133+ cells, muscle-derived stem cells (MDSC), side population (SP) cells and mesoangioblasts have been tested in animal models (16,26-32).

A study that used adult muscle mononuclear cells (AMMCs) in sarcoglycan null dystrophic mice found that AMMCs were 35 times more efficient at restoring sarcoglycan compared to cultured myoblasts (33). Similar studies were carried out using side population (SP) cells (34).

A study carried out to track the fate of bone marrow derived stem cells (BMSC) in mouse models of muscular dystrophy using green fluorescent protein-positive (GFP+) demonstrated that transplanted BMSC differentiate into muscle cells via repopulation of the muscle stem cell compartment (35). Similar test was carried out using 3H-thymidine labeled human bone marrow derived MSCs (36). Embryonic stem cells (ESC) have also shown its potential in muscle regeneration. On injecting wild type ESCs into the mdx blastocysts, mice with improved pathology and function were produced (37-39). However, due to ethical issues and immune rejection not many studies have been carried out on humans using ESCs. Experimental studies have also been carried out where human umbilical cord blood (HUCB) cells have shown to differentiate into muscle cells (40,41).

Clinical studies

Around 20 studies are published demonstrating the effect of stem cell therapy in different types of muscular dystrophies. Various types of stem cells are being explored such as autologous bone marrow mononuclear cells, allogenic umbilical cord stem cells, bone marrow mesenchymal cells, muscle precursor cells, muscle derived CD133+ cells, myogenic cells.

Torrente et al [42] tested the safety of autologous transplantation of muscle derived CD133+ cells in eight boys with Duchenne muscular dystrophy in a 7-month, double-blind phase I clinical trial. No local or systemic side effects were observed in all treated DMD patients. Treated patients had an increased ratio of capillary per muscle fiber with a switch from slow to fast myosin-positive myofibers. On the other hand, donor myoblasts injected into muscles of 12 patients with DMD, did not show any significant difference in muscle strength between arms injected with myoblasts and sham-injected arms [43]. 4 patients however, had low levels of donor dystrophin. In another study, 3 DMD patients received injections of myogenic cells [44]. Dystrophin-positive myofibers were observed 4 weeks later in all the patients. In another single case report, donor-derived dystrophin was found in the muscles, of a patient with DMD, that were injected with allogenic muscle precursor cells [45].

Double transplantations of autologous bone marrow mesenchymal stem cells and umbilical cord mesenchymal stem cells was tested for safety and efficacy in progressive muscular dystrophy [46]. Treatment efficacy was observed in 68 of 82 patients (82.9% efficacy) and was found to be safe. In another single-blinded study, 11 DMD patients received umbilical cord mesenchymal stem cells [47]. Stabilisation or improvement in muscle strength was found in all the treated patients while

control group showed a decline in muscle strength at 1 year follow up. An 11-year-old DMD boy underwent umbilical cord blood stem cell transplantation [48]. Serum creatine kinase levels declined from 6000 U/L to 2200 U/L post intervention together with improvements in walking, turning the body over, and standing up, 6 weeks post intervention. Umbilical cord-derived hematopoietic stem cell transplantation on the other hand was not found to be efficacious in DMD [49].

Intrathecal and intramuscular transplantation of autologous BMMNCs showed symptomatic and functional improvements in 130 of 150 patients with MD [50]. Stabilisation or improvement in muscle strength and function was found in all 65 patients with LGMD following intrathecal and intramuscular transplantation of autologous BMMNCs [51]. Intrathecal and Intramuscular transplantation in 10 separate case reports demonstrated improvement in function and muscle strength in different MD variants [52-61].

Side Effects

None of the studies encountered any serious side effects. Some minor procedural side effects were noted, such as pain at the site of injection and bone marrow aspiration in case of autologous transplantation. These side effects were self limiting and resolved within a week with medications. Allogenic umbilical cord derived hematopoietic stem cell transplantation resulted in the graft rejection during first transplant which was resolved during subsequent transplants.

Our results

Published data

A study was carried out on 150 patients diagnosed with Muscular Dystrophy. On a mean follow up period of 12 months ± 1 month, 86.67% cases showed symptomatic and functional improvements, with 6 patients showing muscle regeneration and decrease in fatty infiltration on Musculoskeletal Magnetic Resonance Imaging (MSK MRI) and 9 showing improved muscle electrical activity on Electromyography (EMG). 53% cases showed increase in trunk muscle strength, 48% an increase in upper limb strength, 59 % an increase in lower limb strength and about 10 % showed an improved gait pattern.

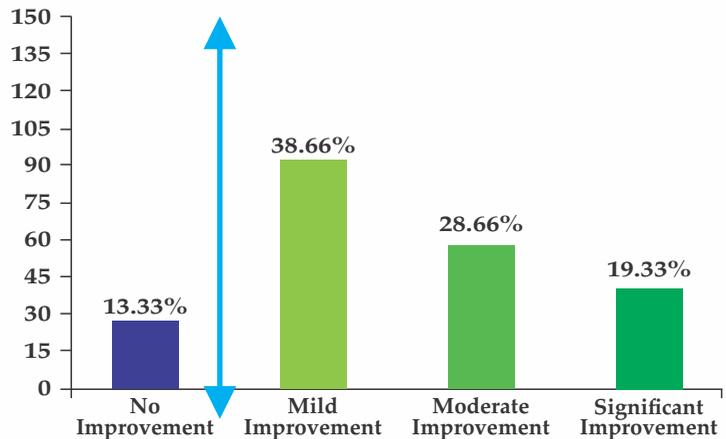


Figure 4: Graph showing improvements in muscular dystrophy patients after stem cell therapy. y-axis = number of patients (n = 150).

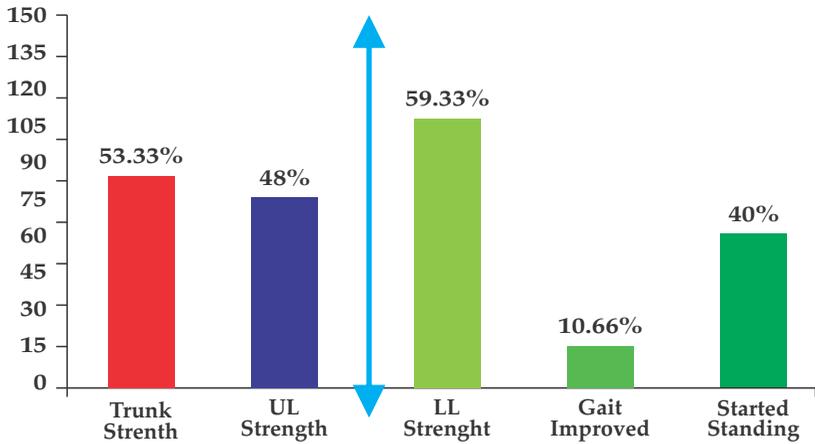


Figure 5: Graph showing symptomatic improvements in muscular dystrophy patients after stem cell therapy. Number of patients showing improvements in trunk strength, upper limb (UL) strength, lower limb (LL) strength, gait pattern, and standing function are shown. y-axis = number of patients (n = 150).

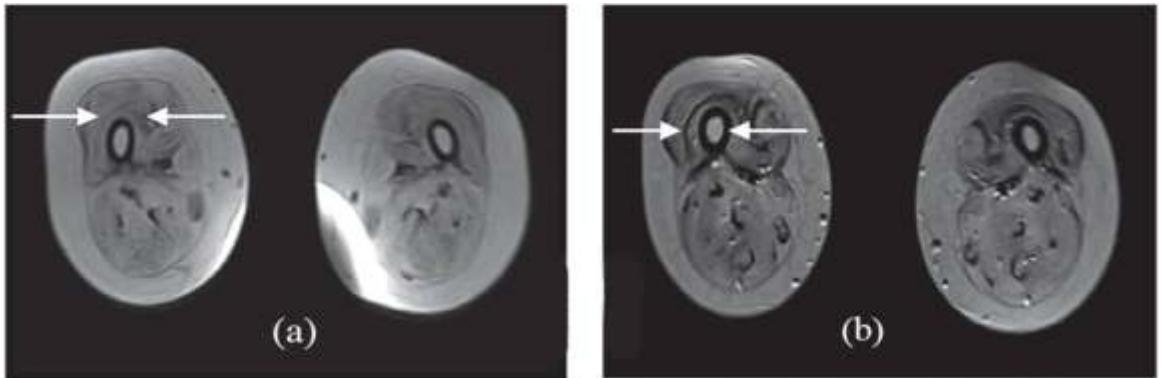


Figure 6: Figures a & b shows the Musculoskeletal MRI images before and after stem cell therapy, respectively. Figure b shows regeneration of muscle in vastus medialis and vastus lateralis.

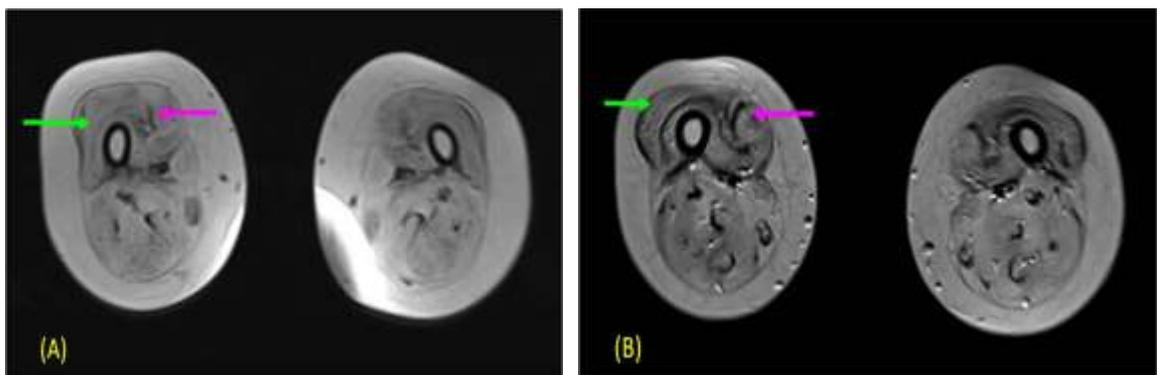


Figure 7: Figures, A & B show MRI Musculoskeletal images before and after stem cell therapy. Regeneration of muscles is seen in Fig B.

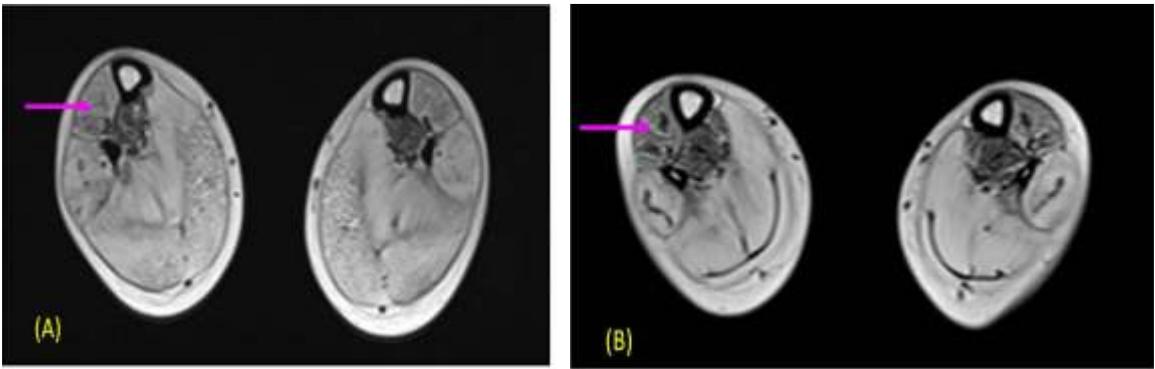


Figure 8: The figures A & B show MRI Musculoskeletal images before and after stem cell therapy, respectively. Regeneration of muscles is seen in Figure B.

An individual study comprising of LGMD patients was also published. This study included 65 patients diagnosed as LGMD that underwent autologous bone marrow mononuclear cells intrathecally and intramuscularly. While 59 cases completed the study, 6 were lost to follow up. These cases were divided into 3 groups depending on the number of transplantations administered. Group 1 included the cases who underwent one stem cell therapy (N=31). Group 2 included cases who underwent two transplantations (N=24). Group 3 included cases who underwent 3 transplantations (N=4). In group 1, 97% of the patients showed improved function. The total percentage of strength improvement ranged from 84% to 100% in all the muscles. In group 2, 96% of the patients showed an improved function. The total percentage of strength improvement ranged from 90% to 100% in all the muscles. In group 3, of the four patients, one patient deteriorated in his FIM score, whereas two patients improved, and one maintained functional status. Most of the patients had maintained muscle strength.

Unpublished data

512 patients diagnosed with muscular dystrophy were analyzed. Symptomatic analysis was done for the core symptoms of the disease. These included changes in ambulatory status, hand functions, balance, stamina/fatigue, trunk activation and standing. They were graded as no change, mild, moderate and significant change. On follow up, out of 332 patients, 85.74% of patients showed improvements while 14.25% of patients remained stable without deterioration in any of the symptoms. Mild improvements were observed in 20.31% of patients, moderate in 35.74% of patients, whereas, 29.68% of patients showed significant improvements.

Duchenne Muscular Dystrophy

Total of 139 boys detected with DMD underwent autologous bone marrow mononuclear cell intrathecal and intramuscular transplantation. Mean age of the group was 11 years, ranging from 3 to 23 years. 39 boys were below the age of 10 years at admission, 77 were between 10 to 15 years and 23 boys were over the age of

15 years. 57 boys were ambulatory at assessment and 81 were non-ambulatory. Genetic testing was available for 64 boys, 38 of which showed distal rod (45-55) exon deletions, 7 showed proximal rod (3-21) exon deletion, 2 showed both proximal and distal rod, 4 showed deletion of exons in other regions and 13 patients showed no deletions but mutations.

Functional status and muscle strength were assessed using, functional independence measure (FIM) scale, Brooke and Vignos scale and manual muscle testing. In addition to these outcome measures the time till ambulation was compared with 35 age matched patients that chose not to undergo stem cell therapy after initial consultation.

The changes in the scales were analyzed statistically using matched pair Wilcoxon Sign Rank test (Table 1 and 2). There was no statistically significant deterioration in these scales suggesting the delayed progression of the disease. Kaplan-Meier Survival Analysis was used to compare the age at loss of ambulation (Figure 1, Table 3). There was a statistically significant difference in the time till loss of ambulation for children that underwent stem cell therapy from those that did not. The average predicted age at the time till loss of ambulation was 142 months for children that did not undergo stem cell therapy; whereas it was significantly higher, 204 months, in children that underwent stem cell therapy. Percentage analysis was performed for the symptomatic improvement in these children (Table 4, Figure 2). This analysis suggested that majority of the patients had shown improvement or halting of the progression in postural deviations, neck weakness, bed mobility, trunk activity, gross and fine motor function, functional upper limb activity, walking and standing. The pre and post therapy measurements were performed at a median follow up duration of 6 months.

Outcome measure	Pre Therapy Mean Score	Post Therapy Mean Score	Statistical Significance
Functional Independence Measure	71	76	0.001
Brooke Scale	3.07	3.27	0.076
Vignos Scale	6.5	6.8	0.245

Table1. Matched pair Wilcoxon Sign Rank test analysis of outcome measures pre and post therapy

Muscle Group	Pre Therapy Mean Score	Post Therapy Mean Score	Statistical Significance
Hip flexors	6	6.69	0.001
Hip Abductors	5.42	6.08	0.001
Hip Adductors	4.21	5	0.001
Knee Flexion	9.1	9.48	0.004
Knee Extension	5.26	5.69	0.003
Shoulder Adduction	5.26	6.02	0.04
Shoulder internal rotation	7.23	7.79	0.001
Biceps	7.96	8.32	0.01
Upper Abdominals	3.8	4.21	0.005

Table2. Matched pair Wilcoxon Sign Rank test analysis of modified manual muscle testing scale

	Comparison Group	Intervention Group	Test statistics
Total no. of patients	35	42	-
Percentage of patients currently non- ambulatory	65%	23%	-
Predicted time till loss of Ambulation	142 months	204 months	0.004

Table3. Kaplan-Meier analysis of time till loss of ambulation for patients with and without stem cell therapy

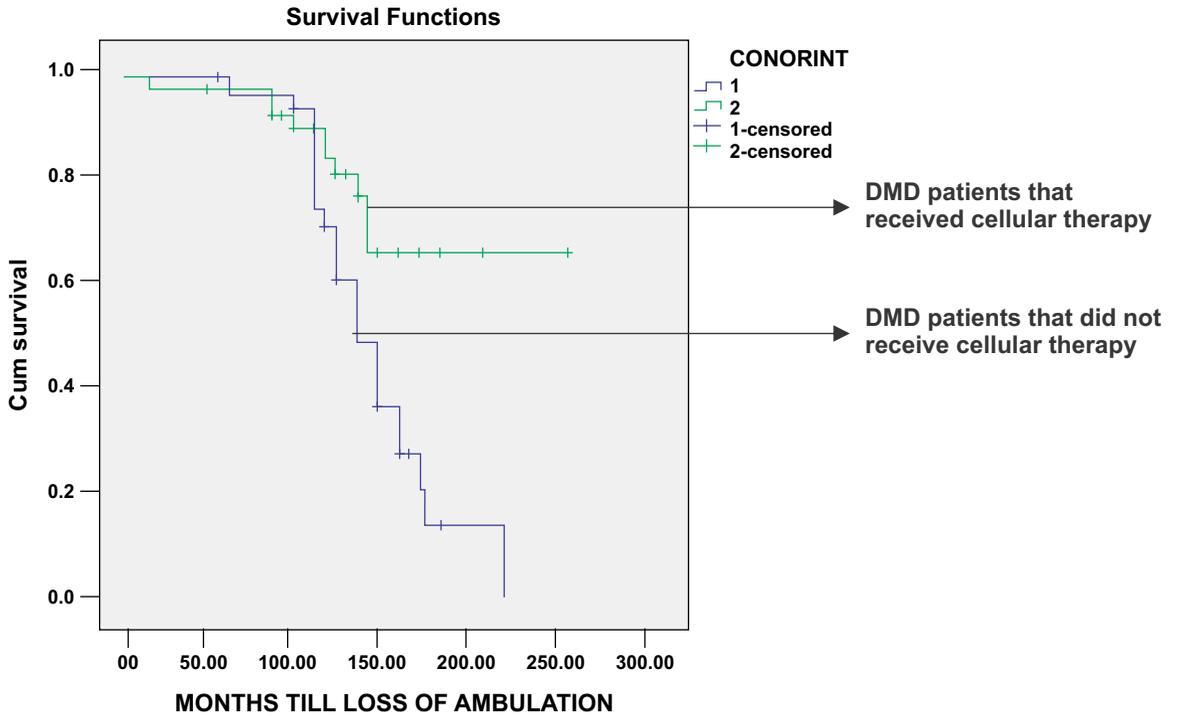


Figure 9: Kaplan-Meier curve analysis of time till loss of ambulation in patients with and without stem cell therapy

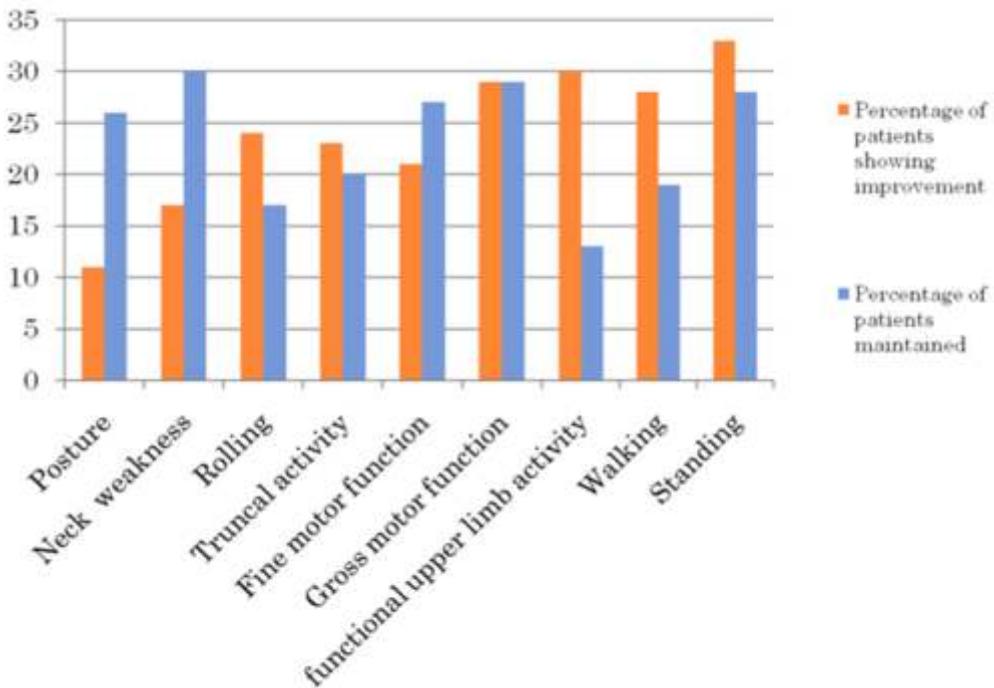


Figure 10: Percentage analysis of symptomatic improvement in the patients with stem cell therapy

Muscle		Percentage of patients with improved muscle strength	Percentage of patients with deteriorated muscle strength	Percentage of patients with muscle strength maintained
Hip	Flexors	29	15	55
	Extensor	37	13	50
	Abduction	32	8	60
	Adduction	41	6	53
Knee	Flexor	29	5	65
	Extensor	29	10	60
Ankle and Foot	Peronei	27	10	63
	Tibialis Anterior	27	10	63
	Tibialis Posterior	26	12	63
	Plantar Flexors	9	3	88
	EHL	14	9	77
	EDL	14	8	78
Shoulder	Deltoids	28	17	55
	Adduction	24	10	65
	Internal Rotation	24	4	72
	External rotation	26	5	69
Elbow	Biceps	19	8	73
	Triceps	26	14	60
Wrist and Fingers	Wrist Flexors	10	4	86
	Wrist Extensors	12	3	86
	Supinators	12	4	85
	Pronators	5	4	91
	Palmar Interossei	12	12	77
	Dorsal Interossei	10	12	78
	Lumbricals	10	5	85
Trunk	Upper abdominals	36	8	56
	Lower abdominals	26	18	56

Table4. Percentage analysis of modified manual muscle testing scale

All the studies have demonstrated stem cell transplantation to be safe. Since, muscular dystrophy is a group of genetic disorders; stem cell therapy is not a cure. However together with neurorehabilitation, it can be effective and may slow down the disease progression. Though, MD is mainly a disease of the muscle, dystrophin-glycoprotein complex is also a component of the neurons and glia of the brain. Also due to synaptic abnormalities, neuromuscular junction is affected. Stem cell therapy should therefore target the nervous system, the innervating nerves and muscles. Since, muscular dystrophy maybe accompanied by cardiac involvement, regular screening and treatment of cardiomyopathy is essential. Along with this regular evaluation of pulmonary function and care are required. It is important that stem cell therapy along with multidisciplinary care is done at an early stage before the disease process has caused much damage.

Although evidence is still limited, stem cell therapy together with multidisciplinary care improves the quality of life and shows promise as a treatment option in muscular dystrophies.

Future Directions

In disorders involving muscular damage, the side population (SP) cells are responsible for production of fibro-adipogenic precursors (FAPs), fibroblasts and ultimately adipocytes as a response to the injury (58). Hence, fibrosis and fat deposition is observed in most chronic muscular dystrophies. This may hinder the repair and regenerative potential of the transplanted stem cells which may decrease the efficacy of intervention. Hence, the future research should be focused on manipulating the cells so as to bypass the fat generation and to stimulate muscle regeneration. Since, MD is a genetic disorder; leading to low production of dystrophin or other muscle proteins, genetic correction plus utilization of the regenerative ability of stem cells to restore the already damaged muscles maybe the key to treatment of MDs. A combination of gene therapy and cellular therapy may thus be a powerful tool. Studies should be directed at assessing which cell types are most beneficial and also the route of administering these cells to the target muscles and innervating nerves. Also, studies carried out so far lack the inclusion of imaging techniques. MSK MRI maybe a useful, non-invasive tool to track disease progression and can serve as an objective measure for assessing efficacy of a treatment.

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"Neurosurgeons would be happy if they could make the spinal cord regenerate thus helping thousands of paraplegics all over the world. Sustained efforts in this direction are the Immediate need of the future."



- Dr. B. Ramamurthi
-Founding father of Neurosurgery in India

11

Role of Stem Cells In Spinal Cord Injury

Along with neurorehabilitation, stem cells can aid in faster functional recovery in Spinal Cord Injury.

A spinal cord injury (SCI) is damage to the spinal cord caused due to trauma such as road traffic accidents (RTAs), fall from height or non-traumatic events such as infection, loss of blood supply, compression by a cancer or through slow degeneration of the spinal bones (vertebrae). It often results in a severe neurological deficit. There could be complete disruption or contusion, compression or penetration of the spinal cord leading to necrosis, demyelination, axonal loss and glial scarring. (1) The demyelination of axons may lead to a permanent loss of sensorimotor functions affecting the quality of life of these patients (2). A severe cervical spinal damage results in quadriplegia, whereas an injury to the thoracic or lumbar spine leads to paraplegia.

Complete recovery of the damaged spinal cord is very difficult, as it does not have the ability to regenerate lost or damaged neurons and re-establish the neural connections. The scar also consists of axonal growth inhibitors which further limit the repair and regeneration process. (3) As a result, there is no cure for SCI available presently.

The current treatment for SCI includes surgical interventions, medicines and rehabilitation. Their main goal is to stabilize the spine and prevent any secondary complications.

Unmet Medical Needs

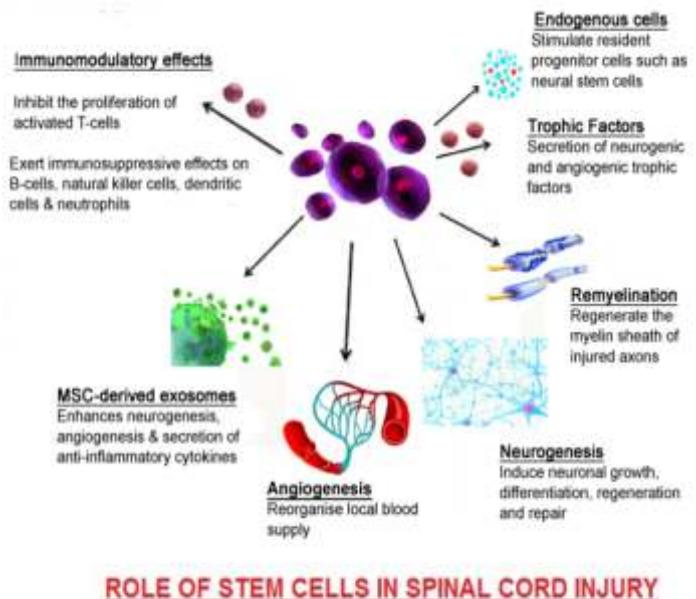
The available treatments for SCI fail to repair the underlying pathology completely, leaving behind some neurological deficits. Presently, all modalities aim at repairing the vertebral column but no surgery or medication repairs the spinal cord. None of the treatments help in neuronal or axonal regeneration. Due to loss of functions, the SCI patients have a high level of dependency on the care taker. Rehabilitation and assistive devices are used to improve functions like ambulation and hand functions but while functional improvements are seen in the patients, residual disability always remains. Affected sensations, loss of bladder bowel control and altered muscle tone, are some of the major complications observed in SCI. The currently available treatment modalities fail to improve these complications in cases of severe injuries.

It is also important to track the cellular changes occurring in the spinal cord over the period of any intervention. However, there is no potent investigative monitoring tool available currently to record these changes. Since, there is a global increase in the incidence of spinal cord injuries, establishing a standard treatment is the need of the hour.

Stem cell therapy in spinal cord injury

Stem cell therapy is a potential treatment for spinal cord injury (SCI), and a variety of different stem cell types have been evaluated in animal models and humans with SCI.

Extensive research has been carried out in the past few decades in order to develop stem cell transplantation as a therapeutic intervention for SCI. It mainly focuses on replacing the lost or damaged cells and promoting axonal growth and remyelination of axons. The cells migrate to the site of injury and initiate the repair process. They release trophic factors to stop neuronal degeneration and stimulate angiogenesis. These factors also activate the quiescent cells and recruit them to the injured site. Experimental models have demonstrated the formation of functional neuronal circuits promoting functional recovery. (4-6)



Pre-clinical studies

Various animal studies have been conducted in the past to establish role of stem cell therapy in SCI. A number of different kinds of stem cells have been tested in basic research to study the safety and efficacy. (7-45) The signaling pathways, protein interactions, cellular behavior, and the differentiated fates of experimental cells have been studied extensively in vitro. Moreover, the survival, proliferation, differentiation, and effects on promoting functional recovery of transplanted cells have also been examined in different animal SCI models. (46-54) These pre-clinical studies have helped translate the use of stem cells in humans initiating an array of human clinical studies.

Clinical Studies

Author	Sample size	Type of cells used	Route of administration	Patients improved	Demonstrated safety
Huang , et al	300	fetal olfactory ensheathing cells (OEC)	direct injection	117	no
Kumar, et al	297	Autologous Bone marrow mononuclear cells	Intrathecal	97	yes
Frolov, et al	202	autologous peripheral hematopoietic SC	intrathecal	116	yes
Huang, et al	171	olfactory ensheathing cells	Direct Injection	171	No
Sharma, et al	110	Autologous Bone marrow mononuclear cells	intrathecal	100	yes
Huang. et al	108	olfactory ensheathing cells	Direct Injection	32	yes
Ravikumar, et al	100	Autologous Bone marrow stem cells	intrathecal	18	yes
Sharma , et al	56	Autologous Bone marrow mononuclear cells	intrathecal	50	yes
Cristante, et al	39	Autologous peripheral blood stem cells	intra-arterial	26	yes

Yoon, et al	35	autologous Bone marrow cells + GM-CSF	Direct Injection	7	yes
Saberi, et al	33	Autologous Schwann cells	Direct Injection	0	yes
Pal , et al	30	Autologous Bone marrow mesenchymal cells	intrathecal	25	yes
Liu, et al	22	umbilical cord mesenchymal stem cells	intrathecal	13	yes
Frolov et al	20	autologous peripheral hematopoietic SC	intrathecal	12	no
Syková, et al	20	Autologous Bone marrow stem cells	Intravenous and intra-arterial	6	yes
Lima, et al	20	autologous olfactory mucosal autografts	Direct Injection	15	yes
Abdelaziz, et al	20	autologous adult bone marrow stem cells	intrathecal	6	yes
Jiang , et al	20	autologous bone marrow-derived mesenchymal stem cell	intrathecal	15	yes
Dai , et al.	20	autologous bone marrow mesenchymal stem cells	Direct Injection	10	no
Chernykh , et al	18	Autologous Bone marrow stem cells	Direct injection and intravenous	12	yes
Rabinovich, et al	18	cells from fetal nervous and hemopoietic tissues	intrathecal	6	yes

Kakabadze, et al	18	Autologous Bone marrow mononuclear cells	intrathecal	9	yes
Huang , et al	16	fetal olfactory ensheathing cell (OEC)	direct injection	0	yes
Oh, et al	16	Autologous Mesenchymal stem cells	Intramedullary and subdural	2	yes
Rabinovich , et al	15	Cells from fetal nervous and hemopoietic tissues	Subarachnoidal	11	yes
Mendonca, et al	14	Autologous Bone marrow mesenchymal cells	Direct Injection	14	yes
Derakhshanrad, et al	12	Autologous Peripheral nerve grafts	Direct Injection	11	yes
Wu, et al	11	fetal olfactory ensheathing glia transplantation (OEGT)	direct injection	11	yes
et al	11	autologous BMC transplantation	intrathecal	5	yes
	10	Autologous Bone marrow mesenchymal cells	Direct Injection	6	yes
Deda, et al	9	autologous Bone marrow derived hematopoietic progenitor stem cell transplantation	Direct injection, IV, intrathecal	9	yes

Geffner, et al	8	Autologous Bone marrow stem cells	multiple routes: directly into the spinal cord and the spinal canal, and intravenous.	8	yes
Ra, et al	8	Autologous Adipose tissue-derived mesenchymal stem cells	intravenous	1	yes
Moviglia, et al	8	Autologous Bone marrow mononuclear cells and neural stem cells	Intravenous and intra-arterial	7	yes
Knoller, et al	8	autologous macrophages	Direct Injection	3	yes
Rao , et al	8	autologous olfactory ensheathing cell	Direct Injection	5	yes
Yazdani, et al	8	bone marrow mesenchymal stromal cell and Schwann cell	Direct Injection	3	yes
Satti, et al	8	Autologous Bone marrow derived mesenchymal stromal cell	intrathecal	Safety profile established	yes
Lima, et al	7	olfactory ensheathing mucosa cells	direct Injection	7	yes
Zhou, et al	6	Autologous Activated Schwann cells	Direct Injection	6	yes
Mackay-Sim, et al	6	autologous olfactory ensheathing cells	Direct Injection	1	yes

Yazdani, et al	6	Bone marrow mesenchymal stem cell (MSC) and Schwann cell(SC)	intrathecal	1	yes
Park, et al	5	Autologous Bone marrow cells + GM-CSF	Direct Injection	5	yes
Saito, et al	5	Autologous Bone marrow stromal cells	intrathecal	5	yes
Subbaiah, et al	5	Autologous Bone marrow stem cells	Direct Injection	2	no
Chhabra, et al	5	Autologous olfactory mucosal cells	Direct Injection	5	yes
Attar, et al	4	Autologous Bone marrow stem cells	Direct Injection	3	yes
Saberi, et al	4	autologous Schwann cell	Direct Injection	1	yes
Feron, et al	3	autologous olfactory ensheathing cells	Direct Injection	0	yes
Ha , et al	3	autologous bone marrow cells + GM-CSF	Direct Injection	3	yes
Tabakow , et al	3	autologous mucosal olfactory ensheathing cell (OEC)	Direct Injection	3	yes
Moviglia, et al	2	autologous Bone marrow neural stem cells	intra-arterial	2	yes
Jarocho, et al	1	bone marrow nucleated cell (BMNC) and mesenchymal stem cell (MSC)	Intravenous and intrathecal	1	yes

Saito, et al	1	Autologous Bone marrow stromal cells	intrathecal	1	yes
Kang, et al	1	umbilical cord blood cells	intrathecal, Direct injection	1	no
Ichim, et al	1	allogeneic umbilical cord blood ex-vivo expanded CD34 and umbilical cord matrix MSC	intrathecal	1	yes
Guest. Et al	1	fetal olfactory bulb derived cells	direct injection	1	no
Vaquero et al	10	autologous mesenchymal stromal cells	Subarachnoid	10	no
Vaquero et al	12	autologous bone marrow mesenchymal stromal cells	intrathecal	12	yes
Levi AD et al	29	Human Neural Stem Cells	Intramedullary	8	yes
Thakkar UG et al	10	autologous adipose tissue derived neuronal differentiated mesenchymal stem cells and hematopoietic stem cells	Intrathecal	10	yes
Shroff et al	11	Human embryonic stem cells	Intramuscular and intravenous	11	yes
Shroff et al	226	Human embryonic stem cells	intravenous	158	yes
Shroff et al	15	Human embryonic stem cells	intravenous		yes
Ghobrial et al	17	human CNS-derived NSCs	intramedullary		

So far 60 studies have been conducted demonstrating a beneficial effect of stem cell therapy in more than 1000 patients. (55-108) Stem cells from different lineages and sub-types have been used in these studies which include autologous bone marrow derived mononuclear stem cells, bone marrow derived mesenchymal stem cells, bone marrow derived stromal cells, adipose derived mesenchymal stem cells, hematopoietic progenitor cells, peripheral hematopoietic cells, activated Schwann cells, olfactory ensheathing cells and umbilical cord mesenchymal stem cells. Improvements have been observed clinically in the form of improved functions and objectively in the form of improved ASIA scores and MRI changes. Post therapeutic adverse events were observed in a few patients such as fever, headache, nausea and vomiting, tingling sensations, spasms, neuropathic sensory symptoms including burning and pain sensations.

Our Results

Published data:

1. A detailed analysis of chronic thoracolumbar SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells (BMMNC's) followed by neurorehabilitation was conducted. The study sample included 110 thoracolumbar SCI patients. The outcome was recorded at a mean follow up of 2 years ±1 month. The outcome measures were Functional Independence Measure (FIM) score, American Spinal Injury Association scale (ASIA) and detailed neurological assessment. Data was statistically analyzed using McNemar's Test to establish significance between the change in symptoms and the intervention.

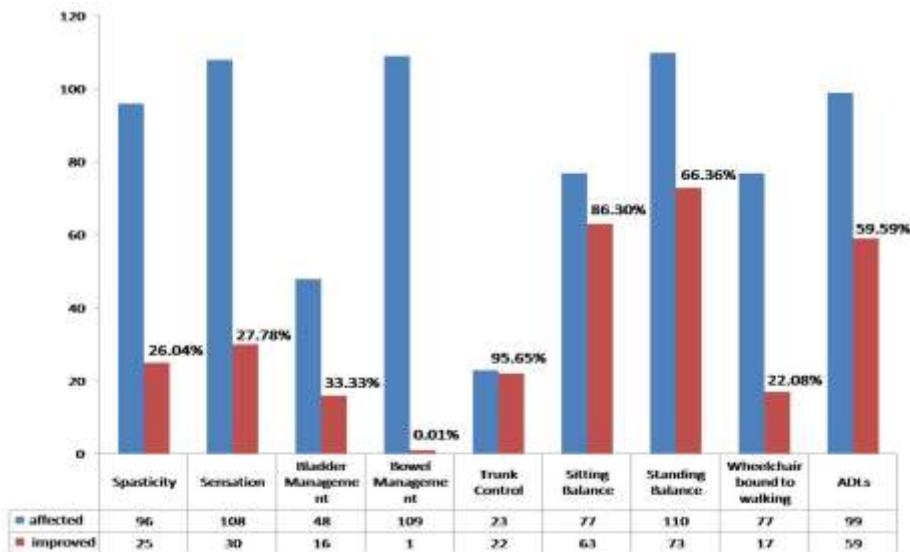


Figure 1: Symptomatic improvements in patients with spinal cord injury after stem cell therapy. The x axis denotes symptoms presented in the patient population and the y axis denotes the number of patients. (ADLs - activities of daily living).

100 out of 110 (91%) patients showed improvements which are shown in the graph below. A statistically significant association of these symptomatic improvements with the cell therapy intervention was established using McNemar's Test. On electrophysiological studies, 2 showed improvement and 1 showed change in functional MRI. (98)

Symptom/ Function	No. of Affected patients (out of 110)	No. of patients improved	Chi-Square Value †	P Value #
Spasticity	96	25	23.04	<0.0001
Sensation	108	30	28.033	<0.0001
Bladder Management	48	16	14.06	0.0002
Bowel Management	109	1	0	1.000*
Trunk Control	23	22	20.045	<0.0001
Sitting Balance	77	63	61.016	<0.0001
Standing Balance	110	73	70.014	<0.0001
Wheelchair bound to walking	77	17	15.059	0.0001
ADLs	99	59	57.017	<0.0001

Table 1: Statistical significance for each symptomatic/functional change using McNemar's test

	Nerve/Sites	Amplitude 2-4 mV (before)	Amplitude 2-4 mV (after)
Patient 1	R Tibial (knee)-AH- Ankle	3.5	5.4
	R Tibial (knee)-AH- Knee	2.7	5.1
	L Tibial (knee)- AH- Ankle	4.1	5.7
	R Tibial (Knee)- Gastrocnemius- Knee	7.2	14.8

	L Tibial (Knee)- Gastrocnemius- Knee	10.2	11.7
Patient 2	L Comm Peroneal- EDB- Ankle	0.8	3.0
	R Comm Peroneal- Tib Ant- Fib Head	1.6	3.4
	L Comm Peroneal- Tib Ant- Fib Head	1.8	4.3
	R Tibial (knee)-AH- Ankle	7.0	8.0
	L Tibial (knee)-AH- Ankle	7.9	8.3
	R Tibial (knee)- gastrocnemius-Knee	6.2	18.7
	L Tibial (knee)- Gastrocnemius- knee	2.5	17.2

Table 2: Objective improvements evident on electromyography (A) and functional magnetic resonance imaging (B) after stem cell therapy in selected patients

2. A detailed analysis of chronic cervical SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells followed by neurorehabilitation was conducted. (99) This study includes 50 patients of chronic cervical SCI. The outcome was recorded at a mean follow up of 2 years \pm 1 month. The outcome measures were Functional Independence Measure (FIM) score, American Spinal Injury Association scale (ASIA) and detailed neurological assessment. Data was statistically analyzed using McNemar's Test to establish significance between the change in symptoms and the intervention. 37 out of 50 (74%) showed improvements. A statistical analysis using McNemar's test established a significant association of these symptoms with the intervention. 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.

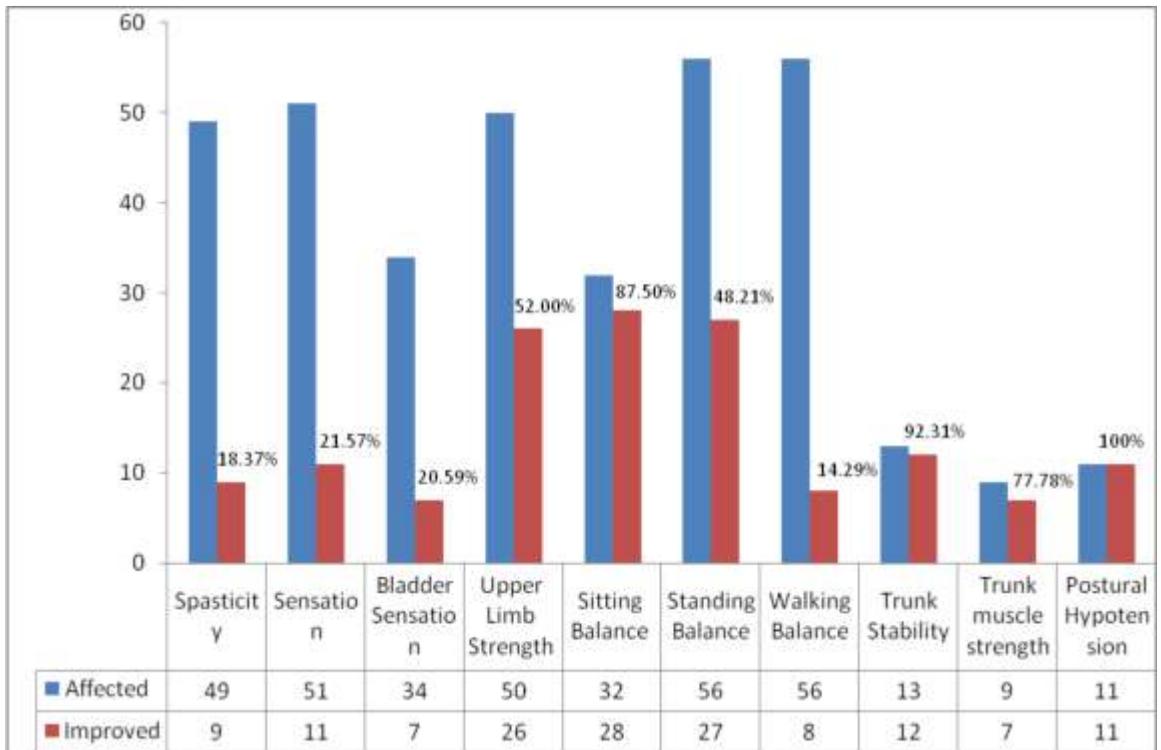


Figure 2: Graph demonstrating symptomatic improvements in chronic cervical SCI patients after cell therapy

Symptom	No. of patients affected	No. of patients improved	McNemars test value	P value
Spasticity	49	9	7.11111	*0.00766
Sensation	51	11	9.09091	*0.00257
Bladder Sensation	34	7	5.14286	*0.02334
Upper Limb Strength	50	26	24.03846	*<0.000001
Sitting Balance	32	28	26.03571	*<0.000001
Standing Balance	56	27	25.03704	*<0.000001
Walking Balance	56	8	6.125	*0.01333
Trunk Stability	13	12	10.08333	*0.0015
Trunk muscle strength	9	7	5.14286	*0.02334
Postural Hypotension	11	11	9.09091	*0.00257

*significant at p value ≤ 0.05

Table 3: McNemar's test: Table demonstrating the statistical analysis for each symptomatic improvement in cervical SCI using McNemar's test.

Factors		Percentage improvements
Age	<18 yrs	100%
	18-35 yrs	41%
	>35 yrs	42%
Cause of Trauma	RTA	37.20%
	Non-RTA	30%
Chronicity	1-3 yrs	47.82%
	3-5 yrs	33.33%
	>5 yrs	44.44%
Rehabilitation	Done	36.84%
	Not Done	55.55%

Table 4: Percentage analysis of improvements: Table demonstrating a detailed analysis of various factors and the improvements.

Symptoms improved	Cervical SCI	Thoracolumbar SCI
Spasticity	18.37%	26%
Sensation	21.57%	28%
Bladder Sensation	20.59%	33%
Bowel Sensation	5.66%	0.9%
Sitting Balance	87.50%	81.81%
Standing Balance	48.21%	66.36
Trunk Stability	92.31%	95.65%
Postural Hypotension	100.00%	100%

Table 5: Comparison between Cervical SCI and Thoracolumbar SCI: Table comparing the outcome of cell transplantation in cervical SCI and Thoracolumbar SCI.

Published case reports

We have published 5 case reports of different types of Spinal cord injuries. (109-113) These cases underwent autologous bone marrow mononuclear cell transplantation intrathecally. They demonstrated various functional and neurological improvements in the form of improved symptoms. Improvements were seen in

bladder and bowel sensations, back and abdominal strength, limb strength, gait, balance and mobility. They showed improved scores on FIM scale indicating improved ability to perform daily tasks. It was observed that their quality of life had improved significantly.

Unpublished data

Thoracic Spinal Cord Injury:

We analyzed 184 patients with chronic thoracic spinal cord injury to study the effect of stem cell therapy. Changes were recorded in symptoms like muscle tone, lower limb activity, sensory changes, bowel/bladder function, trunk activity, balance, standing, ambulation and activities of daily living. Analysis revealed that out of 184, 96.19% patients showed improvements while 3.80% showed no improvements in any of the symptoms. Mild improvements were observed in 15.21% of patients, moderate in 56.52% of patients, whereas, 24.46% of patients showed significant improvements

Improvements in Thoracolumbar Spinal Cord Injury After Stem Cell Therapy

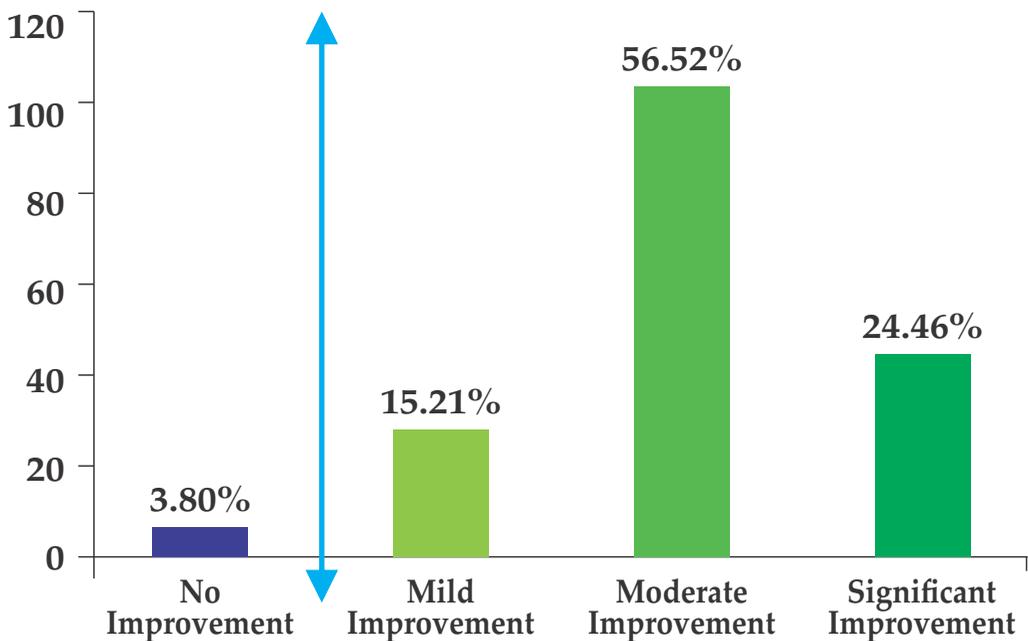


Figure 3: Improvements seen in thoracolumbar SCI after intrathecal administration of autologous BMMNCs.

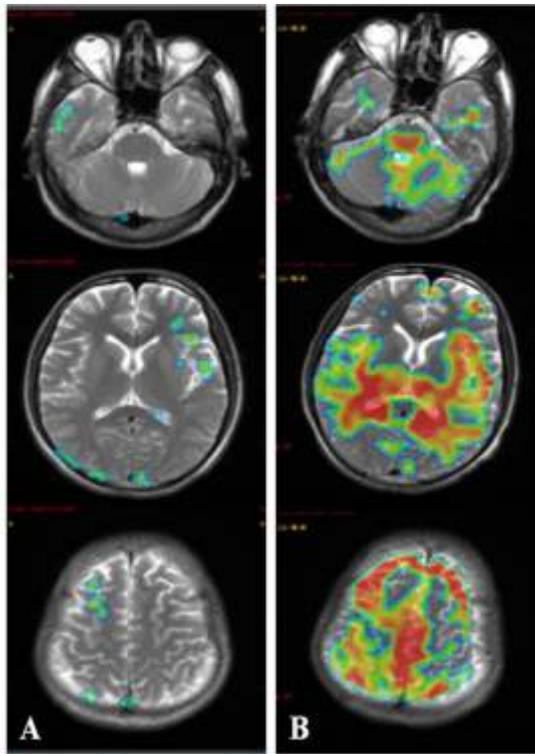


Figure 4:

A. fMRI-T2 weighted image showing the brain activation during the task before the cell therapy

B. fMRI-T2 weighted image showing the brain activation during the task after the cell therapy. The brain show increased activation in motor cortex and associated regions

Cervical Spinal Cord Injury:

104 patients with diagnosis of cervical spinal cord injury were included in the analysis. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant improvements. The symptoms included were muscle tone, upper limb activity, lower limb activity, sensory changes, bowel/bladder function, trunk activity, balance, standing, ambulation and activities of daily living. Analysis revealed that out of 104 patients, 96.19% patients showed improvements while 3.80% did not show any improvements. Mild improvements were observed in 15.21% of patients, moderate in 56.52% of patients and 24.46% of patients showed significant improvements.

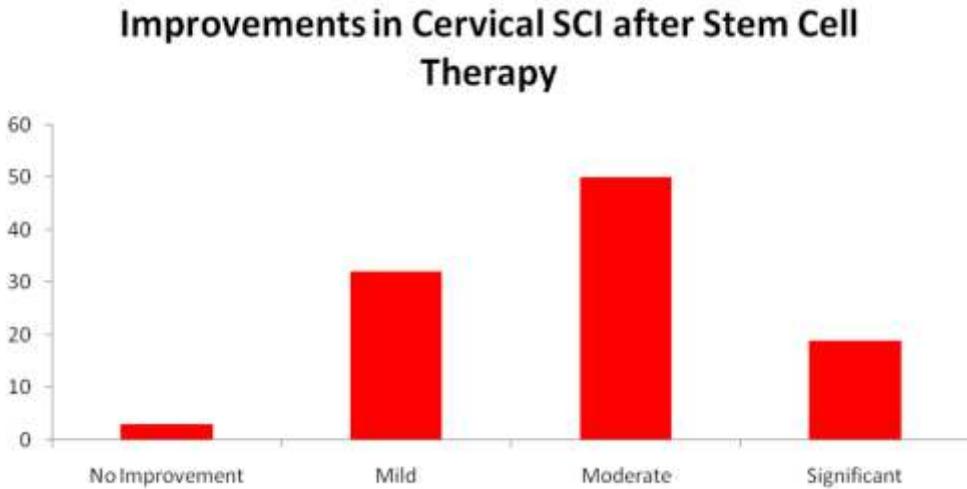


Figure 5: Improvements seen in cervical SCI after intrathecal administration of autologous BMMNCs.

Conclusion

It has long been considered that spinal cord injury is an irreversible condition causing permanent debilitation. However, recent advancements in the field of regenerative medicine have disproved that assumption. While stem cell therapy is not a cure for SCI, the neuro-regenerative and reparative effect of stem cells enhances the outcome of the currently available rehabilitative and medical treatments. The safety and efficacy of stem cell therapy has been established in the clinical studies done so far. Factors that can influence the outcome of stem cell therapy in SCI are (A) Level of injury (patients with thoraco-lumbar level of injury have a better prognosis than those with cervical level of injury) (B) Incomplete SCI have a better prognosis than complete injury (C) The earlier the treatment initiated the better the prognosis (D) Rehabilitation is essential for maximizing functional recovery in patients. Thus the benefits of stem cell therapy are clear to see in the clinical studies conducted so far and the results seen have been very encouraging and offer much hope for the future.

Future Directions

In SCI, rapid loss of the oligodendrocytes is recorded. The quiescent endogenous ependymal cells which are activated after the injury are unable to differentiate into the required cells of oligodendrocyte lineage failing to limit the damage. Also, the microenvironment of the injured spinal cord prevents neuronal differentiation of the transplanted cells due to the pro-gliogenic signals. Hence, future research should focus on manipulating the cells before transplantation or infusing neurotrophic growth factors in order to stimulate the endogenous cells and modulate them towards producing more oligodendrocytes. (114) The future of

regenerative medicine in SCI lies in combining the use of stem cells with nanodrug delivery systems. (115) Recently, the stem cells are being co-transplanted with nanospheres improving the cell survival and neurological functions in the animal models. However, their long term safety needs to be assessed. Cells of varied origin such as dental pulp, adipose tissue and other induced pluripotent cells are being studied extensively to test their potency, safety, feasibility and efficacy in SCI. (116-118) Nanofiber scaffold formulations have been used successfully for targeted cell delivery into the body organs. (119) Neuralstem has developed NSI-566 neural stem cells which it seeks to deliver directly into the spinal cord gray matter in SCI patients. These cells are expected to integrate with the patient's neural tissue and potentially form new neural circuits to connect and bridge axons above the site of injury with neuron segments below. (120)

Many clinical trials are being conducted in the USA, China, India, Switzerland to optimize the intervention, find the appropriate time and frequency of injection, type of cells, route of administration, etc. (121)

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Our natural power is sapped by the parasites of the centuries: fear, superstition, a view of reality that reduces life's wonders to creaking machinery. If we starve these parasitic beliefs they will die. But we rationalize our fatigue, our inertia; we deny that we are haunted.

Our choice, is between the painful but confidence instilling process of coming to know who and where we are and the immensely appealing but finally empty alternative of continuing to drift, of acting as if we know what we are doing when both the mounting evidence and our most honest fears indicate that we do not....In government, as in other relationships, we have the capacity to deceive ourselves, to shape the realities by which we live, so that our prime focus is on our comfort rather than the truth"

- Marilyn Ferguson

12

Stem Cell Transplantation In Stroke

Angiogenesis, synaptogenesis and neurogenesis functions of stem cells help in recovery of Stroke.

Stroke is classically characterized as a neurological deficit attributed to an acute focal injury of the central nervous system (CNS) by a vascular cause, including cerebral infarction, intracerebral hemorrhage (ICH), and subarachnoid hemorrhage (SAH), and is a major cause of disability and death worldwide. (1) It is caused by a sudden interruption of the blood supply to the brain leading to reduced oxygen and nutrient supply in that area. The two major types of stroke are ischemic and hemorrhagic. In ischemic stroke, decreased or absent circulating blood deprives neurons of necessary substrates. Intracerebral hemorrhage originates from deep penetrating vessels and causes injury to brain tissue by disrupting connecting pathways and causing localized pressure injury. The extent of neurological involvement may range from mild motor deficit to gross involvement of various function namely sensorimotor, perceptual, emotional, behavioral, memory intelligence, speech and language function, ultimately affecting the activities of daily living.

Acute medical management is based on the type of stroke where, ischemic stroke is treated by thrombolysis or anticoagulation medications. For hemorrhagic stroke, management is focused at the underlying cause of bleed, that is reduce blood pressure or treatment of aneurysm etc. Medical and surgical strategies aim at prevention of recurrence of stroke. Stroke rehabilitation remains the cornerstone for patients with stroke, and should be initiated as early as possible. Most return of function is seen in the first few months, and then improvement falls off with the

"window" considered officially by U.S. state rehabilitation units and others to be closed after six months, with little chance of further improvement.

Unmet medical needs

With the current treatment approaches, medical, surgical or rehabilitative, the pathophysiological processes and the resultant damage occurring at the microcellular level cannot be reversed. This permanent change in the structure of CNS leads to long lasting physical impairments, seen as residual problems, which translate gradually into activity limitation and restricts these individuals to participate in the community. There have been many advances in the medical management of acute stroke but, little has changed to address the residual deficits of chronic stroke. A treatment approach, which changes the physiology at the neuronal level, is the need of the hour. Cell therapy offers hope for stroke patients, especially for those who have missed the "window".

Role of stem cell therapy in chronic stroke:

Stem cells impersonate the natural process of recovery after stroke, which is mobilization of stem cells, originally present in the bone marrow, to the area of injury in the brain. This occurs with the release of certain factors. This mobilization of stem cells to the injured brain initiates the process of neurorestoration. These stem cells secrete various growth factors like VEGF, bFGF and BDNF which support and amplify angiogenesis, neurogenesis and synaptic plasticity at the penumbral region. Along with the above neuroreparative processes, the stem cells also decrease the glial scar formation and promote glial-axonal remodeling which is seen in chronic stroke (2-5). The number of stem cells mobilized after acute stroke starts decreasing as chronic stage approaches. Therefore as time passes by the rate of recovery also reduces in the chronic stage. This forms the rationale that if more number of stem cells are supplied to the injured area in the chronic stage, it may hasten and increase the chances of recovery.

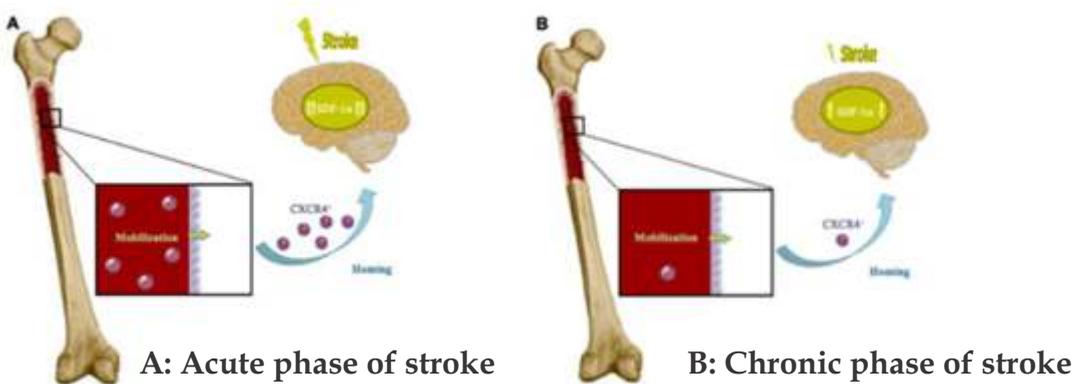


Figure 1: Natural process of mobilization of endogenous bone marrow stem cells after stroke.

Courtesy: Borlongan CV, Glover LE, Tajiri N, Kaneko Y, Freeman TB. The Great Migration of Bone Marrow-Derived Stem Cells Toward the Ischemic Brain: Therapeutic Implications for Stroke and Other Neurological Disorders. *Progress in neurobiology*. 2011;95(2):213-228. doi:10.1016/j.pneurobio.2011.08.005.

Stem cell therapy in Stroke

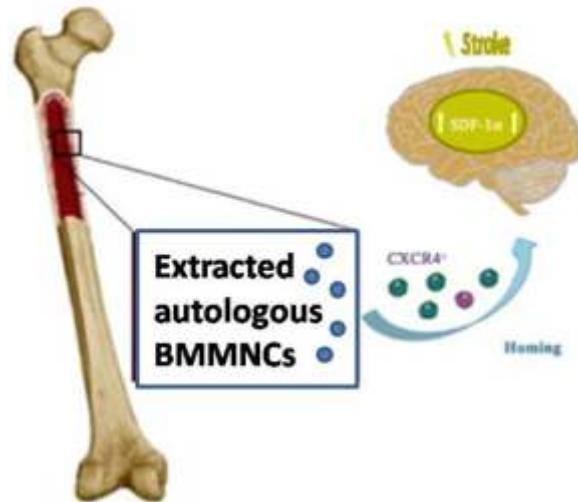


Figure 2: The transplanted autologous BMMNCs provide large number of stem cells in the chronic phase which mimic the natural pathway (depicted in figure 1) to repair the damaged brain areas.

Pre-clinical studies:

There are various studies performed on animals, to assess the effects of stem cell therapy in improving the outcomes post stroke. The findings of these studies included increased angiogenesis at the site of the infarct, increased modulation of neurotrophic growth factors, and reduction in the infarct volumes. They exhibited improved functional performance and restore neurological deficits (6-14).

A systematic review and meta-analysis of the pre-clinical studies using MSCs for stroke was conducted by Quynh et al. 2016. They identified 46 relevant studies that analyzed the effect of MSCs transplantation for Ischemic stroke. Out of these, 44 studies showed a significant improvement in the neurological severity score and other motor function tests. There was also a significant reduction in infarct size of the treated rats. Greater improvements were noted in rats treated with intracerebral routes followed by intra-arterial and intra-venous routes. The study did not find any significant change in the outcomes when the cells were delivered within 24 hours of the stroke or after 24 hours of the stroke. The mechanism of action in acute stage is presumed to be reduction of ongoing injury to neural tissue whereas in subacute stage stem cells promote neural repair (15).

Clinical studies

Reference	No of patients	Patients improved	Stem cell type	Route of administration	Advere effects
Kondziolka D et al.	12	6	Neuronal cells	IntraCerebral	None
Bang OY et al.	5	5	Autologous MSCs	Intravenous	None
Rabinovich SS et al.	1	1	Immature nervous and hematopoetic cell suspension	Subarachnoid	None
Kondziolka D et al.	14	6	Human Neural stem cells	IntraCerebral	Seizure and syncopal event
Savitz SI et al.	1	1	Fetal procrine cells	IntraCerebral	Seizure
Mendonça MI et al.	5	5	Autologus Bone Marrow Mononuclear cells	Intraarterial	None
Man Y et al.	12	8	Allogenic Bone Marrow Mononuclear cells	IntraCerebral	None
Suárez-Monteagudo C et al.	6	5	Autologous MSCs	IntraCerebral	None
Lee JS et al.	16	12	Autologous MSCs	Intravenous	Seizures
Honmou O et al.	12	12	Autologous MSCs	Intravenous	None
Bhasin A et al.	6	6	MSCs	Intravenous	None

Battistella V et al.	6	6	Autologous Bone Marrow Mononuclear cells	Intraarterial	None
Han H et al.	1	1	HUCB MSCs	Intrathecal	None
Savitz SI et al.	10	10	Bone Marrow Mononuclear cells	Intravenous	Pulmonary infarct
Moniche F et al.	10	10	Bone Marrow Mononuclear cells	Intraarterial	Isolated partial seizures
Friedrich MA et al.	20	14	Bone Marrow Mononuclear cells	Intraarterial	None
Bhasin A et al.	40	40	Bone Marrow Mononuclear cells	Intravenous	None
Prasad K et al.	58	0	Bone Marrow Mononuclear cells	Intravenous	None
Li ZM et al.	60	60	Bone Marrow Mononuclear cells + MSCs	IntraCerebral	None
Chen L et al.	120	120	OEC, NPC, UCMSC, Schwann cells	IntraCerebral	None
Jiang Y et al.	4	4	UCMSCs	Intraarterial	None
Banerjee S et al.	5	5	Autologous CD34+	Intraarterial	None
Sharma A et al.	24	24	Bone Marrow Mononuclear cells	Intrathecal	None
Prasad K et al.	120	0	Bone Marrow Mononuclear cells	Intravenous	None
Moniche F et al.	8	8	Bone Marrow Mononuclear cells	Intraarterial	None

Qiao LY et al.	18	18	NSPCs and MSCs	Intravenous	Low grade fever
Moniche F et al.	10	7	Bone Marrow Mononuclear cells	Intraarterial	None
Taguchi A et al.	12	12	Bone Marrow Mononuclear cells	Intravenous	Pneumonia
Zhu J et al.	110	110	Bone Marrow Mononuclear cells + MSCs	IntraCerebral	None
David C Hess, Lawrence R Wechsler	129		adult progenitor cells	Intravenous	None
Bhasin A1, Kumaran SS2, Bhatia R1,	12	6	autologous MSCs	Intravenous	None
Dheeraj Kalladka, John Sinden, Kenneth Pollock,	11	11	Neural stem cells	Intracerebral	None
Ashu Bhasin a M.V. Padma Srivastava	20	20	Bone Marrow Mononuclear cells	Intravenous	None

Total 33 studies were published demonstrating the benefits of stem cell therapy in stroke. Different cell types studied in these articles were Autologous bone marrow mononuclear cells (BMMNCs), Autologous and allogenic mesenchymal cells (MSCs), Fetal porcine cells, Neural stem cells (NSCs), Immature neurons and hematopoietic cells, Olfactory ensheathing cells (OECs). Different routes of administration studied in the articles were Intracranial (IC), Intravenous (IV), Intraarterial (IA), Intrathecal (IT), Subarachnoid.

All these studies unanimously suggested that the transplantation of various types of stem cells was safe using various routes of administration. Although some adverse events were noted in these studies none of them were related to cell transplantation. Many studies showed that there was a statistically significant difference in the pre and post measures of Barthel Index, Modified Rankin Scale, National Institute of Health Stroke Score, Functional Independence Measure and Frugal Myer scale after stem cell therapy. Along with the functional improvements

there were improvements in the metabolism as measured on PET-CT scan and some studies showed reduction in the lesion size on MRI. (16-43)

Our results

Published data

We performed a study to demonstrate the effect of intrathecal administration of autologous bone marrow mononuclear cells (BMMNCs) on the recovery process of patients with chronic stroke. (43) 24 patients diagnosed with chronic stroke were administered cell therapy, followed by multidisciplinary neurorehabilitation. They were assessed on functional independence measure (FIM) objectively, along with assessment of standing and walking balance, ambulation, and hand functions. Out of 24 patients, 12 improved in ambulation, 10 in hand functions, 6 in standing balance, and 9 in walking balance. Patients with age less than 60 years showed a higher percentage of improvement in the areas of ambulation, hand functions, and sitting and standing balance, as compared to the patients with age more than 60 years. They also showed improvement in the FIM scores. Time since the stroke episode also seemed to have an effect on the recovery of patients. The percentage of improvement was higher in patients, whose episode of stroke was less than 2 years old, as compared to those patients whose stroke was older than 2 years. Patients with ischemic type of stroke had better outcomes in all the mentioned areas, as compared to those with hemorrhagic stroke. Also, patients with right brain involvement showed higher percentage of improvement in area of ambulation, standing balance, and walking balance, as compared to the left brain. There was a statistically significant difference ($p < 0.05$) seen in FIM scores before and after the cell therapy.

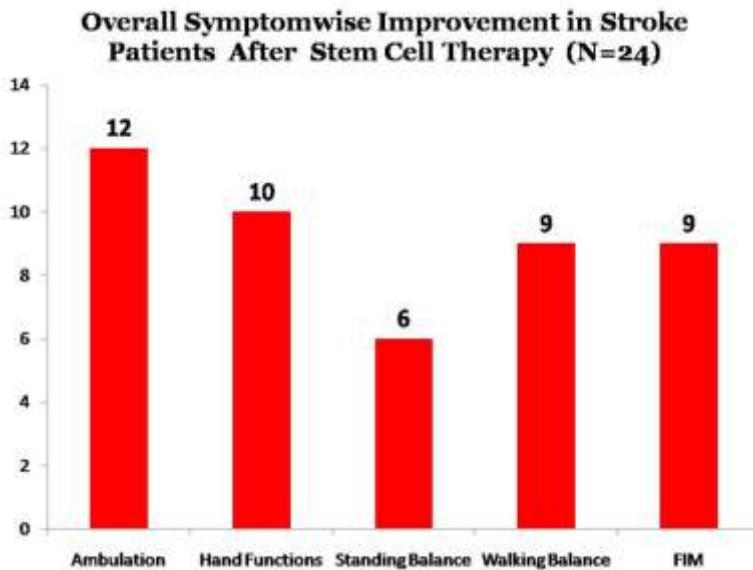


Figure 3: Symptom-wise improvements seen in patients of stroke after intrathecal administration of autologous BMMNCs.

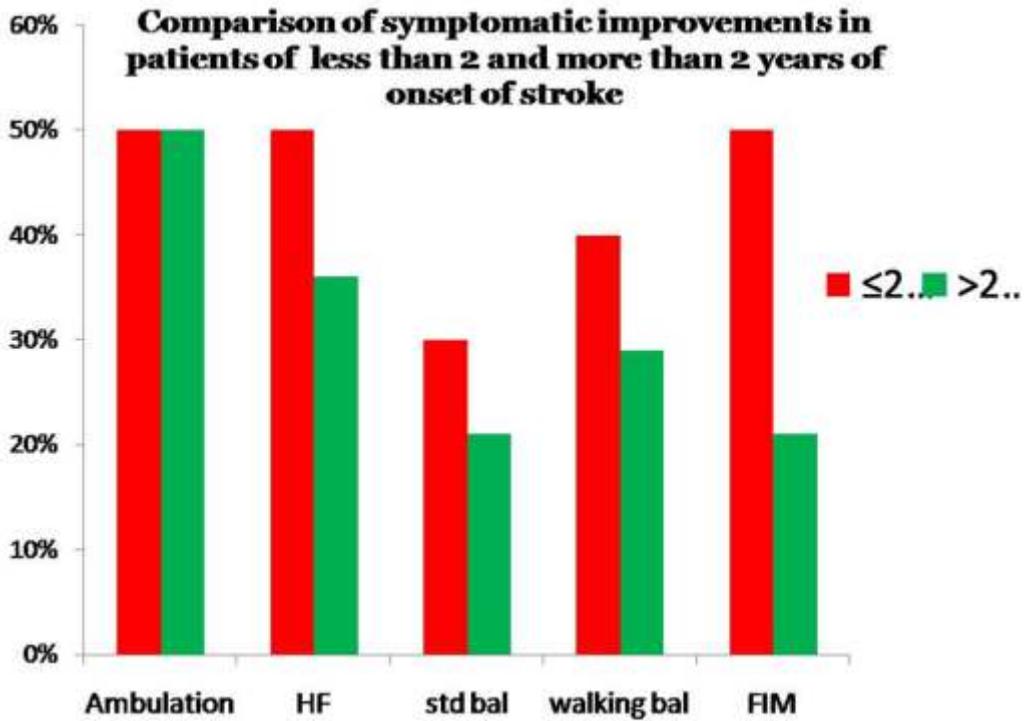


Figure 4: Comparison of symptomatic improvements in patients according to duration since onset of stroke.

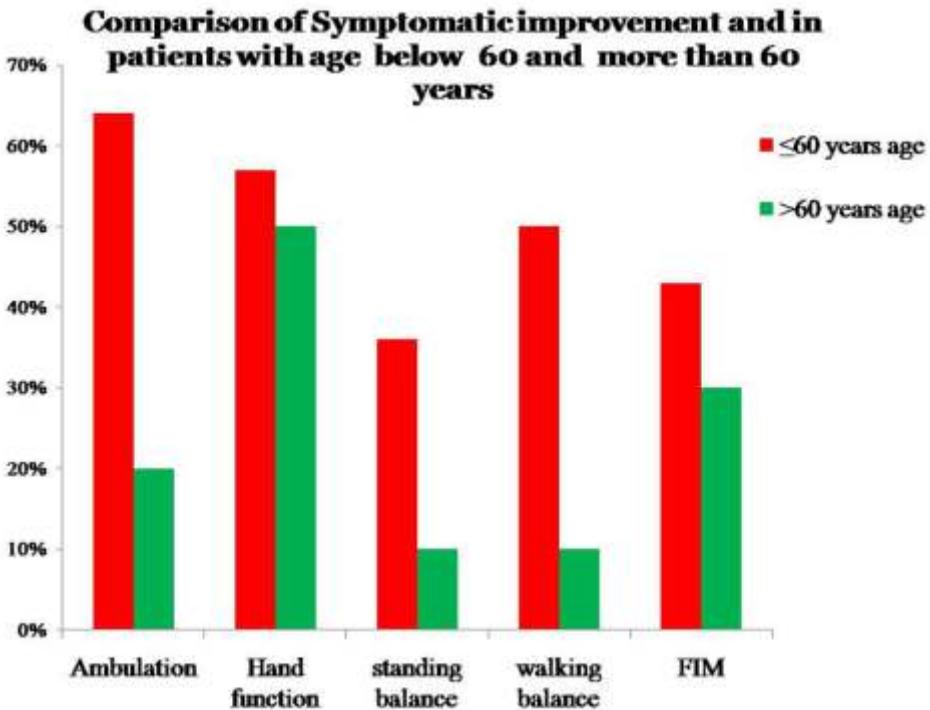


Figure 5: Age-wise comparison of symptomatic improvements in patients of stroke.

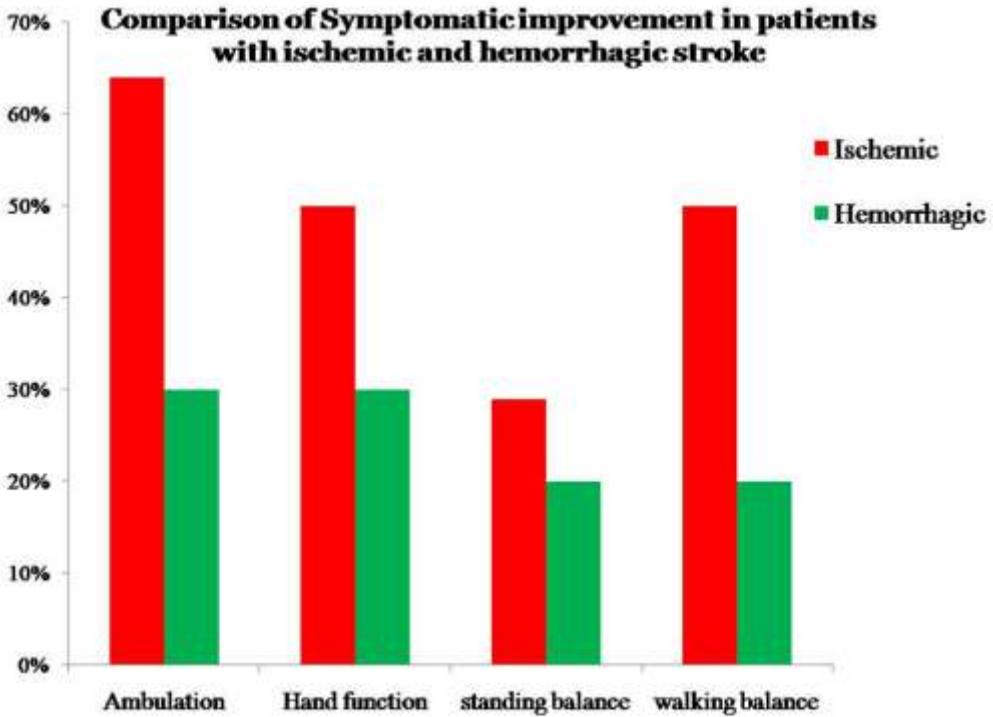


Figure 6: Comparison of symptomatic improvements according to the type of stroke.

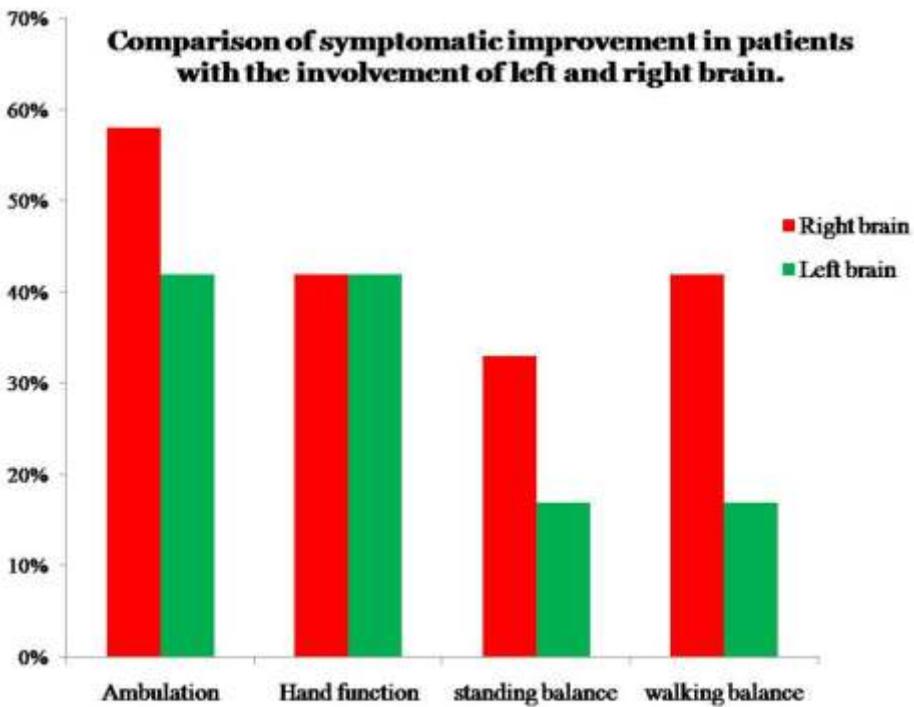
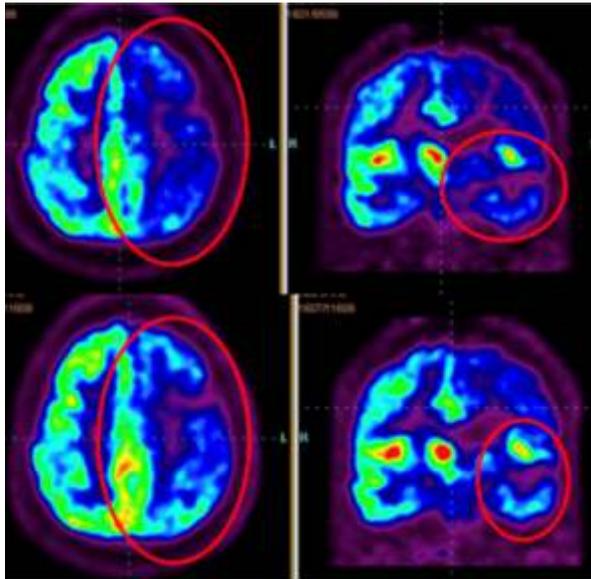


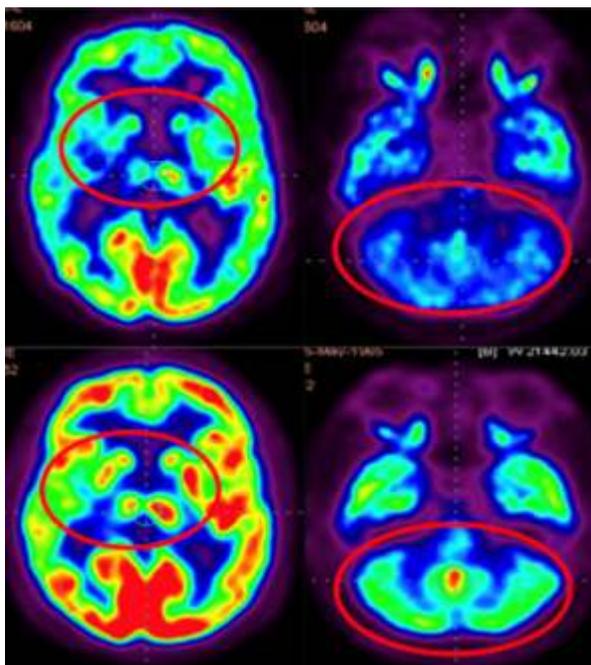
Figure 7: Comparison of symptomatic improvements according to side of the brain involved.

We published 4 case reports of patients with different types of stroke in various peer reviewed journals. These cases underwent intrathecal autologous bone marrow derived mononuclear cell transplantation. All these patients showed functional and neurological improvements on follow up. Improvements were seen in symptoms such as walking and standing balance, hand grip, voluntary control, spasticity, speech, mobility, etc. These patients also showed improved brain metabolism on PET CT scan brain. (44-47)

Objective improvement on PET-CT scan seen in patients treated with autologous BMMNCs



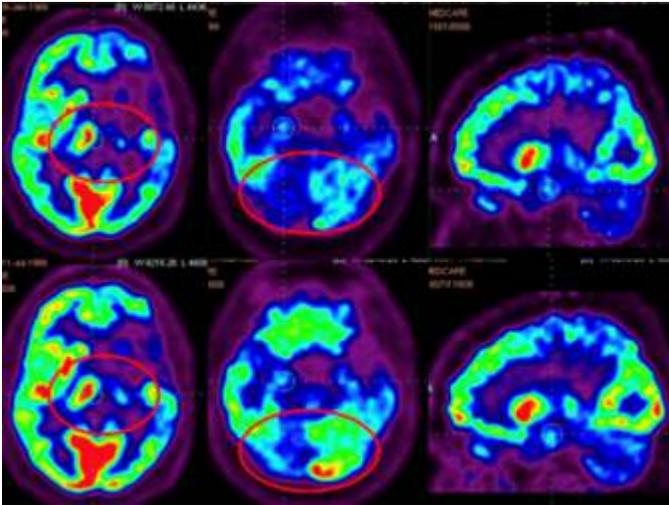
Post PET scan following cellular therapy showed significant improvement in Right superior frontal cortex, sensory motor cortex, bilateral posterior cingulate, right temporal cortex



Before Stem Cell Therapy

Significant improvement in metabolism is observed in right thalamus, basal ganglia and cerebellum

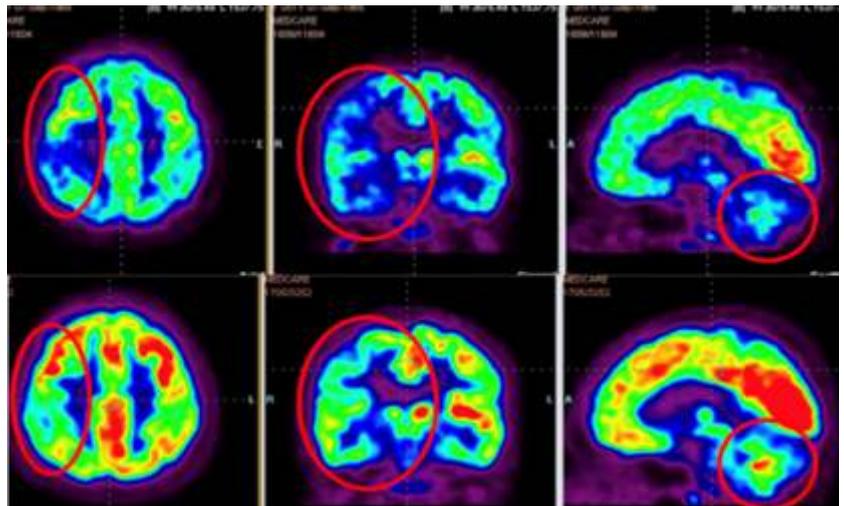
After Stem Cell Therapy



Post PET scan following cellular therapy showed significant improvement in Left thalamus, bilateral medial temporal cortex and cerebellum

**Before
Stem Cell
Therapy**

**After
Stem Cell
Therapy**



Significant improvement in metabolism is observed in bilateral frontal-parietal cortex, right thalamus, temporal cortex and cerebellum

Future directions

There are many areas which needs to be analyzed in depth, to gain the best outcomes out of cell therapy. The question of best cell type for transplantation with stroke needs to be addressed. To optimize cell therapies in stroke, it is also necessary to elucidate the molecular mechanisms controlling the interaction of the grafted cells with the ischemic brain, as the post ischemic environment can affect the function of transplanted stem cells, which in turn can modulate the inflammatory response and the local microenvironment. Timing of transplantation in different time windows needs to be assessed in detail, as most of the studies takes into account acute, sub acute and chronic stroke. This is crucial to analyze the effect of cell therapy at various stages. Appropriate dosage remains unclear. A dose-

response correlation is an important aspect of cell therapy. Routes of administration are an important area which decides the intensity of effect of cell therapy. Objective imaging needs to be introduced into clinical trials, to get an insight into the physiological processes occurring at the cellular level after cell therapy, to strengthen the results obtained.

Conclusion:

Autologous bone marrow mononuclear cells facilitate recovery after stroke by facilitating neuroregeneration and enhancing neural repair through various paracrine mechanisms like neoangiogenesis, immunomodulation, stimulation of resident stem cells, secretion of various neurotrophic factors and growth factors. It mimics the natural recovery process in the body. Autologous bone marrow mononuclear cells intrathecal transplantation combined with multi-disciplinary neurorehabilitation can provide significant clinical benefit in acute as well as chronic stroke patients. It is a safe treatment option and improvements in various symptoms of stroke as well as activities of daily living can be noted however, it is not a curative treatment. Various factors may influence this recovery some of which are like age, time since stroke and type of stroke may influence the process of recovery.

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Do not fear to defend new ideas even the most revolutionary, your own faith is what counts most. But have the courage also to admit an error as soon as you have proved it to yourself, that your idea is wrong. Science is the graveyard of ideas. But some ideas that seem dead and buried away may at one time or another rise up to life again more vital than ever"

-Louis Pasteur

13

Role of Stem Cells In Motor Neuron Disease / Amyotrophic Lateral Sclerosis

“Stem Cell Research is the KEY to developing cures for degenerative conditions like Parkinson’s and Motor Neuron Disease from which I and many others suffer.” - Stephen Hawking

Motor neuron disease [MND] is a progressive disorder characterized by weakness of muscles and selective degeneration of motor nerves [1]. The disease has poor prognosis and has poor life expectancy. The disease affects only motor neurons and sparing the sensory system. There is no conclusive evidence about the causative or risk factors for the disease [1]. Motor neuron disease connotes a group of diseases that can lead variety of upper and lower motor neuron symptoms like cramps, fasciculation, dysarthria and dysphagia.

Amyotrophic Lateral Sclerosis [ALS] is a form of MND which is characterized by fast progression and presence of both UMN and LMN symptoms in the trunk, extremities and bulbar regions. The life expectancy of patients with ALS is very poor of up to 3 to 5 years since the onset of the disease [2,3,4,5]. The onset of the disease may be from weakness in the extremities or bulbar region. As the disease progresses the weakness spreads to all the regions of the body. The terminal symptoms of the disease are caused due to weakness of respiratory muscles leading to respiratory insufficiency and eventually respiratory failure [1]. Common symptoms of the disease are weakness in the distal muscles of the extremities,

fasciculations and cramps in all the parts of the body, emotional disturbances, dysarthria, dysphagia, fatigue and spasticity. Reflexes may be exaggerated and Hoffmann's sign may also be positive. ALS significantly hampers the quality of life of patients due to increased dependence for performing activities of daily living as the disease progresses. The presentation of the disease is variable and has different rates of progression. Some factors have been identified, which may be responsible for poor prognosis, such as, presence of LMN features, old age, bulbar onset, low forced vital capacity [FVC] and low scores on revised ALS- functional rating scale [ALS-FRSr] [6,7]. Various other neurodegenerative disorders also present with similar UMN and LMN symptoms. The diseases that are categorized as MND, also present with similar symptoms as observed in ALS [8]. ALS/MND is of two types familial (10%) i.e. with known genetic involvement, sporadic (90%) with no underlying genetic abnormality.

However to improve the quality of life and complications developed secondary to prolonged and progressive muscle weakness, multi-disciplinary care is required. Commonly the management includes pharmacological intervention, rehabilitation, nutritional advice, good nursing care, artificial ventilator support in the later stages of the disease and Percutaneous endoscopic gastrostomy[PEG] preventing dysphagia related complications [9].

Pathophysiology of ALS

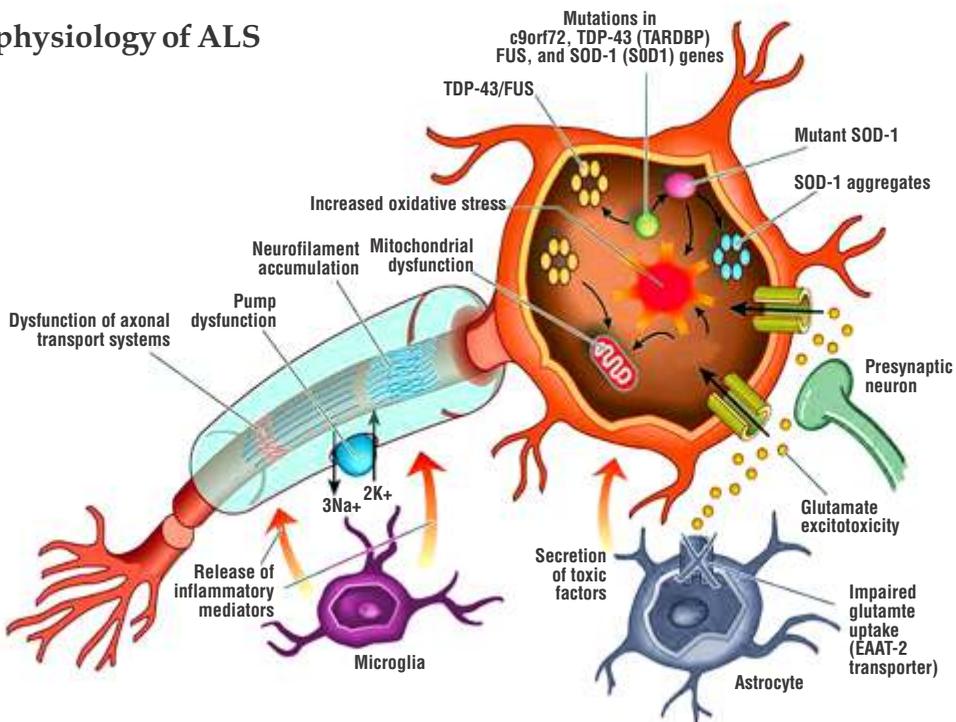


Figure 1: A diagram depicting various pathophysiological mechanisms causing ALS/MND - genetic mutations, glutamate toxicity, inflammation and neurotoxicity due to glial cell response, axonal dysfunction, increased oxidative stress and mitochondrial dysfunction.

Unmet medical needs

The progression of the disease is rapid, presentation of the disease is varied and the causes are not understood. It is therefore challenging to find a medical cure for the disease. All the conventional treatments available manage the symptoms and associated conditions, failing to address the core pathology of ALS. There is a need to develop a treatment strategy to halt or arrest the progression of the disease and eventually a treatment that will reverse the symptoms and cure the disease.

Stem cell therapy for ALS

Stem cell transplantation is a promising new therapy for ALS. Different cell types, routes of administration and different protocols of administration are being studied widely world over.

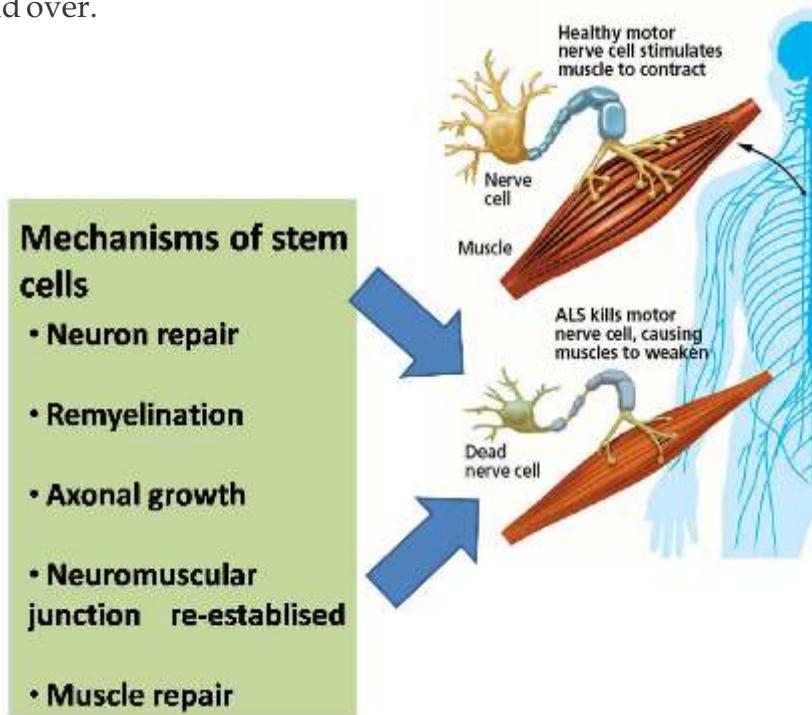


Figure 2: Mechanism of action of stem cells in MND/ALS.

Replacement of degenerated motor neurons is the ultimate goal of transplantation therapy but various factors influence the outcome of the transplanted cells. Survival of the cells in the host environment, their neurogenic potential, actual neurogenesis at the target site and formation of neuronal connections over long distances are some of the factors [32,33]. As the transplantation science evolves these factors could be monitored to gain appropriate outcome but currently the aim of transplantation is to protect the existing motor neurons and attempt to bring out regeneration and repair in the damaged motor neurons. Although stem cells have neurogenic potential their fate is dependent on various factors. They have a neurotrophic influence on the nervous system and can home onto the site of injury

[34]. They further demonstrate immunomodulatory, anti-inflammatory and cytoprotective properties [35]. The factors secreted by these cells bring about neoangiogenesis [36]. These paracrine effects lead to neuroprotection and subsequent alteration in the disease course and progression [37].

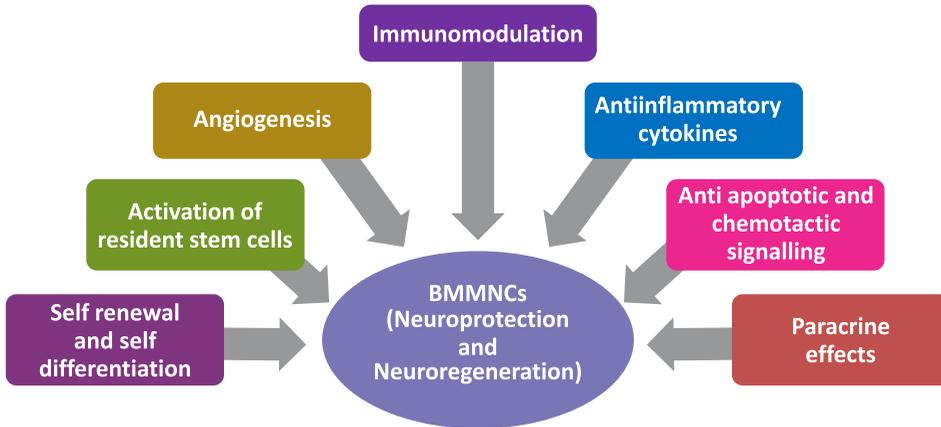


Figure 3: Mechanism of Action of cellular therapy in ALS

Animal Studies

Zhou et al. 2013 [10] have shown that intrathecal transplantation of human bone marrow stromal cells in SOD₁ transgenic mice, reduced the inflammatory glial response and facilitates secretion of anti-inflammatory cytokines.

Pastor D et al. 2013 [11] suggested that bone marrow injected in the muscles may have neuroprotective effects and prevent the death of motor neurons.

Chen B K et al. 2015 [12] carried out the study for safety of the intrathecal delivery of bone marrow mesenchymal stromal cells that showed that stem cell transplantation was safe in the rabbit model of the disease.

Moura et al. 2016 [13] conducted a review and meta-analysis of 10 preclinical studies that studied the effect of cell transplantation on disease progression, survival duration and motor function in mouse model of the disease. Various routes of administration used were intrathecal, intravenous, intraventricular and intraspinal. Histopathologically the nervous tissue obtained from the patients showed better survival of motor neuron and glial cells. It also suggested more proliferation of the glial cells but with lesser density post transplantation. Gliosis of neural tissue was also significantly reduced post transplantation. Clinically the studies suggested slower rate of disease progression and significant survival benefit in rats treated with transplantation. These positive effects can be attributed to the production of neurotrophic factors and the reduction of microgliosis and macrogliosis.

Human studies

In a Phase I clinical trial, human spinal cord-derived neural stem cells were transplanted into the spinal cord of 12 late- to mid-stage ALS patients [14]. There were no long-term surgical complications and importantly, ALS patients tolerated the procedure, gave no indications that the stem cells were injurious to the spinal cord and showed no disease acceleration due to injections. Second part of this Phase 1 trial determined the safety of injecting cells into the C3–C5 cervical region of the spinal cord [15]. Three new ALS patients and three patients that had previously received lumbar injections received human spinal cord-derived neural stem cells. There were some positive effects including slowing of rate of progression, and one patient even showed improvement in clinical status. In an expansion of the study, Mazzini et al. transplanted human fetal brain tissues into the anterior horns of the spinal cord and additionally used a much higher cell dosage [16]. The authors concluded that the procedure was well tolerated and safe

In 2003, Mazzini and colleagues injected variable numbers of mesenchymal stem cells (MSCs) ($7\text{--}152 \times 10^6$ cells) into the thoracic spinal cord of seven ALS patients [17]. Although there was no functional improvement, the transplantation of these cell suspensions into the human ALS spinal cord was safe and well tolerated. Their follow-up studies, lasting more than 4 years post-surgery, showed no signs of toxicity or abnormal cell growth [18,19]. Four patients showed a significant slowing down of the linear decline of the forced vital capacity and of the ALS-FRS score. A phase I/II clinical trial by Karussis and colleagues showed that intrathecal and intravenous administration of autologous bone-marrow-derived MSCs into ALS patients is feasible and safe [20]. Although this study was not designed to detect therapeutic efficacy, encouragingly, it induced immediate immunomodulatory effects in ALS patient and ALSFRS scores remained stable for up to 6 months following treatment.

Although allogeneic hematopoietic stem cells (HSCs) transplanted intravenously in six ALS patients [21] did not provide any clinical benefit, donor-derived cells were found to localize at the sites of pathology, rendering these cells to be particularly suitable for delivering therapeutic molecules.

Olfactory ensheathing cells extracted from human fetal olfactory bulbs were injected into the bilateral corona radiata in 15 ALS patients who were compared to 20 untreated controls [22]. Over a 4-month follow-up period, positive and beneficial results were observed as a five-point difference in the ALSFRS-R. In another study, 327 patients received injection of olfactory ensheathing cells into the spinal cord, the bilateral corona radiata, or both [23]. They reported improved ALS functioning rating scale and normalized electromyographical findings 4 weeks after transplantation, with no differences between the three groups.

Studies have demonstrated safety of intraspinal infusion of autologous bone marrow mononuclear cells [24-26]. Deda et al, demonstrated reinnervation in 7 of 13 patients on electro neuro myography following intraspinal infusion of autologous bone marrow mononuclear cells, 1-year post treatment [26]. Clinical improvements were seen in 10 patients. Intrathecal transplantation of autologous bone marrow mononuclear cells was found to be safe and effective in slowing down disease progression in 5 studies [27-31].

Our results

Published results

This was a retrospective controlled cohort study of 57 patients [27]. Out of these, 37 patients underwent autologous bone marrow mononuclear cell transplantation in addition to standard rehabilitation and Riluzole. The survival duration since the onset of the disease of this group was compared with a control group consisted of 20 patients who did not receive cell transplantation. Survival duration was computed using a Kaplan-Meier Survival analysis and compared using log-rank test. Effect of age at onset, type of onset and lithium on survival duration in the intervention group was analyzed. Mean survival duration of patients in intervention group was 87.76 months which was higher than the control group mean survival duration of 57.38 months (Figure 2) (Table 1). Survival duration was significantly ($p=0.039$) higher in people with the onset of the disease below 50 years of age (Figure 3). Limb onset and lithium also showed positive influence on the survival duration (Figure 4 & 5). Mean survival duration of the intervention group was also higher than the survival duration of ALS patients in previous epidemiological studies (Table 2).

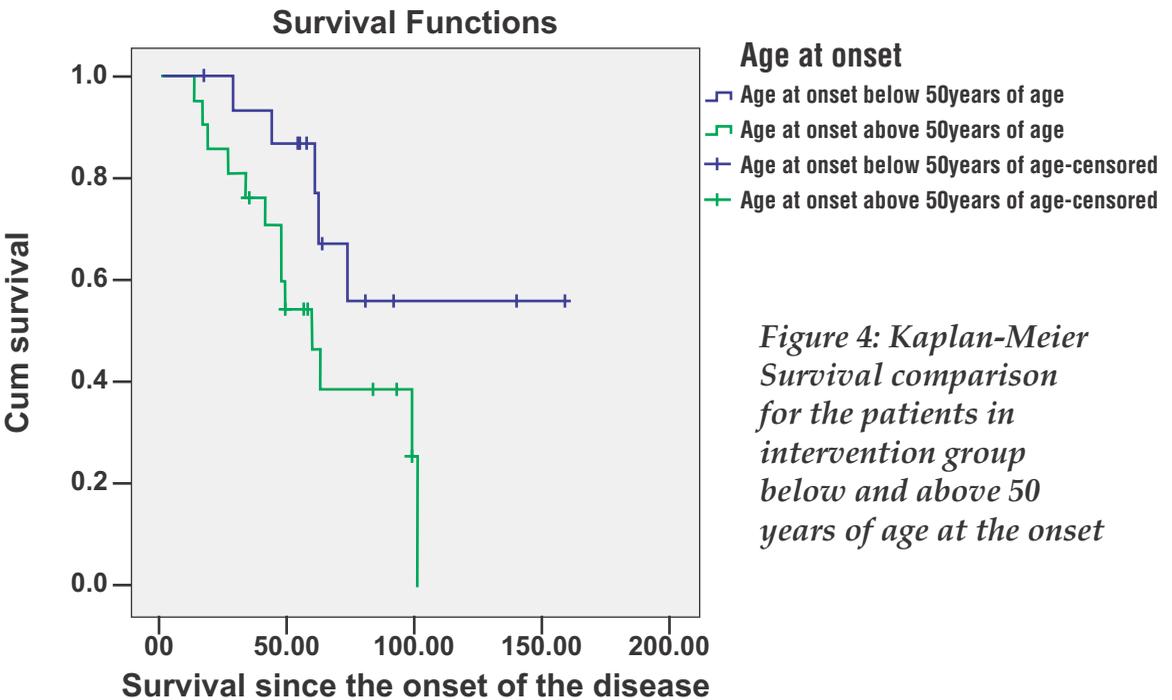


Table 2: Survival analysis

Survival analysis	Intervention group	Control group
Total mortality	48.64%	50.00%
Range of survival duration (months)	13 – 158	26-84
Mean survival duration (months)	87.76 (10.45)	57.38(5.31)

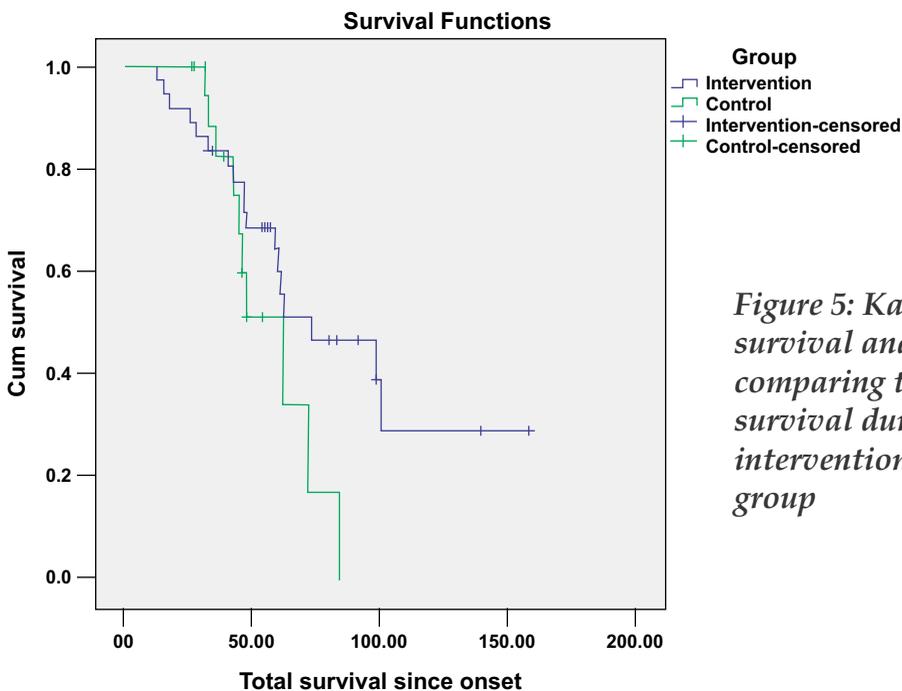


Figure 5: Kaplan Meier survival analysis comparing the mean survival duration of the intervention and control group

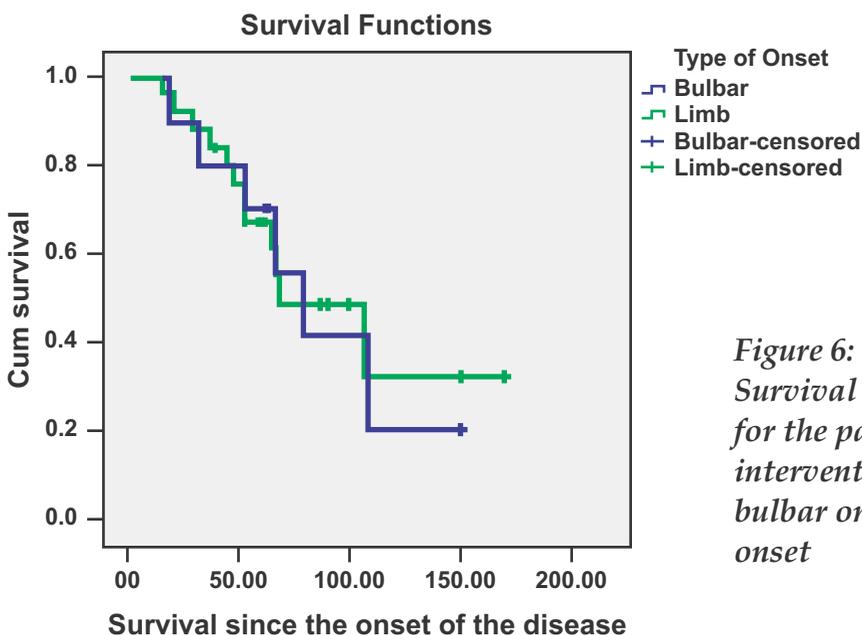


Figure 6: Kaplan-Meier Survival comparison for the patients in intervention group with bulbar onset and limb onset

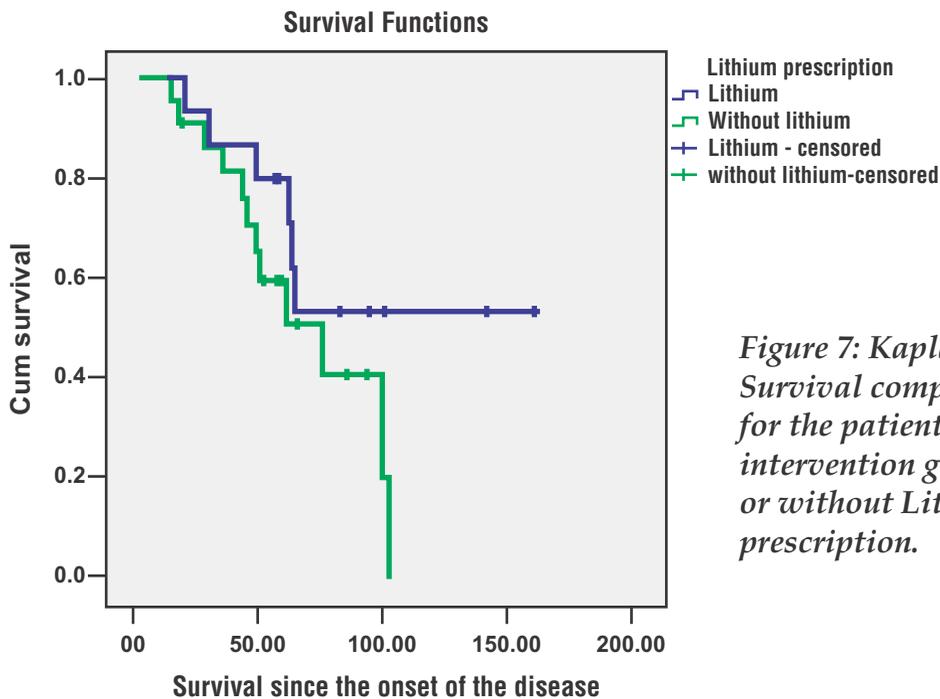


Figure 7: Kaplan-Meier Survival comparison for the patients in intervention group with or without Lithium prescription.

Table 3: Mean and median survival duration as observed in previous epidemiological studies and its comparison with the current findings

Author	Year	Country	Sample size	Mean age of the population in years (SD)	Mean or median Survival since onset	Comparison with the results of our study
Haverkamp et al. [38]	1995	USA	1200	55.7	37.5 months	88.86 months
Forbes et al. [39]	2004	Scotland	1226	Men - 65.2 Women - 67.7	25months 5 year survival probability - 11%	88.86 months 5 year survival probability - 62.3%
Milul et al. [40]	2005	Italy	79	64.4	39.2 months	88.86 months
Osuntukun et al. [41]	1974	Nigeria	92	39.2 (1.6)	Dead - 73 months Alive - 79 months	Dead - Alive -
Radhakrishnan K [42]	1986	Libya	23	51	42	88.86 months

Norris et al [43]	1993	USA	708	Range - 25 -74	24-44 years - 71.5months 45-54 years - 35 months 55-74 years - 32.5 months	24-44 years - 138.7 months 45-54 years- 76.80 months 55-74 years - 57.19 months
Alcaz S [44]	1997	Serbia	58	Men - 56.2 Women - 56.6	2 years survival probability - 62% 5 years survival probability - 27% 7 year survival probability - 27%	2 years survival probability - 89% 5 years survival probability- 62.3% 7 year survival probability - 43.2%
Eisen et al. [45]	1993	USA	246	Not available	< 40 years - 98.4months 61 - 70 years - 31.2 months	< 40 years - 61 - 70 years -
Traynor et al. [46]	2000	Ireland	388	Men - 63.3 Women - 64.4	Definite ALS - 27 months Probable - 30 months	Definite ALS - 88.86 months Probable ALS - Not available

Sorenson et al. [47]	2002	USA	77	63	23 months	88.86 months
Tysnes et al. [48]	1991	Norway		60.9 Range - 34 to 82	28 months	88.86 months
Turner et al. [49]	2003	England	769	Long survivors - 43, short survivors - 57 , others - 62	43months	88.86 months

Conclusion of the study:

In addition to the standard treatment with Riluzole, early intervention with combination of BMMNCs transplantation and Lithium may have a positive effect on the survival duration in ALS. Administration of lithium, younger age and limb onset patients have better outcome after cell therapy. Prospective randomized controlled studies with a larger sample size and rigorous methodology are required for conclusive findings.

Unpublished Data

The survival duration of the ALS patients treated with intrathecal autologous bone marrow mononuclear cells transplantation since August 2008 till August 2015 was analyzed. There was a comparison made between the survival duration of the patients that underwent stem cell transplantation to those that did not. The statistical test used for the comparison was Kaplan-Meier survival analysis. There were total 84 patients in the intervention group and 20 patients in the control group. Both these groups shared similar baseline demographic characteristics. Comparison of the survival duration suggested that the mean survival duration of the patients treated with intrathecal autologous BMMNCs transplantation was longer than those who were not treated [Table 4][Fig.6]. The mean survival duration of the patients who received treatment was 90.96(9.27) months and those who did not was 57.38 (5.31). The difference between the two was statistically significant ($p=0.051$). A clinically significant difference of 34 months in the survival duration suggests the potential benefit of intrathecal autologous BMMNCs transplantation in the treatment of ALS.

Table 4: Survival analysis

Survival analysis	Intervention group	Control group
Total mortality	29.8%	50.00%
Range of survival duration [months]	8 - 158	26-84
Mean survival duration [months]	90.96 [9.27]	57.38[5.31]

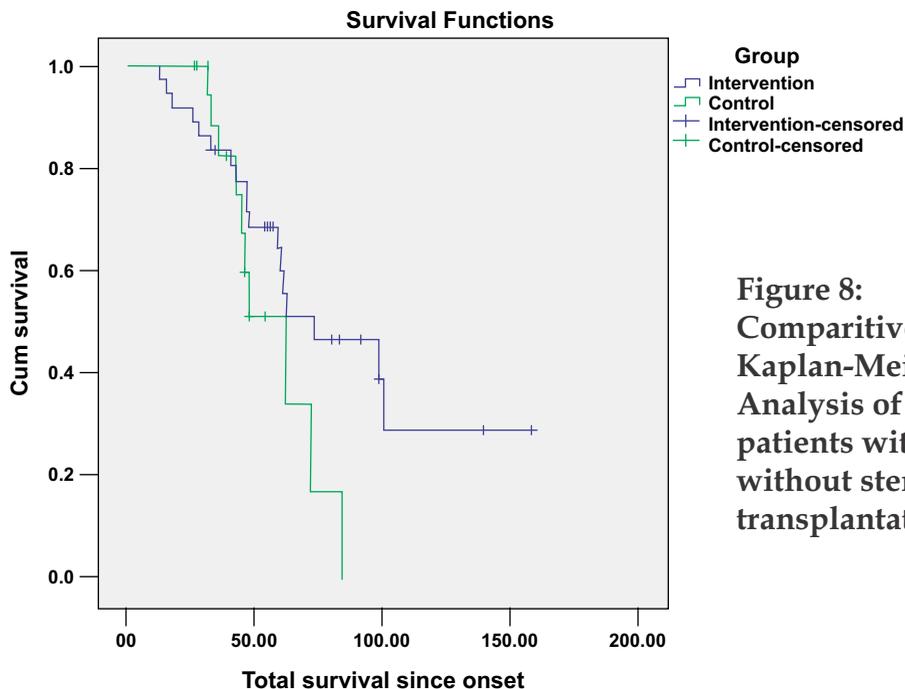


Figure 8:
Comparative
Kaplan-Meier
Analysis of ALS
patients with and
without stem cell
transplantation

Conclusion of the clinical study:

The study suggested increased survival of patients undergoing autologous BMMNCs intrathecal transplantation and riluzole as that of control population. In addition to the standard treatment with Riluzole and neurorehabilitation, there is a possibility that early intervention with combination of BMMNCs transplantation has a positive effect on the duration of survival in ALS.

Neuroprotective effects of female reproductive hormones in combination with cellular therapy

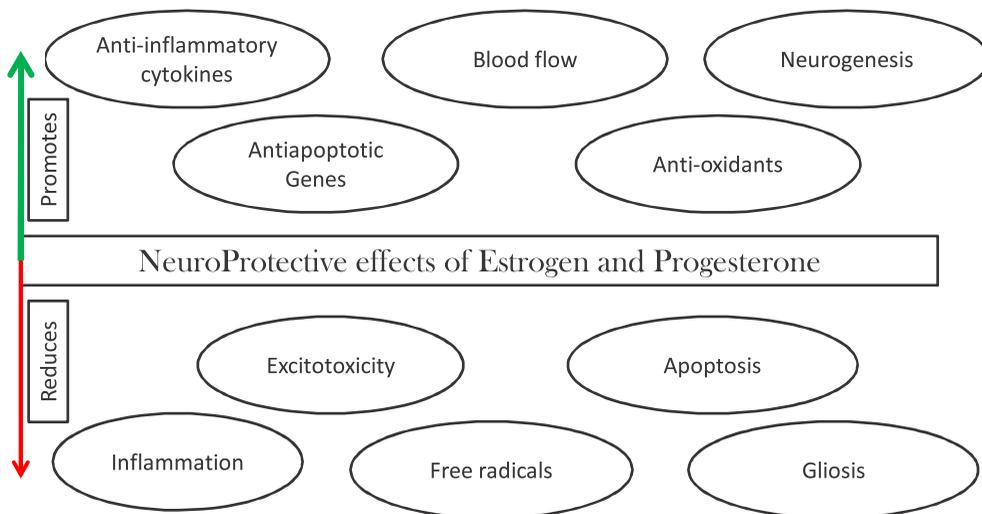


Figure 9: Neuroprotective effects of Estrogen and Progesterone

It has been shown in various studies that female reproductive hormones i.e. estrogen and progesterone have several Neuroprotective benefits. These neuroprotective benefits are due to various mechanisms as shown in Figure 8. We analyzed disease progression of the patients that received autologous bone marrow derived mononuclear cell intrathecal transplantation in various subgroups based on age and hormonal status. The groups were, pre-menopausal women who still have the putative neuroprotection of the reproductive hormones, post-menopausal women who were exposed to the putative neuroprotection of the reproductive hormones but are not currently exposed to it, men below the age of 50 and men above the age of 50. Men were divided into 2 groups based on their age because age is a known prognostic factor and as age advances prognosis of ALS gets worse.

Upon comparing the disease progression and survival percentages we found that, pre-menopausal women have the slowest disease progression and no mortality in the 1.5 years of follow up period. This was followed by men below the age of 50 years, post menopausal women and men above the age of 50 years. The disease progression of the 4 groups is given in Figure 9, Table 5.

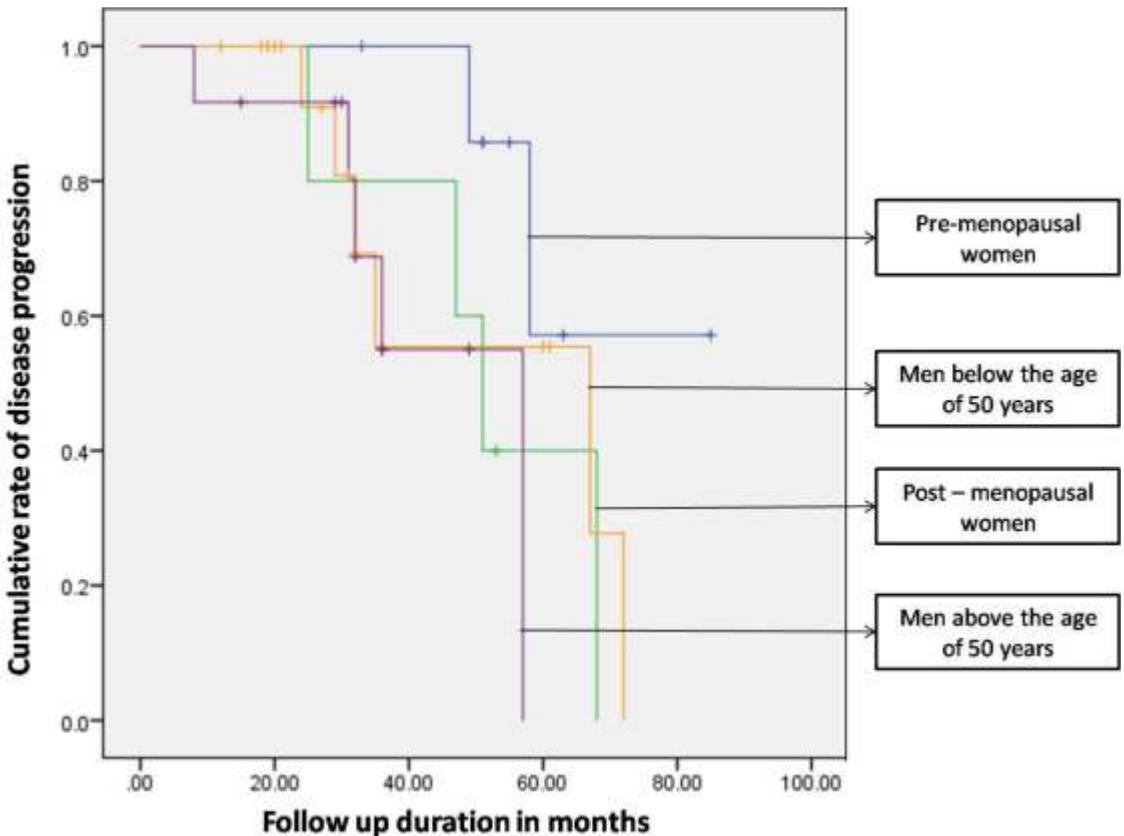


Figure 10: Disease progression of the 4 subgroups of ALS patients treated with cell therapy

Table 5: Disease progression of the 4 subgroups of ALS patients treated with cell therapy

Group	Mean estimated months for ALS-FRSr score to drop to 24	Percentage mortality
Pre-menopausal women	72.13	0
Men below the age of 50 years	52.17	25
Post-menopausal women	51.80	40
Men above the age of 50 years	44.19	55

There is some evidence to suggest that the male reproductive hormone testosterone may also provide a Neuroprotective benefit in ALS and may have a positive benefit for slowing down the disease progression in younger males.

Future directions

Gene therapy

Suspected genetic causality of ALS and some evidence to support the genetic alterations in ALS has led to emergence of gene therapy as a future management strategy for ALS. A clinical trial using Antisense Oligonucleotides to reduce the toxic protein aggregates in ALS is currently being undertaken [50].

Nur-Own cells transplantation

Recently brain stem cell technologies have developed Nur-Own cells. These are adult autologous mesenchymal cells harvested from bone marrow which are differentiated into specialized neuron supporting cells using the technology developed by Brain Storm Cell Therapeutics. Currently a Phase IIa trial is being conducted with 12 participants using intramuscular and intrathecal transplantation of the Nur-Own cells.

In the recently published article the results of the open-label proof-of-concept study in 26 patients who were administered these mesenchymal stem cells (nur own cells) intramuscularly or intrathecally or both. The groups were followed for 3 months before transplantation and 6 months after and it was found out that the rate of progression of ALS-FRSr and Forced Vital Capacity (FVC) was slower after the treatment. Responders were identified as the patients who showed progression to be at least 25% slower than before.

Combination of gene therapy and stem cell therapy

There have been recent discoveries of various genes involved in the possible etiology of ALS and therefore trials are targeting combining the two approaches of gene therapy and stem cell therapy.

Induced pluripotent stem cells (iPSCs)

Progenitors that develop into supporting neurological cells apart from neurons have been developed and tested to in ALS. A trial has been announced by the Cedar-Sinai institute in USA that will administer iPSC progenitor of astrocytes unilaterally and intraspinally in the lumbar cord combined with growth factor treatment. There will be comparison between the disease progression of the two extremities of the same patients to find out the effect of these cells.

Effect of Reproductive hormones

Female and male reproductive hormones provide neuroprotection through mechanisms similar to that of the paracrine effects of the stem cells. It may therefore be interesting to explore the benefits provided by combination of cellular therapy with hormonal therapy.

Conclusion:

At present, there is no proven treatment for ALS/MND. The only available treatment is riluzole which has 3 to 6 months of survival benefit. Therefore, there is a significant unmet medical need. Stem cell therapy has the potential to slow down or halt the disease progression of ALS/MND. A combination of stem cell therapy and Lithium in addition to standard treatment with riluzole and rehabilitation offers a survival benefit of 30 months. In the treated population, premenopausal women have shown 0% mortality and slowest disease progression. The published results of Prabhakar, Deda, Martinez and Meamar as well as the recent review by Moura also shows the beneficial effects of cell therapy in ALS.

These results highlight the potential of stem cell therapy in the treatment of ALS/MND. However, there are many unanswered questions pertaining to stem cell therapy. Further research needs to be focused on exploring different routes of administration, types and dosage of cells for better clinical outcome.

It is likely that a definitive treatment and maybe even a cure will come from a combination of treatments which will include cell therapy, medications like riluzole and lithium and an aggressive rehabilitation program and may be adjuvant treatments like hormone replacement therapy.

Based on our own clinical experience we believe that the following patients are likely to get greater clinical benefit with stem cell therapy

1. Younger patients below the age of 50 years

2. Women, especially premenopausal women
3. Patients in the early stage of the disease
4. Patients who have still not developed swallowing, breathing and speech problems

Cellular therapy provides a promising future in the management of ALS. Prospective trials with rigorous methodology making use of randomization, blinding and larger sample size need to be carried out for conclusive evidence. It is of importance to compare the effects of different cell types. Combination of cellular transplantation with various other neuroprotective regimens should also be studied to find the treatment option that gives best possible results in ALS.

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To believe that, what has not occurred in history will not occur at all, is to argue disbelief in the dignity of man.

- Mahatma Gandhi

14

Role of Stem Cells in Traumatic Brain Injury

Stem cells can migrate towards the damaged areas of the brain and promote the repair process in Traumatic Brain Injury .

Traumatic brain injury (TBI) is the most common cause of death and disability in young people. It is mostly caused by an external physical impact producing an altered state of consciousness resulting in impairment of physical functions or cognitive abilities (1) The damage caused to the brain is either focal or diffused depending on the event causing TBI. The outcome consists of two stages (a) primary insult, which occurs at the time of impact (b) Secondary insult, which is a cascade of events after the primary insult with delayed clinical presentation. (2) There are several significant pathophysiologic sequelae of TBI responsible for the neurobehavioural outcome, including the location and severity of the injury, diffuse effects and secondary mechanisms of injury. Alterations in cerebral blood flow and oxygenation, edema, excitotoxicity, cell death, disruption of the blood brain barrier, and generalized atrophy is commonly observed in TBI. (3) The damage to the brain could result in temporary or permanent behavioral and/or emotional disturbances leading to functional disability.

Unmet medical needs

The past 2 decades have seen great advancements in understanding the molecular and cellular mechanisms of TBI. The standard treatment modalities for brain injury involves medications, physical and behaviour therapy, Hyperbaric Oxygen

therapy(HBOT), and medical management of associated conditions aims at improving the functional abilities and restoring the patient's daily life. However, these strategies have failed to translate into a successful treatment strategy that can address the core neurological damage. Among the numerous barriers to finding effective interventions to improve outcomes after TBI is the severity and heterogeneity of the injury. In chronic TBI, there is high prevalence of residual neurodeficits rendering the patient dependent and many a times bed-ridden. In case of mild TBI, physical recovery is witnessed with the standard treatments, but the memory and behavioural issues persist which may affect the quality of life of the individual. The available pharmacological modalities manage these disabilities, but their effect wears off gradually.

Stem cell therapy in TBI

Due to the brain's limited capacity to regenerate the damaged neurons, the intervention should aim at halting the degeneration and replacing the lost and damaged neurons. (4) In past few years, cell therapy has gained attention as a prospective therapeutic options for neurological disorders. Stem cells migrate towards the damaged areas of the brain and initiate the repair process. They promote angiogenesis, axonal remodeling, neurogenesis and synaptogenesis, which may help reverse the pathology of TBI. (5) These cells differentiate into various cells including neural cells, oligodendrocytes, etc. (6) In TBI, there is loss of myelin which disrupts the signal transduction and damages the axons. The oligodendrocytes help in remyelination of the damaged axons and repair the disrupted neural connections. Bone marrow cells also produce various growth factors and neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), which stimulate the endogenous neuroprotection and repair. (7)

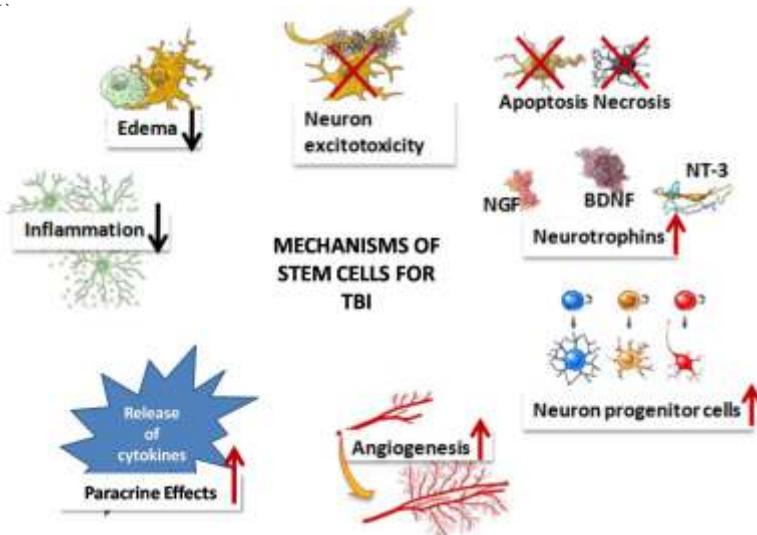


Figure 1: Mechanisms of stem cells for TBI

Animal studies

Various experiments on animal models have been carried out to test the safety and feasibility of different types of cells. Reiss et al, transplanted embryonic cells in experimental rats and recorded dramatic improvements but the safety of administration could not be established. (9) Series of experiments were conducted to study the neural stem cells in TBI which reported improved neurological functions in the injected rat models via various mechanism. (10-15) Bone marrow stem cells were also found to be efficacious in modulating the inflammation-associated immune cells and cytokines in TBI-induced cerebral inflammatory responses. (16,17) Studies also showed that bone marrow derived mesenchymal cells (BMMSCs) migrate to the injured areas and increase expression of neurotrophic factors such as vascular endothelial growth factor (VEGF) and brain derived neurotrophic factor (BDNF). BMMSCs also upregulate the protein expression levels of synaptophysin, a synapse protein which is downregulated after TBI, resulting in neuromotor functional recovery. (18) In an experimental study, rats injected with BDNF gene-modified umbilical cord mesenchymal stem cell (UCMSC) led to improvements in behavior and other neurological functions. (19) In 2016, a study conducted on Wistar rats to investigate the capacity and sensitivity of diffusion-derived magnetic resonance imaging suggested that cell intervention executed at 6 hours accelerates the brain remodeling process and results in an earlier functional recovery.(20)

Few studies were conducted in recently to demonstrate the homing and migration of transplanted cells in rat models of TBI using fluorescence labelling. Dong et al tracked the distribution of human umbilical cord mesenchymal stem cells (MSCs) in large blood vessel of traumatic brain injury -rats. They found that intravascular migration and homing of MSCs in rats which received MSCs transplantation, and new angiogenesis in MSCs-transplanted blood vessels. (21) Guo et al, demonstrated the integration of intravenously injected EPCs into the injured brain tissue. These EPCs enhanced recovery by contributing to neurogenesis. (22.) Lin et al showed that transplanted human neural precursor cells integrate into the host neural circuit, which was detected by fluorometric Ca²⁺ imaging and nerve tracing, and ameliorate neurological deficits in a mouse model of traumatic brain injury. (23.)

Clinical Studies

Not many clinical studies have been conducted on human subjects of TBI showing effects of stem cell therapy. One of the first studies was published in 2008 by Zhang et al. They assessed the safety and feasibility of a combined procedure to deliver autologous mesenchymal stromal cells in 7 patients with traumatic brain injury. (24) They found that neurological functions improved significantly at 6 months after cell therapy. In 2011, Cox et al, performed a phase1 clinical trial to demonstrate the safety and feasibility of autologous BMMNCs in children with severe traumatic

brain injury. 10 children were included in the study. Dichotomized Glasgow Outcome Score at 6 months showed 70% with good outcomes and 30% with moderate to severe disability. (25) Followed by this, they published the results of their phase 2 clinical trial in 2017 which included 25 patients; 15 in the intervention group and 10 in the control group. They recorded positive functional and neurological outcome and also down regulated Inflammatory biomarkers. (26) Similar studies were published by Sharma et al and Liao et al in 2015 demonstrating the positive effect of BMMNCs. Wang et al, in 2013 conducted a study on patients with sequelae of TBI. They administered 40 patients with umbilical cord mesenchymal stem cells and observed improved neurological functions and self-care in these patients as compared to the controls. (27) Vaquero et al in 2017, showed improvement and progressive increase in brain glucose metabolism after intrathecal administration of autologous mesenchymal stromal cells using 18 FDG PET CT, in 3 patients. (28) In 2017, Wang Z et al demonstrated the safety and feasibility of neural stem cell transplantation in patients with severe TBI. They observed that majority of patients experienced improved neurological function on follow up along with increased serum levels of nerve growth factor and brain-derived neurotrophic factor. (29)

Our Published Results

We conducted a study on 14 cases who were administered with autologous bone marrow mononuclear cells, intrathecally. (30) The follow up was done at 1 week, 3 months and 6 months after the intervention. The Functional Independence Measure scale, the SF-8 Health Survey Scoring and the disability rating scale were used as outcome measures.

At the end of six months, a percentage analysis was carried out for improvement in every symptom as discussed in figure 2. Objective improvements were also recorded on PET CT scan at the end of 6 months in the form of improved metabolism of the brain. These changes correlated to the clinical and functional improvements demonstrated by these patients.

Symptomwise Improvements in Chronic TBI After Cell Therapy

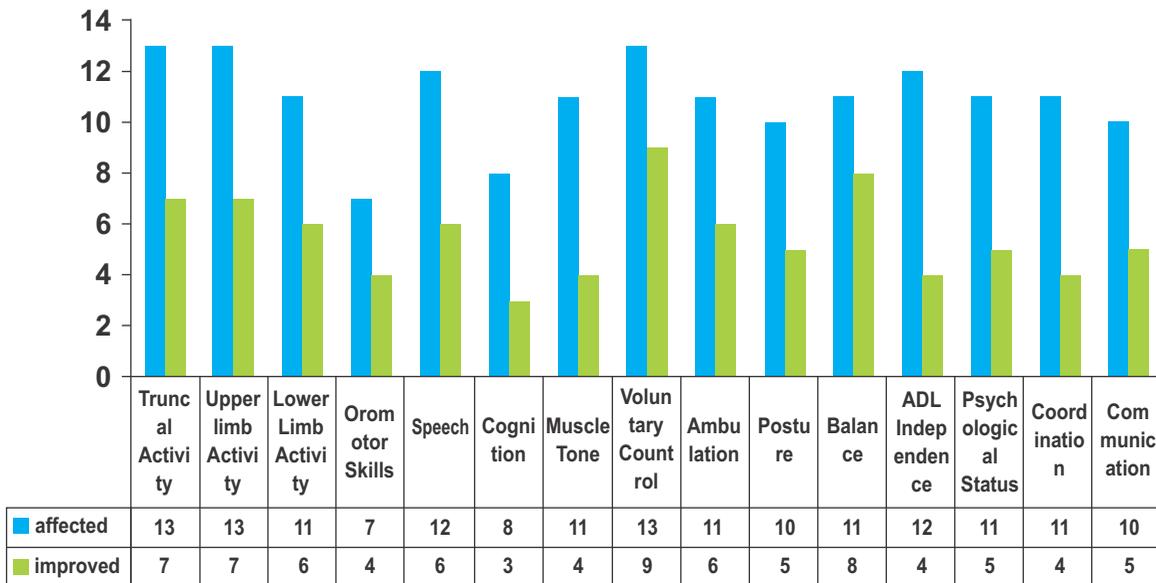


Figure 2: Symptom-wise improvements in chronic TBI patients seen after intrathecal administration of autologous BMMNCs

Table 1: SF8 scores before and after intervention suggesting improved quality of life.

Patient No.	SF8 (Pre)		SF8 (Post)	
	PCS 8	MCS 8	PCS 8	MCS 8
1	28.4	32.5	39.2	40.8
2	44.4	52.1	51.4	59.8
3	47.1	35.4	48.6	43.2
4	33.4	51.5	43	55.9
5	39	64.1	43.1	62.7
6	43.7	58.8	50.1	58.3
7	37.8	44.7	41.5	47.2

Table 2: Areas of the brain showing improved metabolism in PET CT scan and their correlation to the clinical function improvement

Patient	Areas of the brain	Functions improved
Patient 1	Parieto-occipital areas	cognition, speech, sensation, orientation and visual perception
Patient 2	Cingulate gyri	Emotion, attention, cognition, memory
	Amygdala	Emotional responses, memory, attention
	Frontal	Planning, Long term memory, emotions, speech, problem solving
	Temporal lobes	Speech, memory
Patient 3	Amygdala	Emotional responses, memory, attention
	Cerebellum	Coordination, balance
	Cingulate gyri	Emotion, attention, cognition, memory
	Basal ganglia	Voluntary motor control, learning,cognition.
	Occipital lobes	Vision and perception
	Parietal lobe	Movement, orientation
	Temporal lobes	Speech, memory
	Frontal lobes	Planning, Long term memory, emotions, speech, problem solving
	Thalamus	Motor control, sensory functions

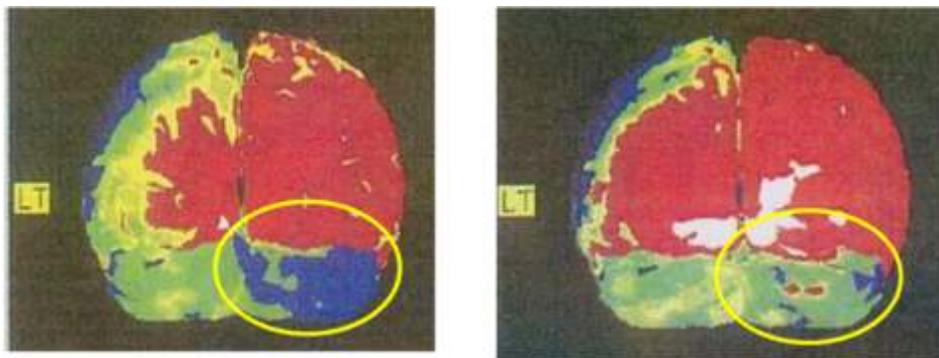


Figure 3: PET CT Scan showing improved metabolic activity which is indicated by decrease in blue areas after stem cell therapy

Unpublished data

To demonstrate the effect of autologous stem cell therapy in TBI, we analysed the data of 44 patients. Symptoms such as balance, voluntary control, memory, upper and lower limb activity , ambulation, posture, muscle tone, speech , cognition and ADLs were analysed. On follow up we found that 72.73 % of patients showed improvements while 27.27% showed no change after intervention. No adverse events were recorded.

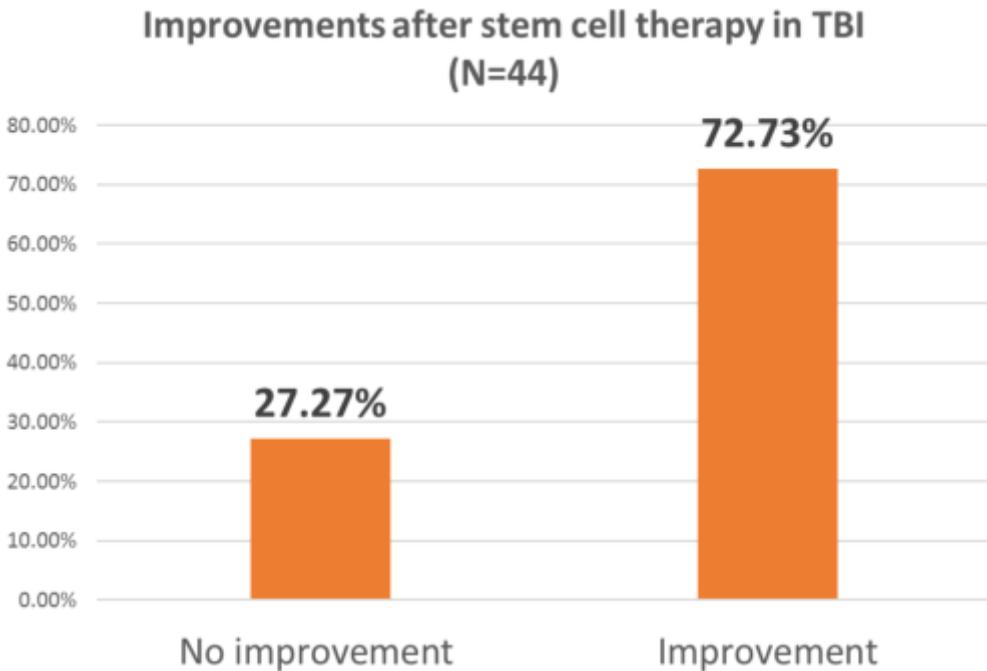


Figure 4: Graph showing overall improvement in the TBI patients after stem cell therapy

PET CT scan as a monitoring tool

18Fluoro-2-deoxyglucose Positron emission tomography (18- FDG PET) is being considered as monitoring tool, to assess the recovery in the damaged brain after cellular therapy in many of the studies. It allows non-invasive quantification of cerebral blood flow, metabolism, and receptor binding. PET CT scan brain is an imaging tool which aids in measuring the brain glucose metabolism using tracer (18F) fluorodeoxyglucose (FDG). Decreased metabolism is suggestive of decreased function of the neurons. An increased uptake of FDG in the previously hypometabolic areas indicate improved function of the neurons.

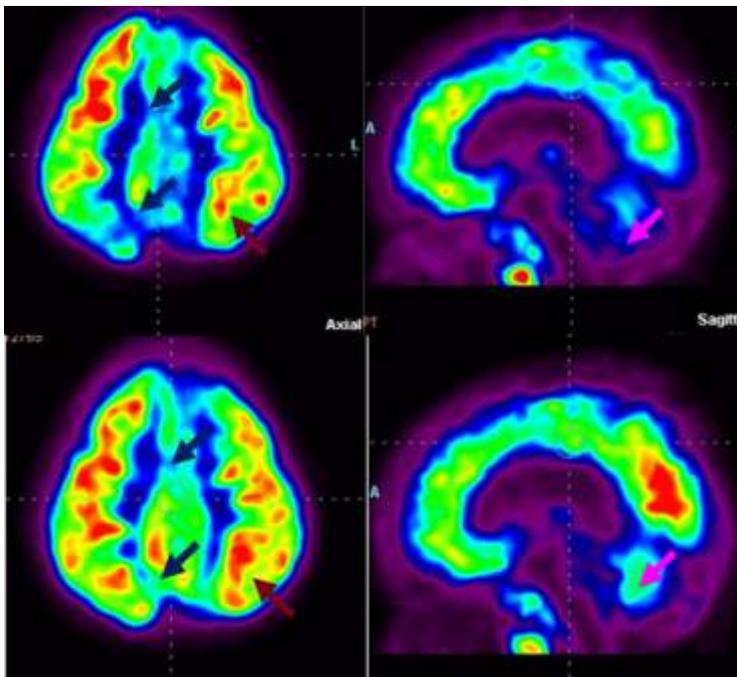


Figure 5: PET-CT scan images pre-stem cell therapy and post stem cell therapy. (Colour code: black- severe hypometabolism, blue-hypometabolism, green-normal metabolism): The areas marked with the arrows depict the areas of the brain which have improved post intervention. Improvement recorded in metabolic activity of cerebellum, cingulate regions, vermis and parietal gyrus.

Conclusion

Cell therapy in combination with neurorehabilitation has a potential to reverse the damage occurring in the brain after chronic TBI. It addresses the diffused nature of injury and the neuronal deficit to the maximum, which current standard intervention may not tackle. The outcome of the cell therapy may be dependent on the age, severity of trauma, time interval between the accident and intervention; and the rehabilitation regime continued after the procedure. It has been observed that the mild TBI cases have a better recovery curve than the severe cases. Chronic TBI patients may require multiple doses of stem cells to accelerate the recovery process. Functional neuroimaging such as PET CT scan of the brain can help to objectify the effects of neuroregeneration in TBI.

Future directions

Studies focusing on combining the efficacy of HBOT and stem cell therapy to target the neurological damage should be established. Future studies should identify the ideal cells for therapeutic use, along with the ideal route of administration. The optimum quantity of cells, frequency of doses and the time interval between consecutive doses should be established to optimize this intervention. The ideal time of injection of stem cells should also be determined as in the acute phase,

inflammation and pathological metabolic changes make the endogenous environment inhospitable for the injected cells. In chronic phase the gliotic changes may affect the efficacy of cell therapy. It is also important to track the changes occurring in the brain after intervention paving way for more research to be conducted on the monitoring tools.

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To believe that, what has not occurred in history will not occur at all, is to argue disbelief in the dignity of man.

- Mahatma Gandhi

15

Stem Cell Therapy for Intellectual Disability

Stem cells have opened new avenues for recovery in cognitive, learning and behavioral deficits of Intellectual Disability.

Intellectual Disability (ID) has been defined as “ a disorder with onset during the developmental period that includes both intellectual and adaptive functioning deficits in conceptual, social, and practical domains” under DSM V. (1)The prevalence of ID is approximately 1-3% with a corresponding intelligence quotient (IQ)< 70. (2) Causes of ID can be heterogeneous which include premature birth, gene mutation and chromosomal abnormalities (Trisomy 21 and fragile X), toxins, prenatal infections and environmental factors (malnutrition, emotional and social deprivation).(3) The clinical presentations in ID include impairment in adaptive behaviour and intellectual functioning, delayed developmental milestones, difficulty in various day to day activities like communication, self-care, home living, social/interpersonal skills, self-direction, academics and safety, inability to meet up with peers of same age at school, and difficulty in solving problems.(4) Currently, there is no treatment available for ID. The conventional management strategies involve behavioural therapy, psychological intervention and occupational therapy which addresses the symptomatic representations in ID. But these strategies seldom answer the core neurological problems of ID. (5)

Pathophysiology

Recent studies indicate that proper synaptic function, and hence normal intellectual

function, depends upon two major components: development of the nervous system, and healthy functioning of the neurons and their network. The underlying mechanism of ID involves abnormalities in dendritic branching and connectivity of the neuronal network which limits its ability to process information, especially in postnatal stage, during which learning and acquisition of intellectual abilities and emotional behaviour occurs. (Fig 1)Neural dysfunction underlying ID may include reduction in neuron numbers, disturbed neuronal migration and alterations in dendritic arborisation and morphology.(6)

Unmet Medical Needs

With increasing awareness, the prevalence of intellectual disability has risen considerably. The population of patients with ID are intellectually and functionally dependent on others and are considered socioeconomic burden in the society. The conventional modalities do not address the residual neuronal deficit which significantly affects the quality of life of the patients and their families. There is a critical need to find new avenues of management of ID which focuses on the underlying cause of the cognitive deficit.

Stem Cell Therapy for Intellectual Disability

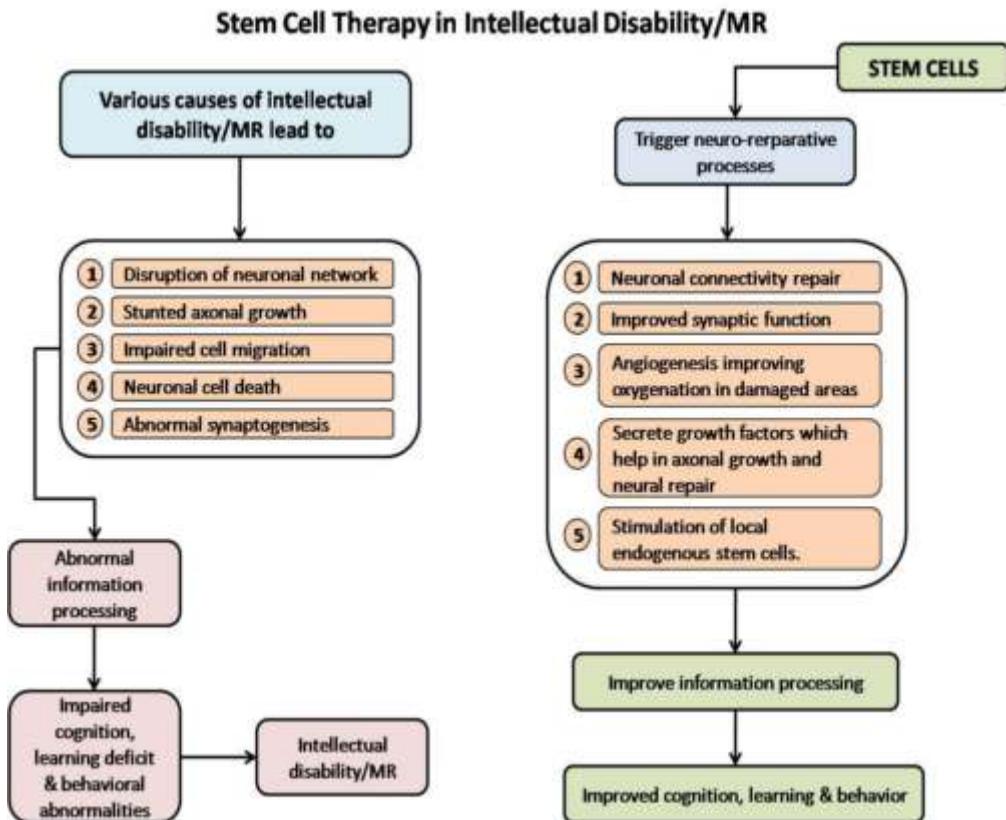


Figure 1: Stem Cell Therapy in intellectual disability

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. [7] To reverse the damage caused to the central nervous system, only a neurorestorative therapy like stem cell therapy would be beneficial.

Stem cells have a unique property of homing and targeting specific damaged areas on administration that require the right combination of signaling molecules from the injured tissue and the corresponding receptors on stem cells. They survive, migrate, proliferate and differentiate into the required cell types. (8) 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. (9) 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

Our Results

Published data

We published the first case study in the world demonstrating efficacy of intrathecal autologous bone marrow mononuclear cell transplantation in individuals with ID. (10) It was an open-label proof-of-concept study which included 58 patients. These patients were divided into two groups: intervention group (n = 29) and rehabilitation group (n = 29). The intervention group underwent stem cell therapy and standard neurorehabilitation while, the rehabilitation group underwent only standard neurorehabilitation.

The results of the symptomatic outcomes were compared between the two groups. The outcome measures used were the intelligence quotient (IQ) and the Wee Functional Independence Measure (Wee-FIM). To compare the pre-intervention and post-intervention results, statistical analysis was done using Wilcoxon's matched-pairs test for Wee-FIM scores and McNemar's test for symptomatic improvements and IQ. The effect of age and severity of the disorder were assessed for their impact on the outcome of intervention. PET CT brain scan was used as a monitoring tool to study effects of the intervention. No major adverse events were

witnessed.

On symptomatic analysis, greater improvements were seen in the intervention group as compared to the rehabilitation group. In the intervention group, the symptomatic improvements, IQ and Wee-FIM were statistically significant.

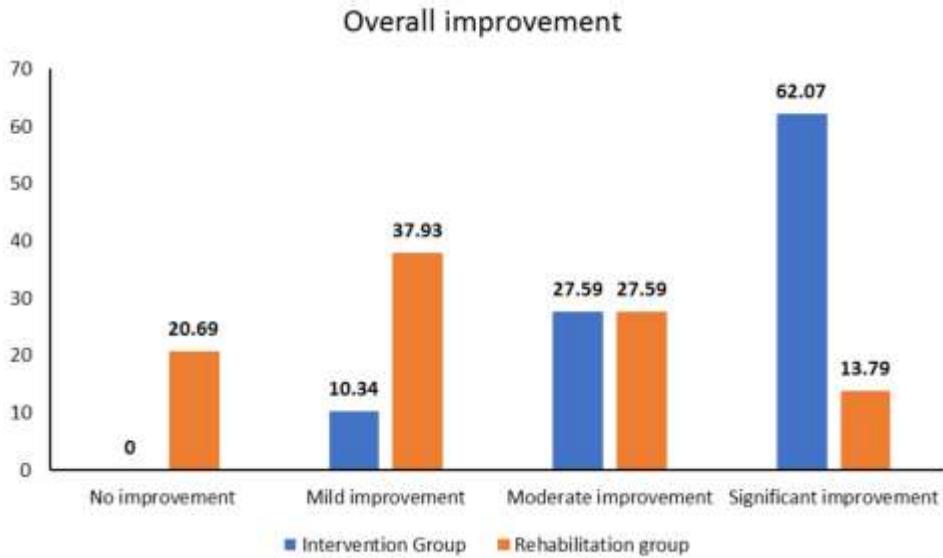


Figure 2: Graph demonstrating a comparison of overall percentage improvements in ID between the intervention group and rehabilitation group.

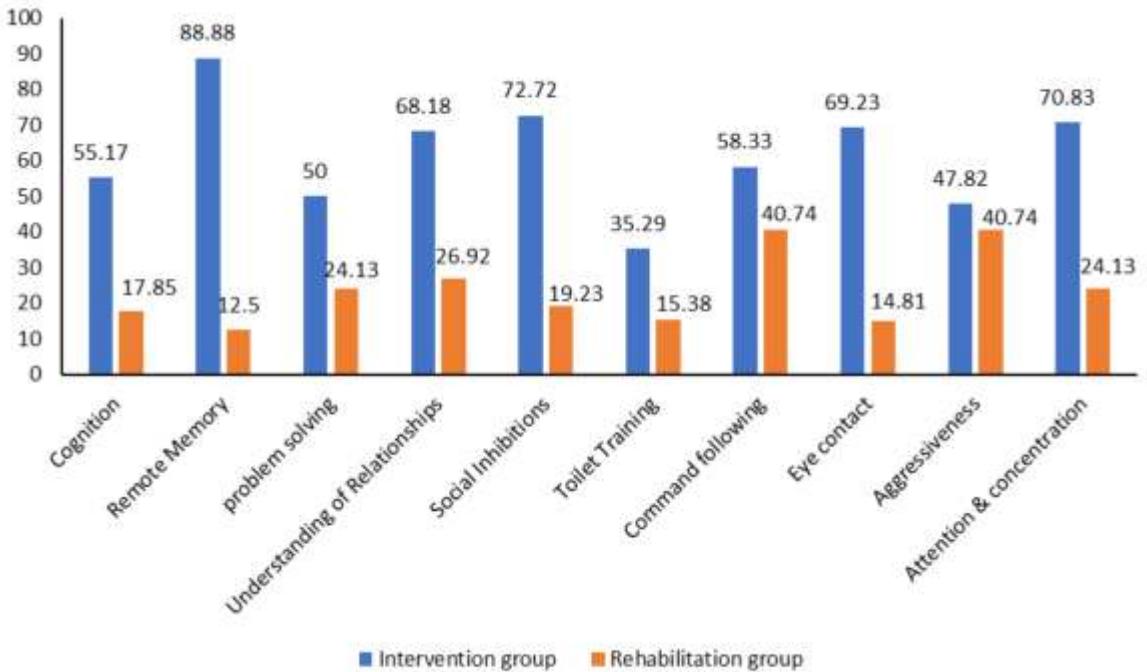


Figure 3: Graph demonstrating a comparison of overall percentage improvements in the symptoms ID between the intervention group and rehabilitation group.

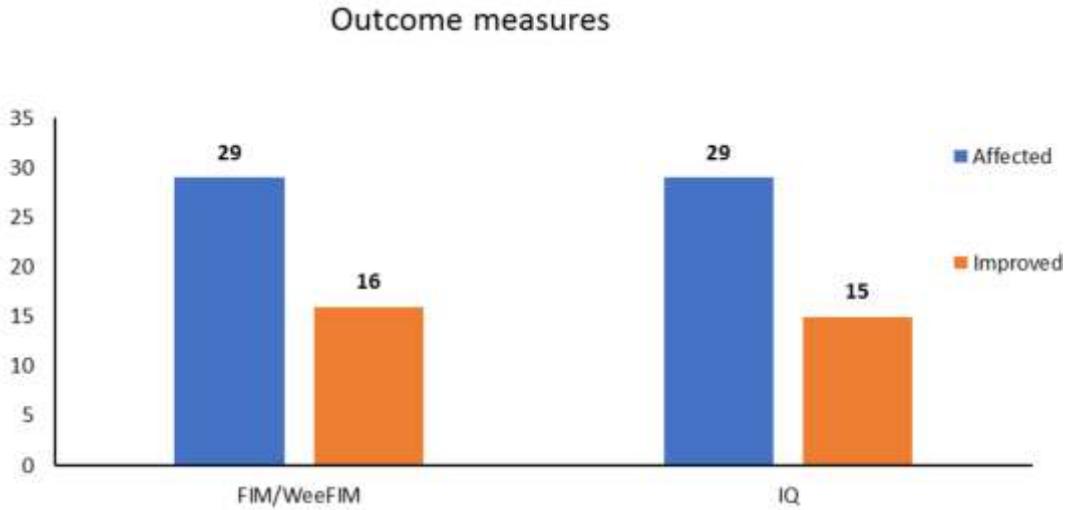


Figure 4: Graph demonstrating improvements in the outcome measures (FIM/WeeFIM and IQ) in patients with ID in the intervention group, six months after cellular therapy

A significantly better outcome of the intervention was found in the paediatric age group (<18 years) and patients with milder severity of ID.

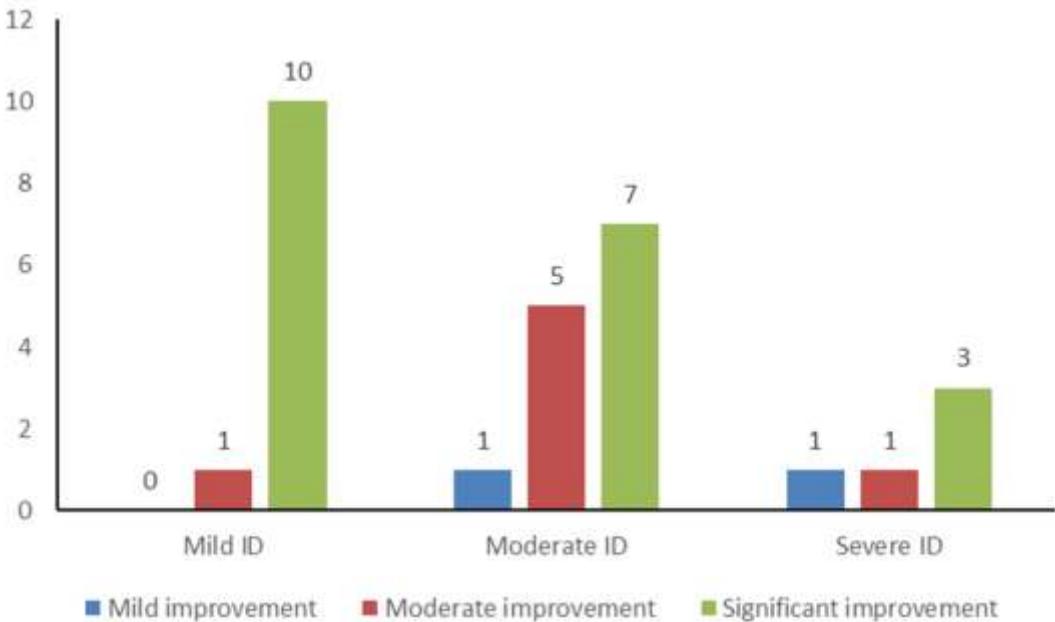


Figure 5: Graph showing the comparison of improvement in patients in the intervention group with severity of ID.

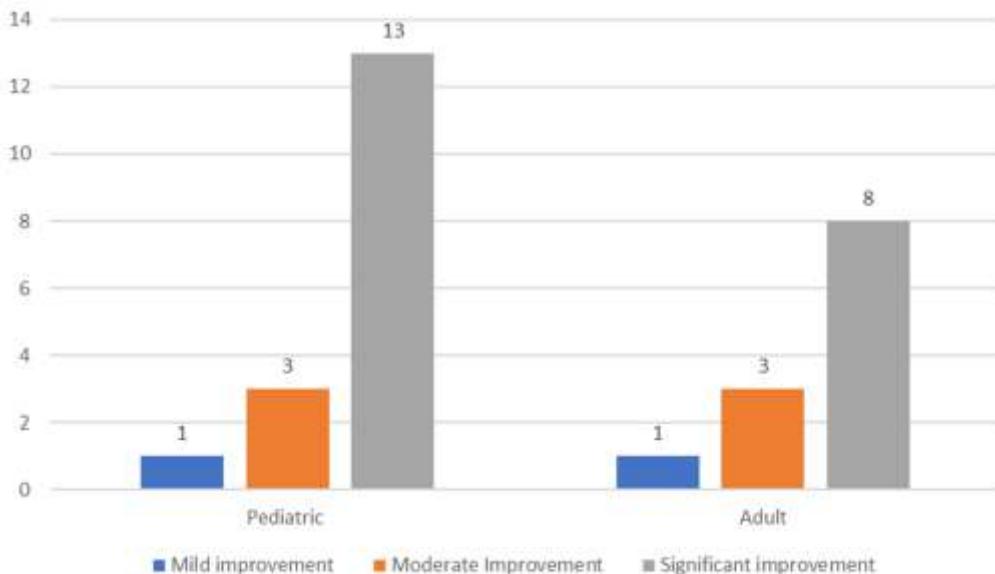


Figure 6: Graph showing the comparison of improvement in patients in the pediatric and adult population of intervention group.

Repeat PET-CT scan in three patients of the intervention group showed improved metabolism in the frontal, parietal cortex, thalamus, mesial temporal structures and cerebellum.

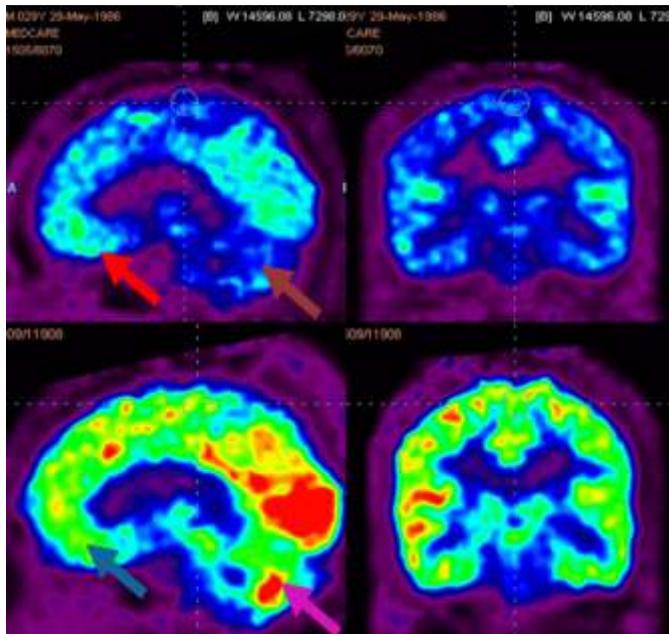


Figure 7: The top row indicates the PET CT Scan image before stem cell therapy showing reduced metabolism in prefrontal, frontal (red arrows), cerebellum (brown arrow); Below row indicates improved 18F-FDG metabolism after intervention in the prefrontal, frontal (blue arrows), and cerebellum (pink arrow).

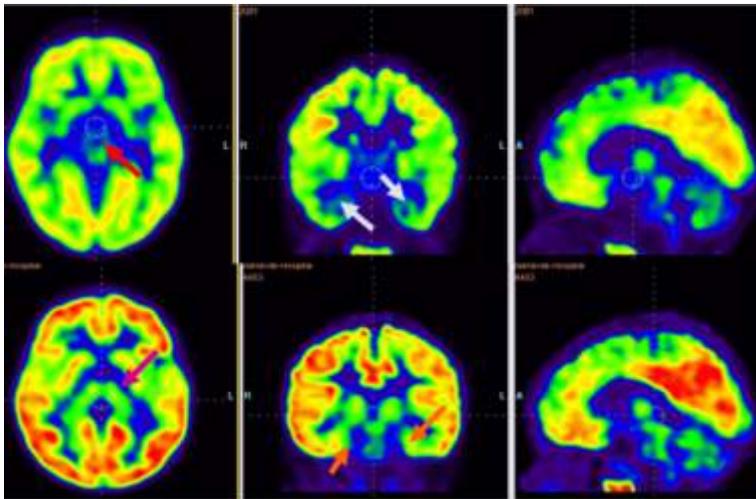


Figure 8: The top row indicates the PET CT scan brain image before stem cell therapy showing reduced metabolism in thalamus (red arrow), mesial temporal structures (white arrow); Below row indicates improved 18F-FDG metabolism after therapy in thalamus (pink arrow), mesial temporal structures (orange arrow).

Case report

We published 2 case reports of individuals diagnosed with moderate ID. They were administered with autologous bone marrow mononuclear cells intrathecally. No major adverse events were recorded in either cases. On follow up they showed improvements in cognition, attention and concentration, behavior (hyperactivity, aggression, temper tantrums had reduced), sitting tolerance, speech, eye contact, etc. These improvements correlated with the improved brain metabolism of the areas responsible. (11,12)

Conclusion-

The multiple mechanisms of action of stem cells promote a reparative process in the dysfunctional brain which can be reflected by symptomatic and functional improvement in the patients with ID. Cellular therapy was found to be safe and effective in repairing the underlying neurological deficits in ID. These cells improve the axon regeneration, brain networking and synaptic arborisation thereby improving the information processing. The outcome may be influenced by the underlying etiology, severity and the time of the intervention. It is well established that rehabilitation promotes the recovery of neurological deficits, and its multidisciplinary approach along with cellular transplantation and special schooling may enhance the recovery process further. Early intervention is advised as the neural circuits, which form the base for learning and behavior, are more plastic during the initial years of life. Stem cell therapy along with current treatment can enhance symptomatic improvements which will help the patient to lead a productive and respectable life in the society.

Future Directions

Due to the heterogeneous nature of the disorder, it is a challenge to find a definite cure for intellectual disability. Genetic factors play a major part in intellectual disability (ID) and gene therapy has provided novel insights in treating the genetic aberrations underlying ID. But gene therapy cannot replace the lost neurons and also poses practical difficulties that have prevented them from being a clinically feasible and viable option for the treatment of ID. Stem cell therapy addresses the core damage occurring in the brain of an individual with ID. The combined approach of gene therapy and stem cell therapy along with standard rehabilitation may help in addressing the gene defect as well as the neuronal dysfunction in the brain. It is also advocated to find the most effective type of cell, number of cells required and the frequency of the doses to effectively treat the underlying neurological deficit. Future studies should consider the use of PET-CT scan as monitoring tool and substantiate the effects of cellular therapy in ID. Large scale, multicentre, and randomized controlled trials are recommended to further establish the safety and efficacy of cellular therapy in ID.

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16

Stem Cell Therapy In Cerebellar Ataxia

With Neuroprotective mechanisms, Stem cells can play a vital role to control disease progression in Cerebellar Ataxia.

Cerebellar Ataxia is a heterogeneous group of neurodegenerative disorders characterized by lack of coordination and imbalance. (1) It is distinguished into a group of hereditary and sporadic ataxias. Hereditary Cerebellar Ataxia can have an autosomal-dominant, autosomal-recessive, X-linked, or mitochondrial mode of inheritance. (2) Sporadic can be symptomatic or idiopathic. (3)

The clinical manifestations of cerebellar ataxia consist of irregularities in the rate, rhythm, amplitude, and force of voluntary movements. Symptoms include gait/postural abnormalities, balance issues, incoordination and involuntary movements, poor fine motor skills, visual abnormalities, increased fatigue, cognitive and mood problems, speech and swallowing difficulties. (4) Presently, there is no effective line of management for treatment of Cerebellar Ataxia. No treatment is available to halt the disease progression.

Unmet medical needs

All the current treatment options focus on symptomatic management. None of the treatment approaches address the underlying pathology of the disorder. A therapeutic strategy is required to stop the degeneration, repair the damaged areas and protect the unaffected areas.

Mechanism of action of Stem Cell Therapy:

Stem Cell Therapy has opened new avenues for the treatment of cerebellar ataxia. Studies have shown that stem cells migrate to the site of injury from the site of injection. They enhance angiogenesis and contribute to neovascularisation by producing signalling molecules such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF2). They impart immunomodulatory and anti-inflammatory effects. Cvetanovic et al, suggested that, reduction of Vascular Endothelial Growth Factor (VEGF) levels observed in Spino Cerebellar Ataxia contribute to its pathology. Stem cells help in regulating the levels of VEGF. (5) The various mechanisms along with paracrine effects of cellular transplantation may help reverse the disease pathology.

Animal studies

Jones et al, analyzed the possibility of using bone marrow-derived mesenchymal stem cells in treating ataxia. They transplanted these cells in animal models and found, two months after the surgical procedure, the treated mice presented significant improvements in the motor behavior tests performed. (6) Zhang et al, administered umbilical mesenchymal stem cells in ataxic mice and found that these cells improved the motor skills of the mice and also alleviated cerebellar atrophy. (7) Chang et al, transplanted mesenchymal cells in mice models of spinocerebellar ataxic mice intravenously or intracranially. They found that intravenous injection delays the onset as well as improves the motor function of the affected mice. (8) Diaz et al and Kemp et al, demonstrated that bone marrow transplantation improves motor activity and stimulates neural repair in ataxia mice. (9,10) Uhlendorf et al and Nuryyev et al in 2017, transplanted human neural progenitor cells in Spastic Han-Wistar Rat models of ataxia. They reported reduced ataxic symptoms and structural and functional improvements. (11,12)

Human Studies

Sr. No	Author	Cells used	Route of administration	Sample size	Patients improved	Adverse events
1.	Jin, Jia-Li, et al. (9)	umbilical cord mesenchymal stem cell	Intravenous, intrathecal	16	16	No
2.	Amariglio, Ninette, et al. (10)	human fetal neural stem cells	Intracerebellar and intrathecal	1	0	brain tumor
3.	Dongmei, Han, et al. (11)	umbilical cord mesenchymal stromal cells	Intrathecal	24	10	No
4.	Yang, Wan-Zhang, et al. (12)	human umbilical cord blood-derived stem cells	Intravenous, intrathecal	30	30	No

5.	Sharma, Alok, et al. (13)	autologous bone marrow mononuclear cells	Intrathecal	1	1	No
6.	Miao, Xingyu et al. (14)	umbilical cord mesenchymal stem cells	Intrathecal	3	1	No
7.	Shroff, G et al (15)	Human Embryonic Stem Cells	Intravenous, Intramuscular	3	3	No

Not many clinical studies have been conducted on human subjects until now. Most of the studies used umbilical cord blood cells (13-16) All of them showed that these cells result in neurological and functional improvements. Amariglio et al transplanted human fetal neural stem cells intracerebellarly and intrathecally in 1 patient with Ataxia Telangiectasia. However, the patient developed host derived brain tumor. (17) Shroff et al transplanted human embryonic stem cells intravenously and intramuscularly in 3 cases of ataxia and reported it to efficacious. (18) Tsai et al, recently published the results of their Phase I/IIa Clinical Study conducted on 6 patients with spinocerebellar ataxia type 3. They transplanted allogenic adipose tissue derived mesenchymal stem cells intravenously. These cells were safe as there were no adverse events recorded and also there was minimal clinical improvement reported. (19)

Published Case report

A 33 year old female with SCA was treated with autologous BMMNCs intrathecal transplantation followed by standard rehabilitation. (20) She had severe impairment of dynamic balance, coordination, speech, gross and fine motor control. Ambulation was dependent; requiring support from two people with an ataxic gait. Functionally, she scored 86 on Functional independence measure (FIM) and 62 on Ataxia rating scale. On follow up at six months after the transplantation there was a significant improvement in handwriting, fine motor activities, standing dynamic balance and intelligibility of speech. There was an improvement in the cerebellar signs and symptoms and outcome measures like Modified International co-operative Ataxia rating scale (MICARS). The MICARS score reduced from 62 to 58.

Our Experience with Cerebellar Ataxia Patients:

We performed a study to demonstrate the effect of autologous bone marrow mononuclear cells in 91 cases of cerebellar ataxia. Symptoms such as co-ordination, ambulation, hand functions, stamina/fatigue, trunk balance and standing were analysed. On follow up, 93.4% of patients showed improvements while 6.6%

showed no improvement. 49.45% patients showed mild improvements, 39.5 % moderate improvements and 4.3% showed significant improvements.

Improvements in Cerebellar Ataxia After Stem Cell Therapy (N=91)

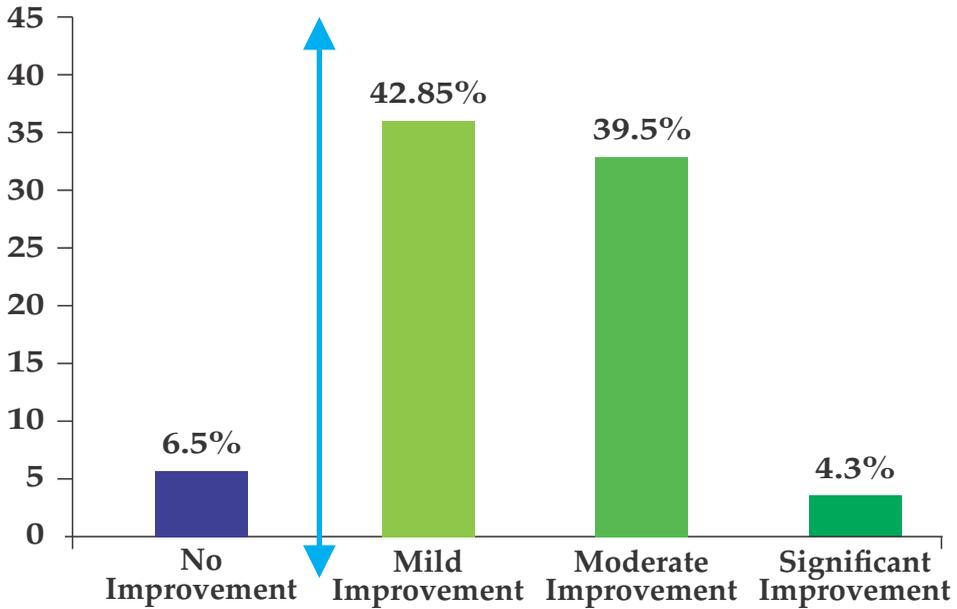


Figure 1: Improvements seen in cerebellar ataxia patients after intrathecal administration of autologous BMMNCs.

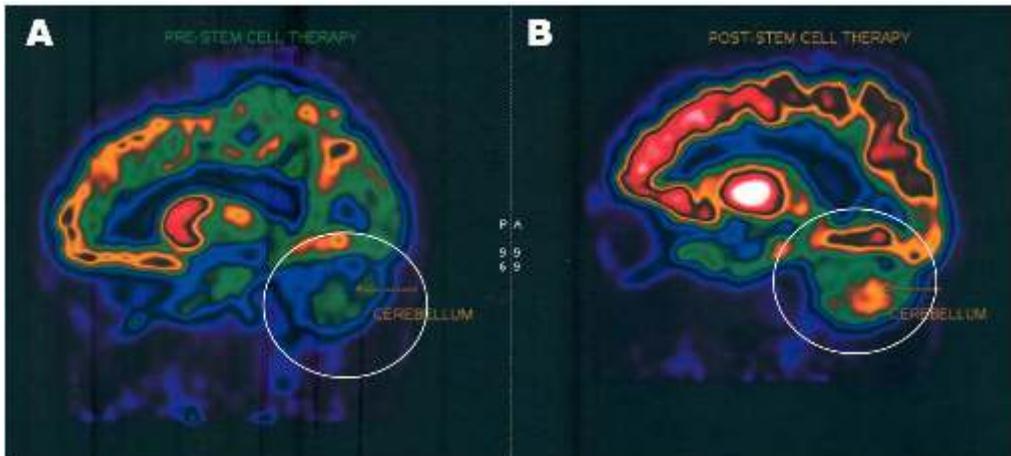


Figure 2: PET CT Scan showing improved metabolic activity which is indicated by increased orange area in the cerebellum after stem cell therapy as indicated by the circles.

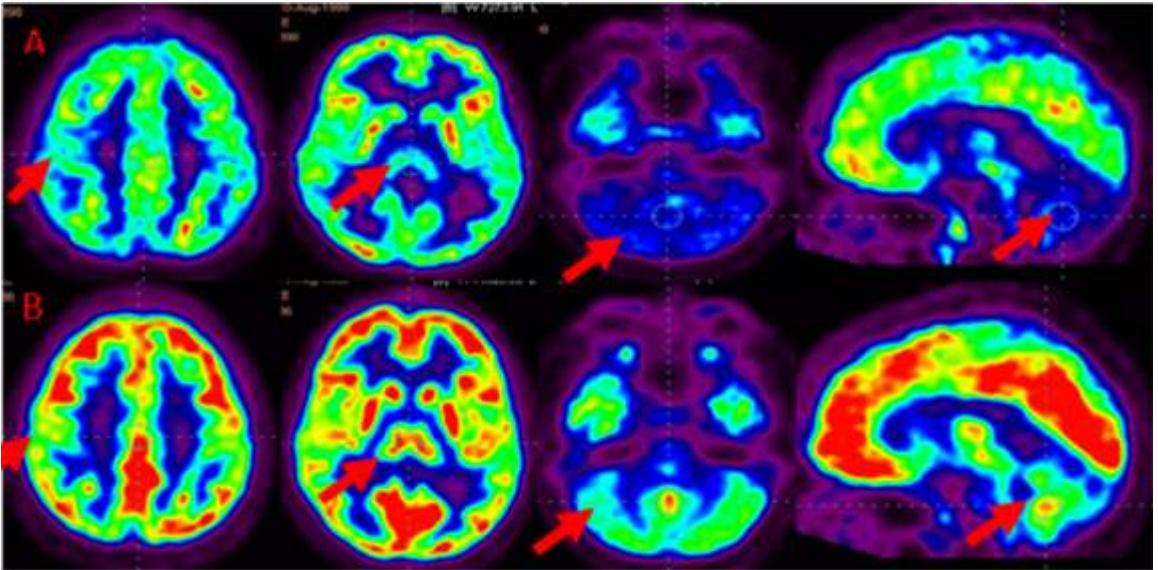


Figure 3: Comparative PET CT scan showing improvements before and after cellular therapy. (A) Pre-PET image before cellular therapy shows reduced metabolism in bilateral sensory motor cortex, parietal cortex, thalamus and cerebellum

(B) Post-PET image of 12-month interval following cellular therapy show improved metabolism in bilateral sensory motor cortex, parietal cortex, thalamus and cerebellum

Conclusion:

Existing literature and our clinical experience highlights the likelihood of stem cell therapy to be a potential treatment strategy for cerebellar ataxia. It does not cure but supplements the effect of other conventional treatments. It may help in alleviating the symptoms and alter disease progression in patients with ataxia making the patients functionally independent and improving their quality of life. In hereditary cerebellar ataxias, gene therapy is being developed to repair the underlying genetic defect. However, stem cell therapy may augment the effect of gene therapy by repairing the already occurred neural damage via neuroprotection. Hence, they can be used in combination for enhancing the therapeutic benefits. Rehabilitation also plays an important role in mobilizing the stem cells and enhancing the effect of transplantation, hence regular rehabilitation is recommended in combination with stem cell therapy. The efficacy of stem cell therapy depends on various factors. Stem cell therapy when done at a younger age gives a better outcome. If done at an early stage of disease, better is the chance of halting the progression and milder the damage, better are the improvements. PET CT scan brain can effectively showcase the metabolic changes occurring as a result of stem cell transplantation, at a cellular level. Hence, it should be further explored as an efficient monitoring tool.

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SECTION C

Important Related Aspects

If you can dream and not make dreams your master, if you can think but not make thoughts your aim; If you can meet with triumph and disaster and treat those two imposters just the same...if you can fill the unforgiving minute with 60 seconds of distant run, yours is the earth and everything that's in it"

- Rudyard Kipling

17

Radiological Imaging in Stem Cell Therapy

Stem cell therapy is the promising novel therapy with a potential to repair the neurological damage and prevent the rapid progressive neuromuscular degeneration. It has been widely explored for the treatment of various incurable neuromuscular disorders. Although there is a large evidence base for the use of stem cell therapy in neurological disorders, the effects observed are either functional or on subjective outcome measures. To develop stronger and more robust evidence base for the effects of stem cell therapy in the treatment of neurological disorders, more objective outcomes are required. The mechanism of action of stem cells is through their paracrine effects on surrounding tissues [1-7]. Although the cells have capability for regeneration, with current technology there are limited structural changes and these may be seen over a long time. The earliest improvements noted are functional improvements. To study these improvements and document the effects of stem cell therapy in detail, modern imaging modalities need to be used. With the use of such sophisticated neuroimaging and musculoskeletal imaging techniques beneficial effects of stem cell therapy have been documented in some of the published reports [8-20]. Various functional imaging modalities that can be used are functional magnetic resonance imaging (f-MRI), Diffusion tensor imaging of the muscles (DTI), Positron emission tomography - computed tomography (PET-CT) scan and structural imaging modalities like Musculo skeletal magnetic resonance imaging (MRI-MSK) and Electromyography.

Structural imaging modalities

Musculoskeletal MRI:

Radio imaging has advanced and various non-invasive techniques can give accurate information for diagnosing and monitoring MD [21]. MRI has several advantages over other imaging techniques used in MD. It is not dependent on the operator and does not use ionizing radiations. There is no need to administer an intravenous contrast for image acquisition. Multiplanar acquisition in MRI makes it easier to be used for the patients with contractures, deformities and severe muscle weakness as seen in MD. MRI also has an inherently high soft tissue resolution and discrimination potential for fat, muscles, fluids, edema and bones. This makes MRI the imaging modality of choice for MD. Musculoskeletal MRI helps to differentiate between the soft tissue and muscles. This allows for grading of the severity of fatty infiltration and muscle atrophy in MD. MRI has been previously used successfully to assess progression of disease in MD [22,23].

Duchenne's Muscular Dystrophy

In a case report published in American Journal of Case reports, a patient of DMD was treated with Autologous BMMNCs intrathecal and intramuscular transplantation and the effects were studied over the period of 3 years using serial MRI-MSKs [24]. Patient had undergone four subsequent transplantations performed at 9, 21 and 33 months after 1st transplantation. Clinical improvements and muscle strength measurements guided the time of subsequent transplantations. There is a 5% increase in the fatty infiltration and 3.9% reduction in the strength of the muscles every year in DMD as suggested by various studies on natural progression of the disease [25,26]. However the patient studied showed no deterioration in the muscle quantity and no increase in the fatty infiltration of the tissues on serial MRI-MSKs (Figure 1A and 1B); which indicated stabilization of the disease. This was also substantiated by the EMG showing better recruitment of the muscles and some new normal motor unit potentials. The patient at the end of 33 months showed improved muscle strength, better endurance, improved quality of the handwriting and was able to walk with the help of push knee splints and walker.

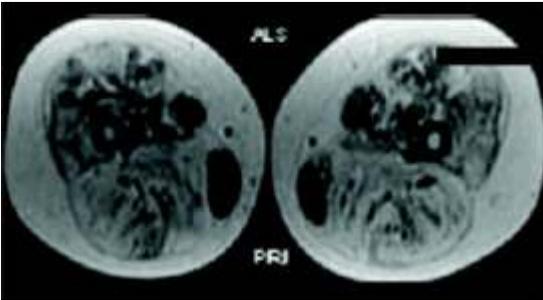


Figure 1A: T1 Weighted Axial Musculoskeletal MRI image before cellular transplantation

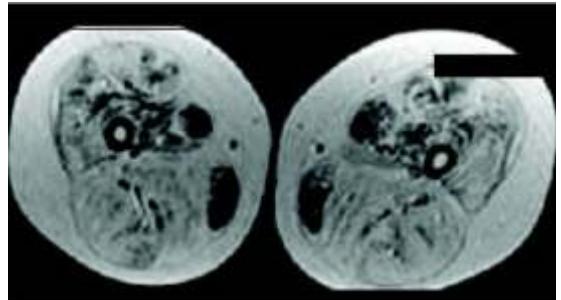


Figure 1B: T1 Weighted Axial Musculoskeletal MRI image 24 months after cellular transplantation showing no reduction in muscle mass and no increase in fatty infiltration suggestive of halting of disease progression in Duchenne Muscular Dystrophy

Becker's Muscular Dystrophy

We studied the therapeutic effect of cellular therapy on BMD using an MRI-MSK over a period of 8 months, as published in the Journal of case reports [27]. There was an increase in the muscle fibers of peronei, gastro-soleus and long, medial and lateral head of triceps with decreased fatty infiltration as observed on the MRI-MSK post 6 months of cellular transplantation (Figure 2A, 2B, 3A, 3B, 4A & 4B). Clinically there was improved standing balance, ability to walk with the help of push knee splints and unilateral human support. There was reduction in calf pain while standing and upper extremity pain while maintaining the quadruped position. All these activities involve the above mentioned muscles. The improved quality of movement may suggest better recruitment of the existing muscle fibers. Post cellular therapy increase in the muscle fibres and reduced fatty infiltration was an improvement that also correlated with the clinical improvements.

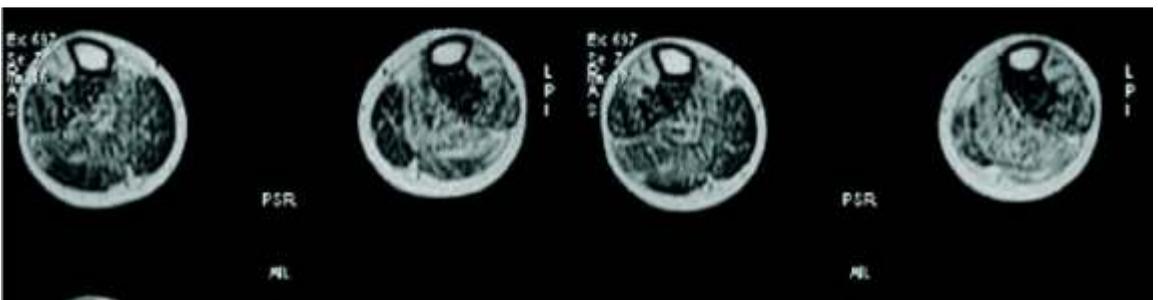


Figure 2A : T1 weighted axial musculoskeletal MRI images of Peroneous Longus and Brevis before Autologous BMMNCs transplantation

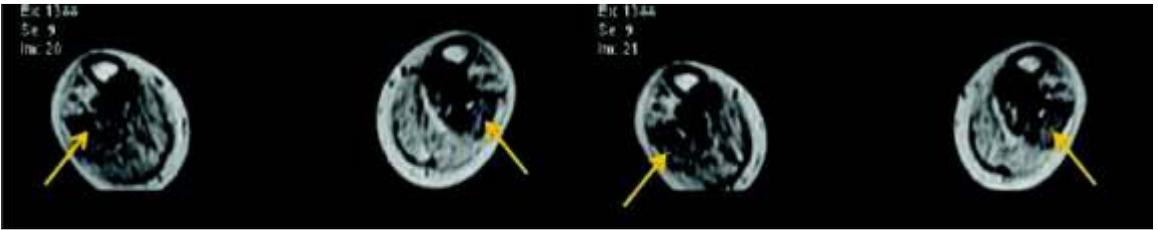


Figure 2B: T1 weighted axial musculoskeletal MRI images of Peroneus Longus and Brevis 6 months after Autologous BMMNCs transplantation, arrows showing muscle regeneration and reduced fatty infiltration

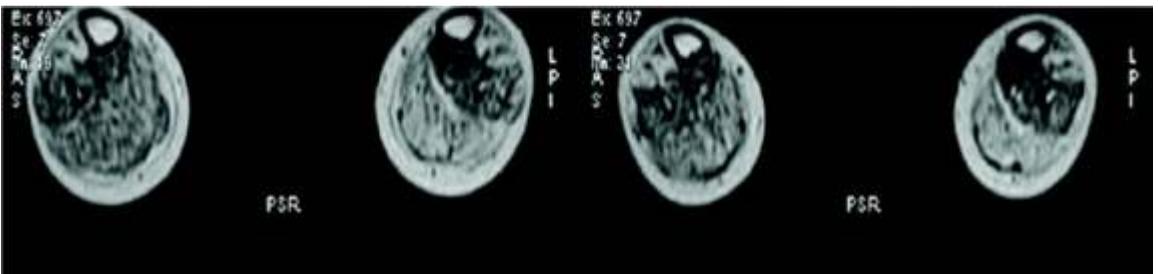


Figure 3B: T1 weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus 6 months after Autologous BMMNCs transplantation; arrows showing muscle regeneration and reduced fatty infiltration

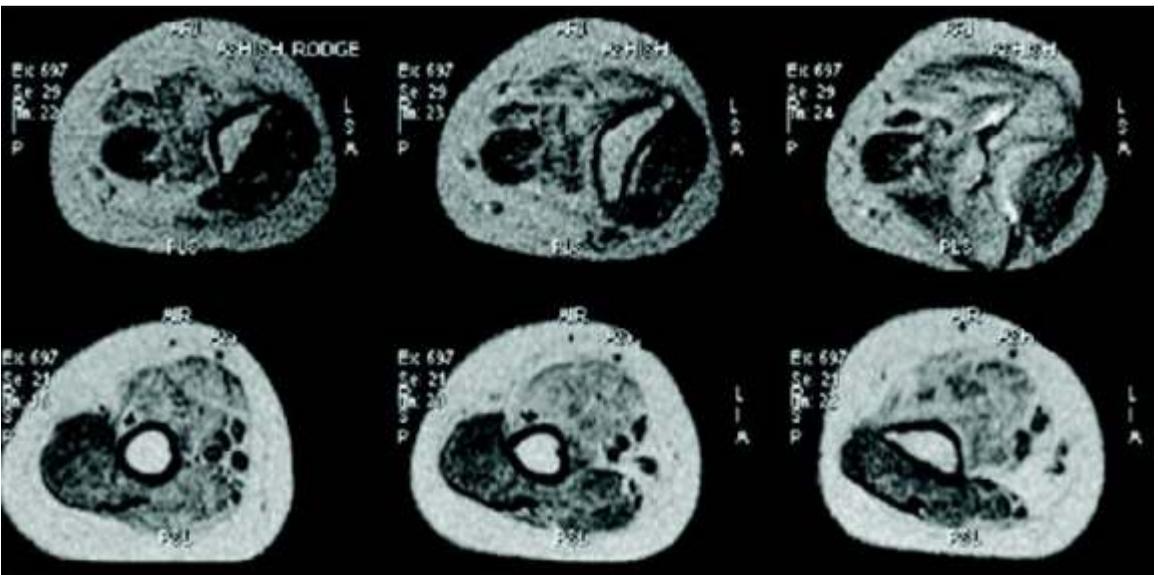


Figure 4A: T1 weighted axial musculoskeletal MRI images of Left and Right Long, Medial and Lateral head of Triceps before Autologous BMMNCs transplantation

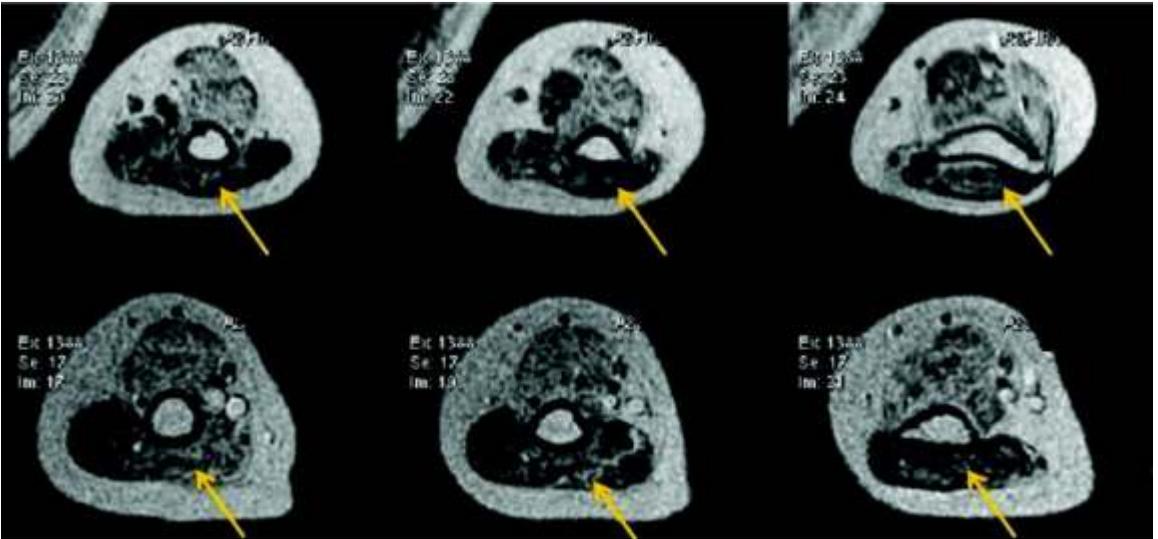


Figure 4B: T1 weighted axial musculoskeletal MRI images of Left and Right Long, Medial and Lateral head of Triceps 6 months after Autologous BMMNCs transplantation; arrows showing muscle regeneration and reduced fatty infiltration

Functional imaging modalities

The underlying principle of functional neuroimaging using PET-CT scan is that the changes in blood flow and energy consumption in the form of glucose are associated with brain metabolism and tissue function [28,29]. F-MRI is another modality that is used for functional neuroimaging. F-MRI measures the blood oxygenation level dependent effects (BOLD) in the brain and spinal cord and provides a diagrammatic representation for the function of the brain and spinal cord [30].

Functional MRI

Spinal Cord Injury

A patient of traumatic paraplegia due to a complete spinal cord injury at the level of 10th thoracic vertebra, underwent autologous BMMNCs intrathecal transplantation one month after his injury. He had complete motor and sensory loss below the level of injury. Bladder and bowel sensory and motor control was absent. He was completely dependent for daily activities. After two serial transplantations 6 months apart and rigorous rehabilitation, he showed some motor recovery in adductors of hip showing strength of grade 1 on manual muscle testing. He also had minimal sensory recovery. Bladder and bowel sensations had improved. This improvement reflected in the functional MRI showing more number of nerve cells being recruited during a particular activity. He also showed improvement in the conduction velocity of motor nerves when tested using EMG.

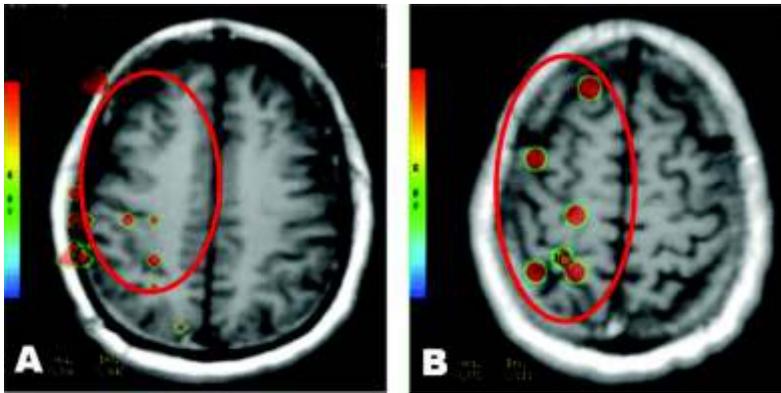


Figure 5: FMRI showing changes after stem cell therapy

Positron Emission Tomography - Computed Tomography Scan

PET-CT scan is a functional imaging technique that represents physiological activity of brain as three dimensional images and reflects the functional processes in the body. The physiological activity of brain is measured using, the FDG (fluorodeoxyglucose) uptake by the brain cells. FDG is an analogue of Glucose. Glucose transporter proteins transport FDG to the brain cell where it undergoes the metabolic processes. Once it has been converted to Glucose 6 phosphate it cannot be metabolized further and is trapped in the cells as the cell membrane is impermeable to this molecule [31]. Metabolism of FDG is due to glycolysis and therefore the trapped glucose 6 phosphate molecules correlate with the metabolic activity of the brain cells. Increased retention of FDG therefore indicates increased metabolic activity of the tissue [32]. PET measures the retention of FDG in terms of standard uptake value (SUV), which is the ratio of the actual concentration of glucose in brain tissue and the hypothetical concentration of the glucose in brain tissue if it was distributed evenly in all the areas of brain. Increased SUV indicates better metabolic activity of the tissue [33]. The following are the PET-CT scan findings that correlated with the functional recovery of the patients with different diagnoses like Autism, Cerebral Palsy, Stroke, Traumatic brain injury, Dementia and Ataxia.

Autism

1. A 6 year old boy diagnosed of Autism was treated with autologous bone marrow mononuclear cell transplantation. He exhibited poor eye contact, hyperactivity, poor communication, poor attention and concentration, repetitive motor behaviour like hand flapping, aggressive behaviours like biting and hitting as well as very poor social interaction. He scored 123 on Indian scale for assessment of autism (ISAA). His PET-CT scan showed hypometabolism in the regions of superior temporal gyrus, amygdale and fusiform gyrus (social brain) as well as bilateral basal ganglia, hippocampus, parahippocampus and cerebellum. 6 months post transplantation metabolism in all the above areas had improved significantly

(Figure 6A, B and C). He showed improvement in non verbal communication, eye contact, social interaction, attention and concentration. Aggressive and repetitive motor behaviour had reduced significantly. His ISAA score also showed improvement, intensity of all symptoms reduced and therefore the score reduced from 123 to 103. In view of these improvements he underwent second stem cell transplantation. 6 months following the stem cell transplantation the ISAA score was maintained and he showed further improvement in speech, communication, hyperactivity, command following and eye contact.

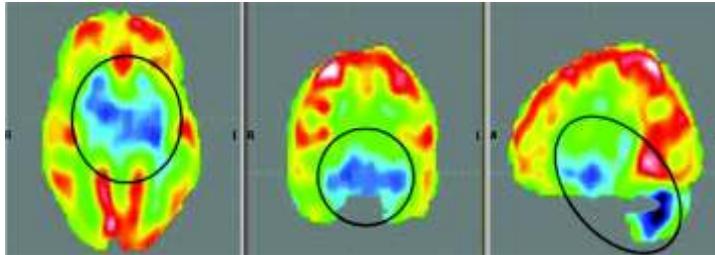


Figure 6A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum

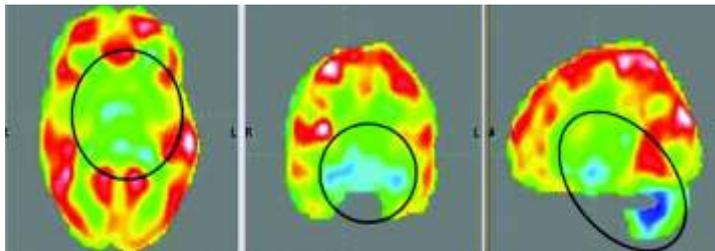


Figure 6B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of superior temporal gyrus, mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum; indicated by reduction in the blue and black areas

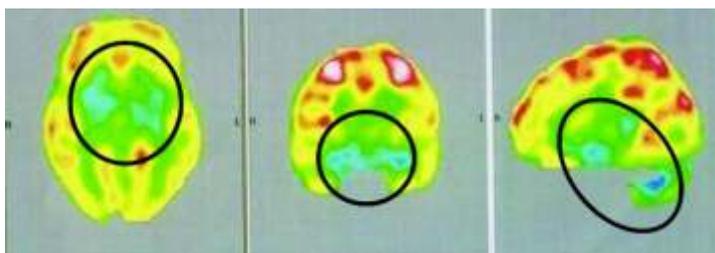


Figure 6C: PET-CT scan 8 months after second cellular transplantation showing maintained increase in the metabolism of superior temporal gyrus, mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum and reduction in the metabolism cortical regions showing the balancing effect

2. A 13 year old male and a known case of Autism was treated with autologous bone marrow mononuclear cells intrathecal transplantation. He was born full term by normal delivery, with history of delayed cry and no neonatal complications. His motor milestones were normal but his speech development was delayed. When he was 2 years old, he was diagnosed to have Autistic features. He presented with symptoms like hyperactivity, restlessness, poor social interaction, poor sitting tolerance, poor command following, temper tantrums, hypersensitivity to touch and fleeting eye contact. After 6 months of the transplantation he showed improvement in sitting tolerance in class, reduction in hyperactivity, reduction in aggressive behaviour, improved eating habits and preference, improved clarity of speech and improved command following. These clinical improvements were reflected on PET-CT scan as increased metabolism in the region of bilateral occipital lobes and mesial temporal structures.

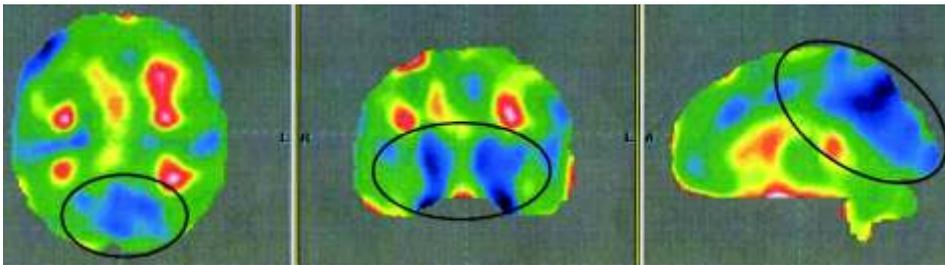


Figure 7A: PET - CT Scan before stem cell transplantation suggestive of reduction in the metabolism of occipital lobes and mesial temporal structures bilaterally

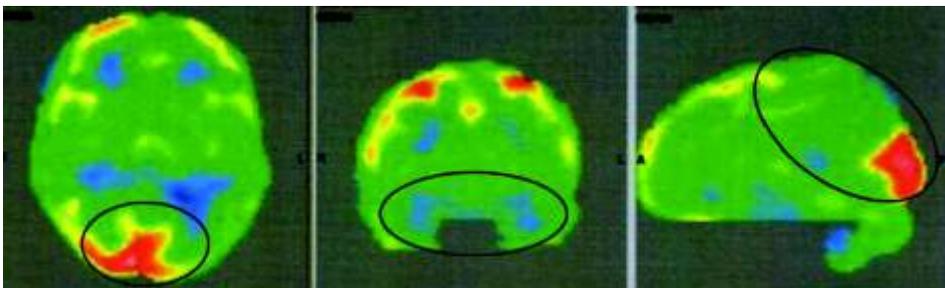


Figure 7B: PET-CT scan after stem cell transplantation showing improved metabolism in bilateral occipital lobes mesial temporal structures

3. A 9 year old girl with Autism underwent autologous BMMNCs intrathecal transplantation. Her main symptoms were poor attention and concentration, social interaction, difficulty in adapting to changed environment, presence of repetitive and strange movements, poor eye contact, irrelevant speech and complete dependence for ADL's. 6 months after the first transplantation, she showed improvements in eye contact, non- verbal communication, and learning, reduction in laughing without reason, improvement in command following, understanding relationships, reduced hyperactivity and started picking up well in ADL training.

These changes also correlated with the PET-CT scan showing improvement in the metabolism of mesial temporal lobes, amygdala, hippocampus, and cerebellum bilaterally (Figure 8B).

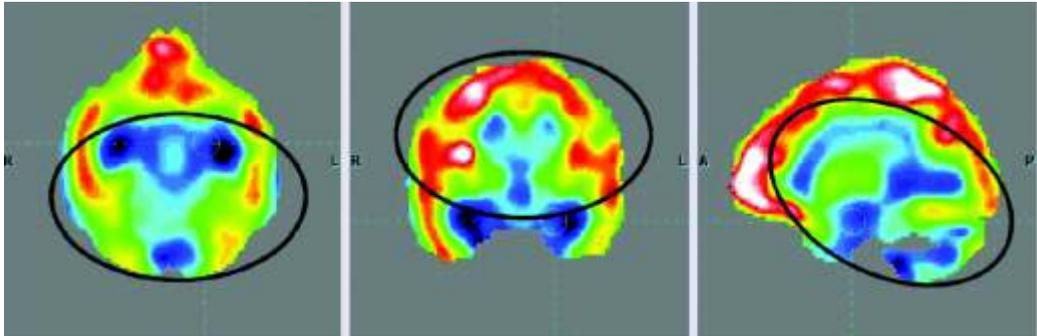


Figure 8A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum

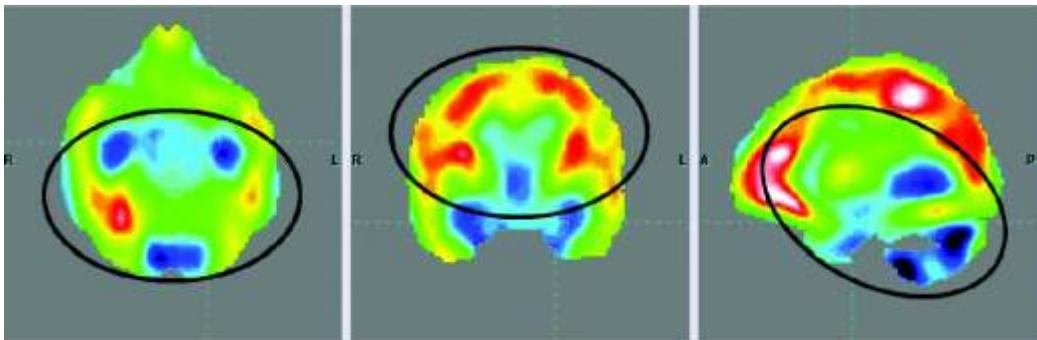


Figure 8B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of mesial temporal lobes, amygdala, hippocampus, and cerebellum bilaterally; indicated by reduction in the blue and black areas

4. 11 year old boy with poor attention and concentration, increased hyperactivity, poor social interaction, with abnormal presence of stereotypical behaviour, poor eye contact, monosyllable speech was treated with cellular therapy. The PETCT scan before stem cell therapy was suggestive reduced metabolism in Temporal lobes, Mesial temporal lobes, Cingulate and paracingulate regions, Cerebellum, Amygdala, Hippocampus and Parahippocampus. Six months after cellular therapy he showed reduction in hyperactivity, he could sit at one place for ½ hour at a stretch. His cognition had improved, he started following written instructions, his sitting posture had improved, his imitation skills had improved, he started following commands and his eye contact also had improved. The PET-CT scan after cellular transplantation also showed increased metabolism in the regions of Temporal lobes, Mesial temporal lobes, Cingulate and paracingulate regions, Cerebellum, Amygdala, Hippocampus and Parahippocampus.

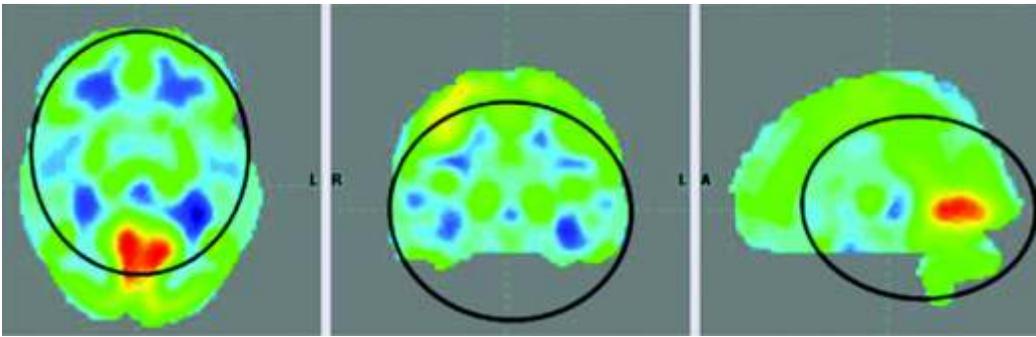


Figure 9A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum

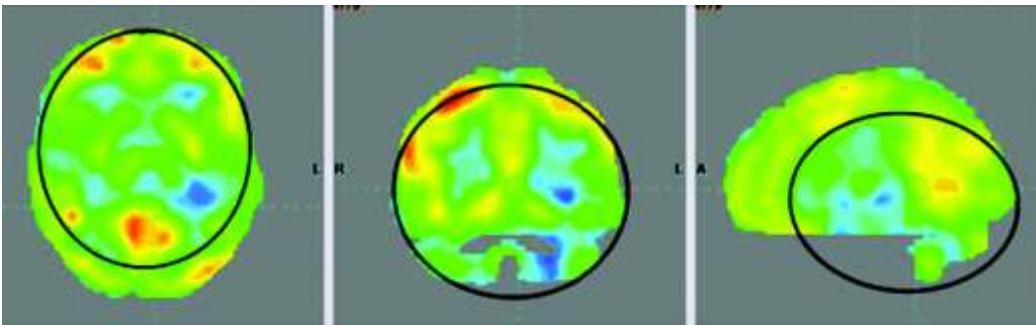


Figure 9B: PET-CT scan after cellular transplantation showing increased metabolism in the regions of mesial temporal lobes, amygdala, hippocampus & cerebellum bilaterally; indicated by reduction in the blue & black areas

5. A 4 year boy with a diagnosis of Autism underwent bone marrow mononuclear cells intrathecal transplantation. He presented with symptoms like poor eye contact, inability to speak, poor cognition, hyperactivity, poor attention concentration and poor social interaction. 14 months after transplantation he showed improvement in eye contact, he could indicate bladder and bowel, he started imitating and repeating words, he was able to write the alphabets, social interaction, attention and concentration improved. These clinical improvements also co related with the changes observed in PET-CT scan findings.

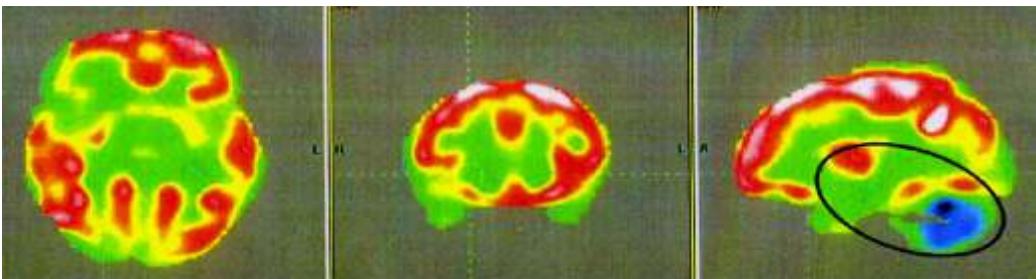


Figure 10A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of bilateral cerebellum

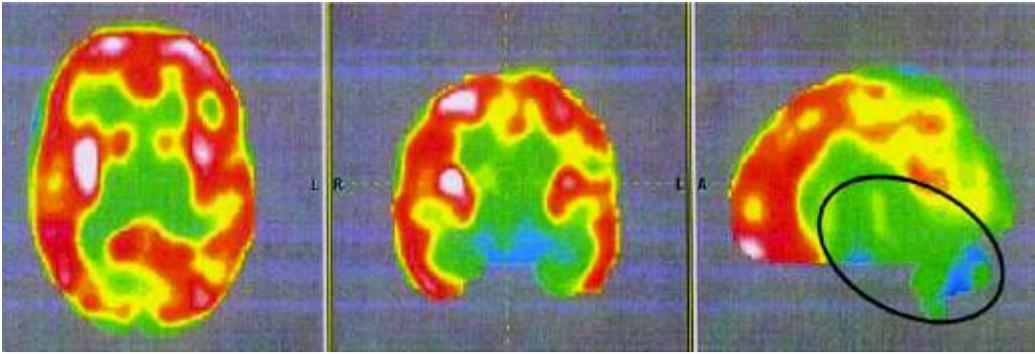


Figure 10B: PET-CT scan after cellular transplantation showing increased metabolism in the regions of cerebellum bilaterally; indicated by reduction in the blue and black areas

6. A case of autism presented with symptoms like poor attention and concentration, fleeting eye contact, poor sitting tolerance, hyperactivity, poor social interaction and impaired speech underwent autologous BMMNCs intrathecal transplantation. The PET-CT scan findings suggested reduced metabolism in mesial temporal lobes, basal ganglia and cerebellar lobes bilaterally. After cellular therapy he showed improvement in attention and concentration, sitting tolerance, command following, improved social engagement, improved vocalisation and speech and reduction in hyperactivity and stereotypical behaviours. PET-CT scan correspondingly showed improved metabolism in basal ganglia, mesial temporal structures and cerebellar lobes bilaterally.

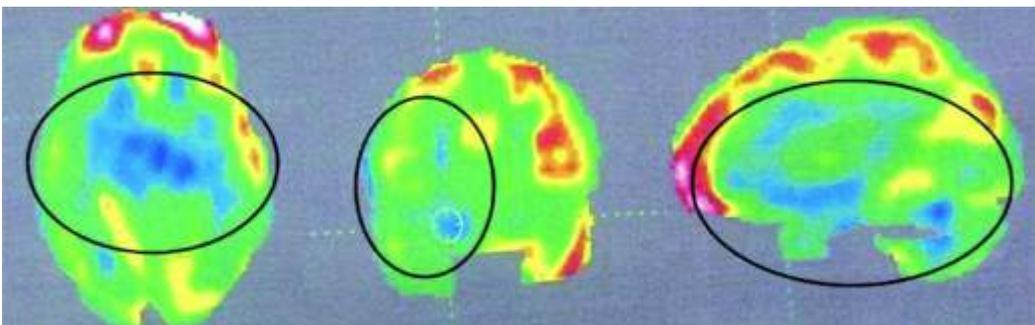


Figure 11A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour and as outlined by the circles in the regions of basal ganglia, mesial temporal structures and cerebellum

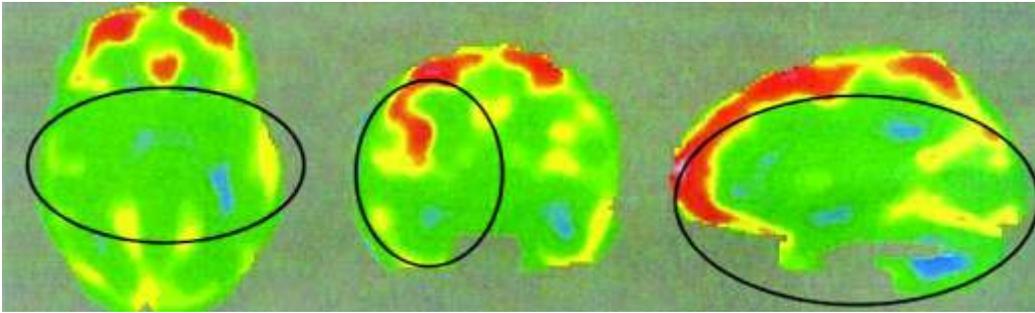


Figure 11B: PET-CT scan after cellular transplantation showing increased metabolism as outlined by the circles in the regions of basal ganglia, mesial temporal structures and cerebellum

7. A 7 year old boy diagnosed with Autism underwent autologous BMMNCs intrathecal transplantation. His main symptoms were poor social interaction, crying spells, hyperactivity, poor eye contact, temper tantrums, poor command following and complete dependence for daily activities. His PET-CT scan (Figure 12 A) suggested diffuse increase in the metabolic activity of the cortical lobes and reduced metabolic activity in bilateral cerebellar lobes. 6 months after the first transplantation he showed improvements in command following, eye contact, attention and concentration, understanding of the relationships, social interaction and reduction in stereotypical behaviour, hyperactivity, temper tantrums and aggressive behavior. These improvements correlated with the PETCT scan changes of balancing of the brain metabolism as shown in the Figure 12B.

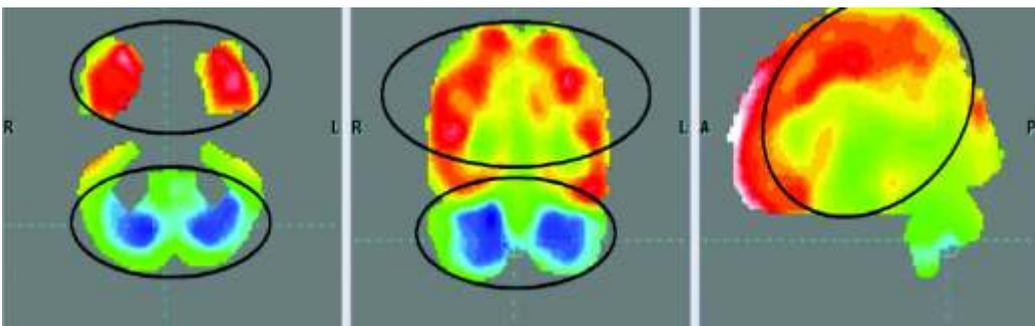


Figure 12A: PET-CT scan before stem cell therapy showing diffuse increase in the metabolic activity of the cortical lobes and reduced metabolic activity in bilateral cerebellar lobes

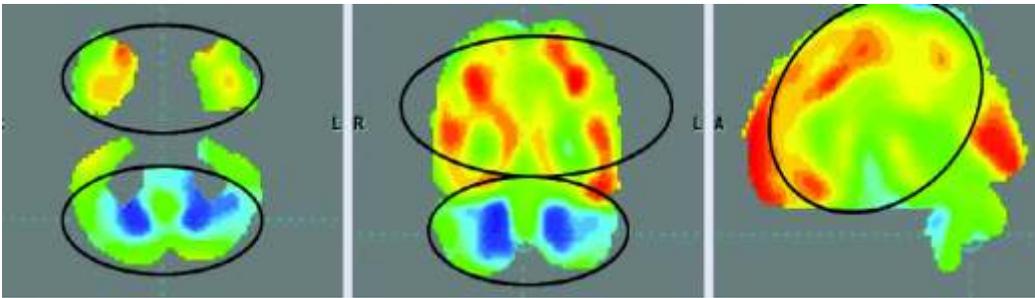


Figure 12B: PET-CT scan after stem cell therapy showing reduction in the metabolic activity of the cortical lobes and increased metabolic activity in bilateral cerebellar lobes hence highlighting the balancing effect of cellular therapy

8. A 4 year old boy with Dopamine Responsive Dystonia underwent Adult Autologous BMMNC's intrathecal transplantation. His main symptoms were severe dystonia and poor trunk control and hand functions ,absence of speech, presence of drooling, presence of rigidity. 6 months after first transplantation, he showed improvements in his neck and trunk control, reduction in drooling, improvements in sitting balance improvement in gross motor skills and tendency for extensor posture has reduced. A comparison PET-CT scan showed increased metabolic activity in bilateral cerebellar lobes and thalami.

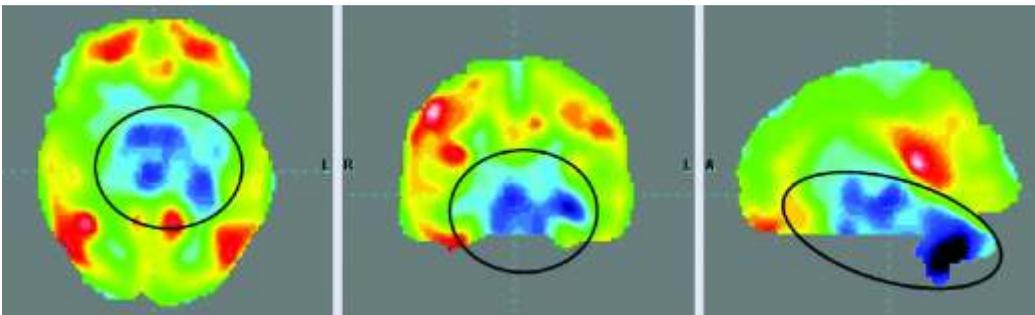


Figure 13A: PET-CT scan before stem cell therapy showing reduction in the metabolic activity of the cerebellar lobes and thalami bilaterally

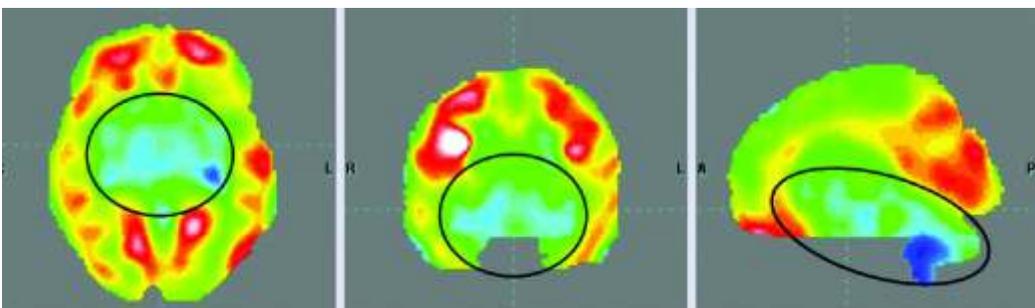


Figure 13B: PET-CT scan after stem cell therapy showing increased metabolic activity in bilateral cerebellar lobes and thalami bilaterally

Cerebral Palsy

1. A 12 year old boy suffering from spastic diaplegic cerebral palsy was treated with cellular therapy. He was hypertonic and hyperreflexic. He had poor hand writing, poor balance in sitting and standing and walked with a crouch gait and was moderately dependent for activities of daily living. There was no sensory or cognitive involvement. The PET-CT scan showed reduced FDG uptake in the cerebellar lobes and mesial temporal structures (Figure 14A). 6 months post cellular therapy he showed significant improvement in fine motor activities, gait and balance. He required only a minimal help for his activities of daily living. PET-CT showed improved metabolism in all the areas of reduced FDG uptake (Figure 14B).

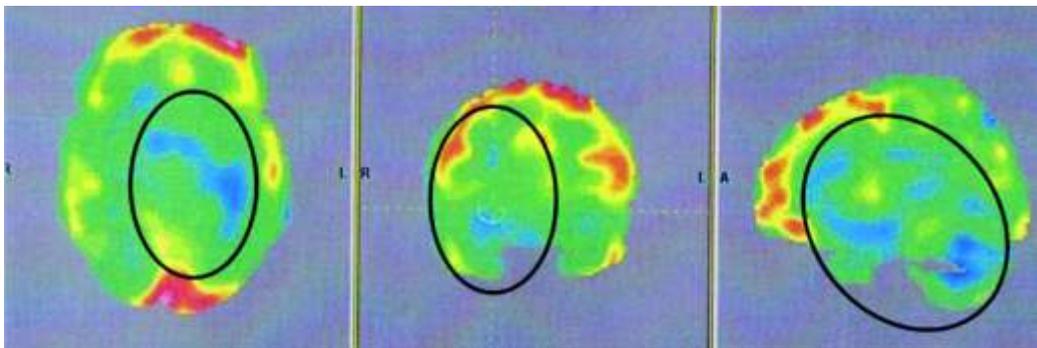


Figure 14A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of cerebellar lobes and mesial temporal structures

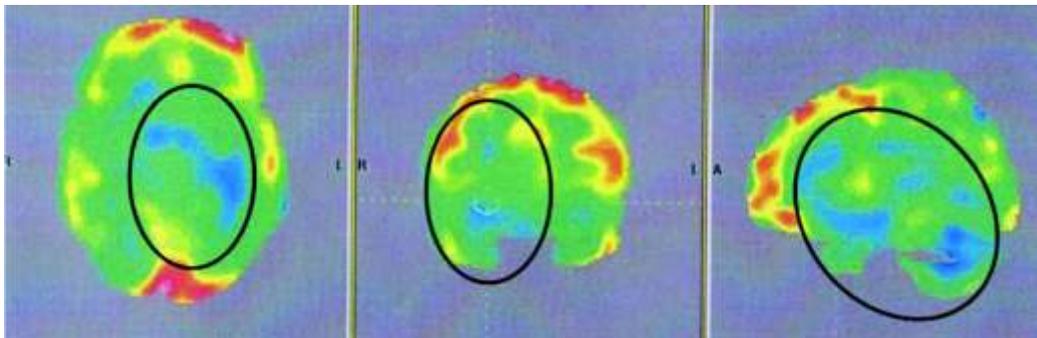


Figure 14B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of cerebellar lobes and mesial temporal structures seen as reduced blue and black areas

2. An 8 year old child born of consanguineous marriage, delivered by caesarean section presented with a history of brain hypoxia due to delayed cry after birth and also had a seizure 5 hours after birth. He was diagnosed with CP and the main symptoms were presence of abnormal reflexes, fluctuating tone, poor voluntary control and poor cognition. His PET-CT scan findings suggested reduced

metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally (Figure 15A). He underwent autologous BMMNCs intrathecal transplantation. There were improvements noted after stem cell therapy in the oromotor skills, voluntary control of upper extremity, reduction of spasticity, improved bed mobility, improved awareness and other cognitive skills, improved eye contact and improved trunk control. These changes also correlated with the improvements in the PET-CT scan suggestive of increased metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally (Figure 15B).

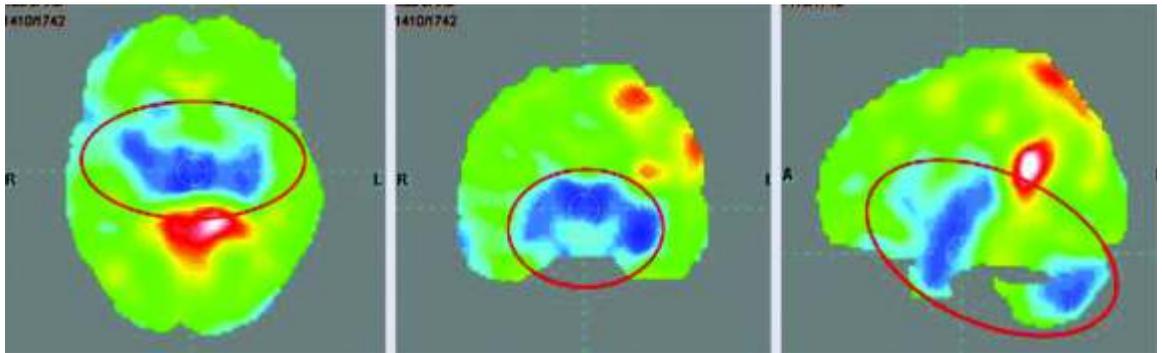


Figure 15A: PET-CT scan before cellular transplantation showing reduced metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally as indicated by the circles

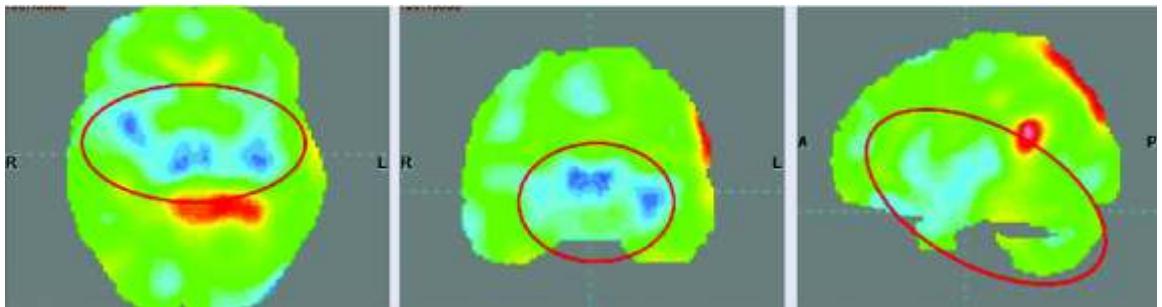


Figure 15B: PET-CT scan 9 months after cellular transplantation showing increased metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally as indicated by the circles

3. A 12 year old boy diagnosed with cerebral palsy was treated with autologous BMMNCs intrathecal transplantation. He presented with symptoms like increased muscles tone, poor voluntary control of bilateral lower extremity and fair fine motor activity of upper extremity. He could walk with elbow crutches in crouch Gait. Post cellular therapy improvements were noted in balance while standing and

performing exercise related activities. Voluntary control of both upper and lower limbs had improved, he could perform all the daily activities independently and his handwriting speed improved. These clinical improvements correlated with PET-CT findings of improved metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes (Figure 16 A&B).

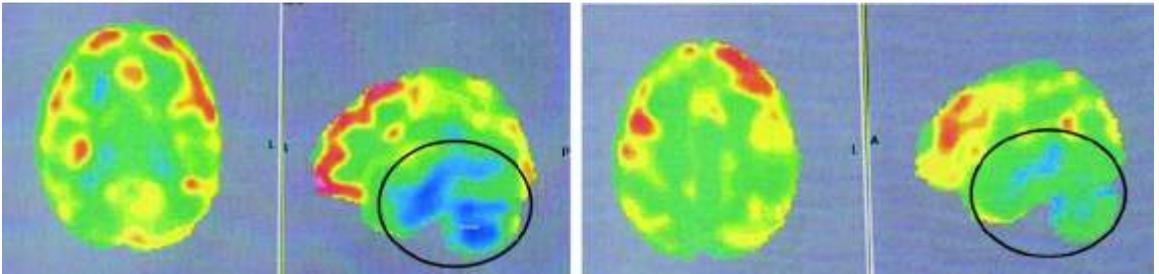


Figure 16A: PET-CT scan before cellular transplantation showing reduced metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes bilaterally as indicated by the circles

Figure 16B: PET-CT scan after cellular transplantation showing improved metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes bilaterally as indicated by the circles

Mental Retardation

1. A 20 year old male suffering from CP and Mental Retardation (MR) was treated with cellular therapy at our center. He had diplegic gait and Intelligence Quotient (IQ) score of 44 with affected fine motor activities, balance, speech and higher functions. PET-CT scan identified frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus as the affected areas (Figure 17A). He was treated with cellular therapy of Autologous BMMNCs intrathecal transplantation followed by multidisciplinary rehabilitation. Six months following therapy, he showed improvement in social behavior, speech, balance, daily functioning and IQ score increased to 55. PET-CT scan showed significant increase in metabolic activity in all four lobes, mesial temporal structures and left cerebellar hemisphere. The clinical improvements correlated with the changes observed in the PET CT scan (Figure 17B).

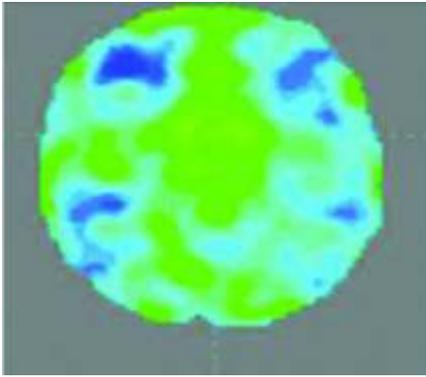


Figure 17A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus

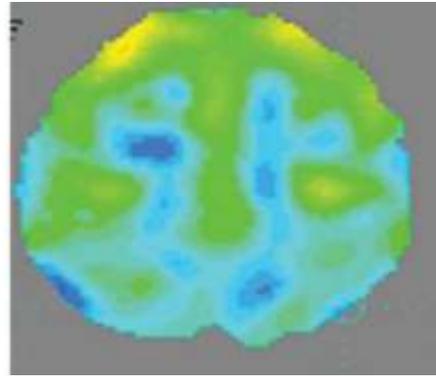


Figure 17B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus; indicated by reduction in the blue and black areas

Ischemic Stroke

1. In a case of chronic stroke caused by ischemia in the territory of right middle cerebral artery Autologous BMMNCs intrathecal transplantation was performed 3 years after the stroke. Upon performing the serial PET-CT scans before and 1 year after transplantation, there was a significant increase in the metabolism of brain in the regions of Parietal lobes. The standard uptake of FDG in parietal lobe increased from 7.01 to 9.51. Clinically he showed improvement in balance, gait and functional independence as well as reduction in spasticity.

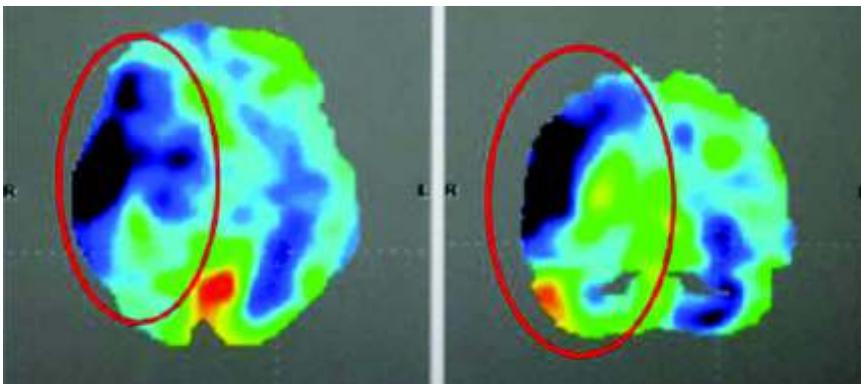


Figure 18A. PET-CT scan before Autologous BMMNCs transplantation suggestive of stroke in the region right MCA territory with black areas suggestive of gliosis where as the blue regions suggestive of penumbra

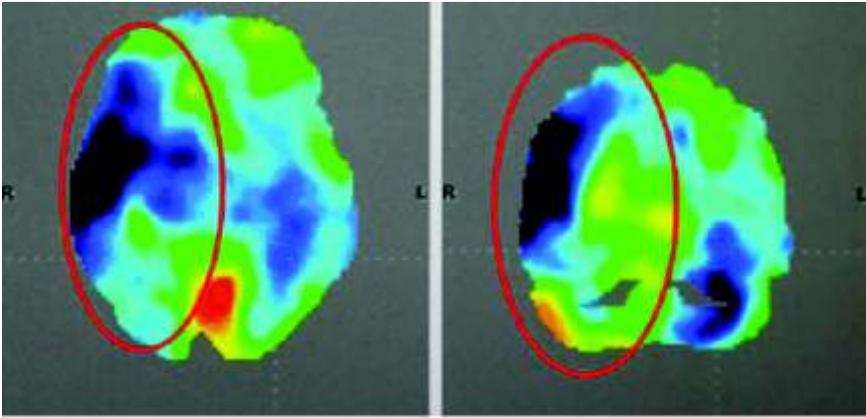


Figure 18B. PET-CT scan 1 year after Autologous BMMNCs transplantation showing improved metabolism in the parietal lobe penumbral regions seen as reduction in the blue coloured areas

2. A 58 year old man with ischemic stroke presented with poor control of right upper and lower extremity, slurred speech, impaired cognition and behavior, increased spasticity in the muscles of upper limb, poor balance while walking and hemiplegic gait pattern. He was treated with autologous BMMNCs intrathecal transplantation 3 years after stroke. 7 months post transplantation he showed improvement in various physical tasks like upper limb overhead activity and fine motor activity, improved gait pattern, improved walking balance, orientation to date, time and place and reduced confusion and emotional outbursts. These changes correlated with the PET-CT scan findings of improved brain metabolism in the region of left frontal lobe, occipital lobe and basal ganglia.

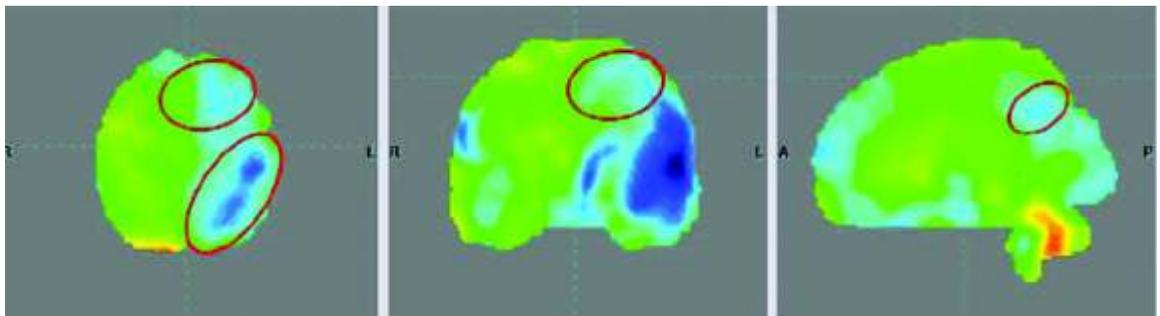


Figure 19A: PET-CT scan before Autologous BMMNCs transplantation suggestive of reduced metabolism in the regions of left frontal lobe, occipital lobe and basal ganglia

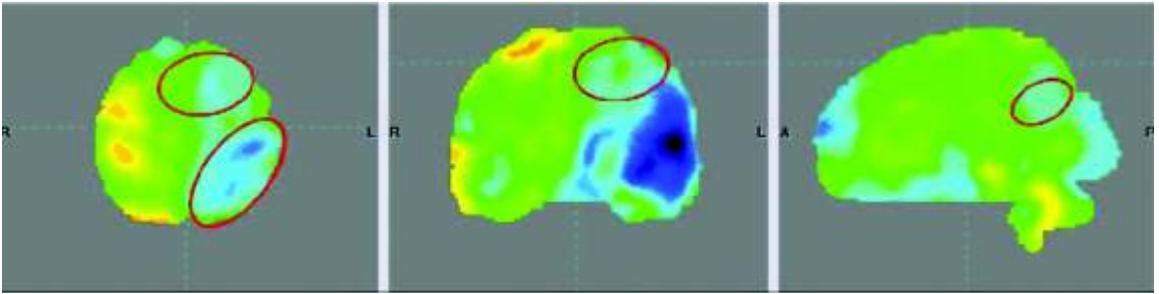


Figure 19B: PET-CT scan after Autologous BMMNCs transplantation suggestive of increased metabolism in the regions of left frontal lobe, occipital lobe and basal ganglia

Hemorrhagic Stroke

Cellular transplantation in the case of chronic hemorrhagic stroke involving frontal, parietal lobes and cerebellum and brainstem was performed 1 year after the transplantation. There was increase in the metabolism of the brain in PET-CT scans. The increase in the metabolism was noted in the regions of Frontal lobe, Parietal lobe and Cerebellum. Clinically this increased metabolism was correlated with improved cognition, balance, motor function, functional independence and speech intelligibility as well as reduction in spasticity.

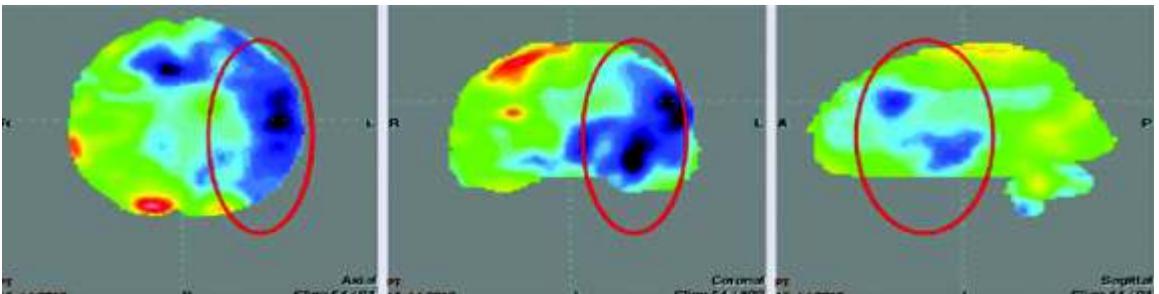


Figure 20A: PET-CT scan before Autologous BMMNCs transplantation suggestive of hemorrhagic stroke in the region left parietal lobe with black areas suggestive of gliotic areas whereas the blue regions suggestive of penumbra

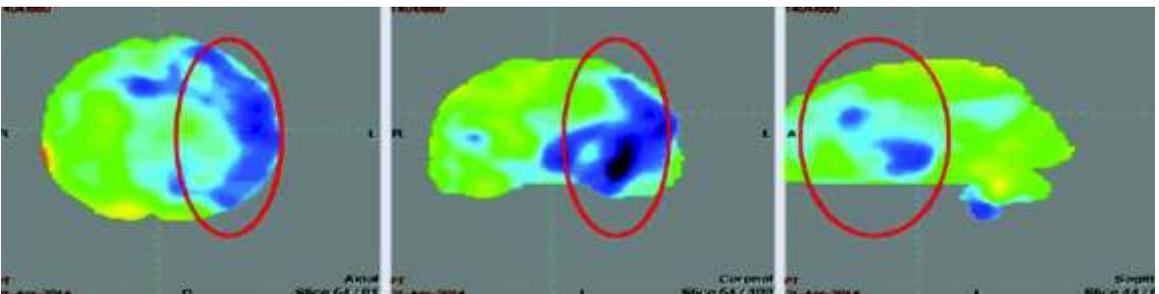


Figure 20B: PET-CT scan 6 months after Autologous BMMNCs transplantation shows reduced blue areas signifying improved metabolism of the penumbral regions

Traumatic Brain Injury

1. 15 yrs old girl presented to us with a history of RTA at age of 8 yrs due to which she suffered a Severe Head Injury and transtentorial herniation with decompressive craniotomy and diffuse axonal injury. Her PET showed Gliotic areas with reduced uptake in bilateral parieto-occipital and right anterior inferior temporal region. She underwent autologous bone marrow mononuclear cell transplantation, with intensive multidisciplinary rehabilitation. Post SCT improvements were seen throughout, with maximum changes occurring by 6 months. Improvements were seen in her in her short term memory, behavior, improved new learning skills, improved understanding and her overall cognition, she also had an improvement in her vision with better perception for moving objects, there was also an improvement seen in her Rt sided gross motor and fine motor co-ordination and reduced neglect on Rt side with an overall improvement in her bilateral co-ordination. There was also significant change seen in her PET scan, there was an increase in the FDG uptake seen in the anterior cingulate gyrus, the middle cingulate gyrus and in the posterior cingulate gyrus. The para hippocampal gyrus uptake has increased. Increased FDG uptake is also seen in the amygdala. There was also increased FDG uptake seen in both frontal lobes, and in the right temporal lobe.

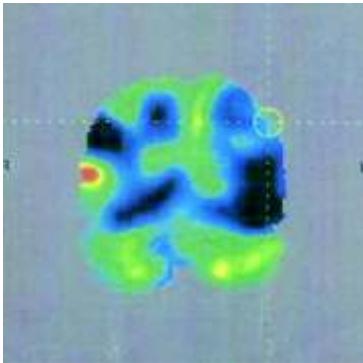


Figure 21A: PET-CT scan before Autologous BMMNCs transplantation showing gliotic areas in black with areas of reduced metabolism in blue seen in bilateral parieto-occipital & right anterior inferior temporal region

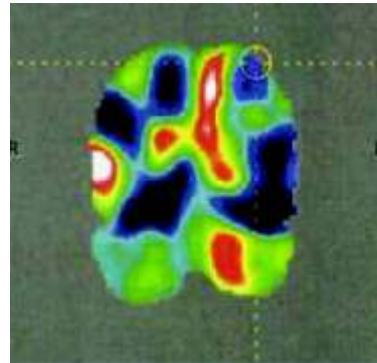


Figure 21B: PET-CT scan after Autologous BMMNCs showing improved metabolism in the regions of anterior, middle and posterior cingulate gyrus, amygdala, hippocampus and parahippocampus

2. A 34 year old patient with traumatic head injury was treated with autologous BMMNCs intrathecal transplantation. Due to the traumatic injury he had developed right hemiplegia, increased muscle tone in right upper and lower extremity, dysarthria, poor sitting and standing balance, inability to walk without

support and subnormal cognition. His PET-CT scan showed reduced metabolism in the region of right cerebellum. After the stem cell therapy he showed improvements in sitting and standing posture, gait pattern, spasticity, oromotor control, speech and higher cognitive functions. This clinical improvement correlated with improved metabolism in cerebellum.

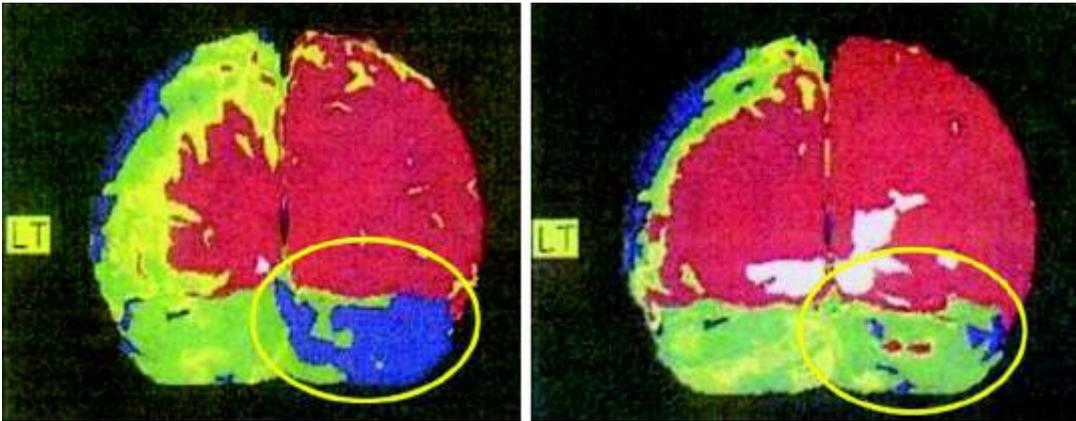


Figure 22: PET CT Scan showing improved metabolic activity which is indicated by decrease in blue areas after stem cell therapy

Dementia

1. A 61 year old, right handed female, presented with a medical history of hypertension and vascular dementia. On the Functional Independence Measure (FIM), she scored 75/126. And on the objective neuropsychological assessment using Mini Mental Status examination, she got a score of 10/30 indicating severe dementia. PET scan showed global hypo metabolism in the brain. Bilateral parietal lobe showed moderately reduced FDG uptake and bilateral frontal and temporal showed mild reduction. She underwent autologous bone marrow derived mononuclear cell transplantation. At follow up assessment, improvements were noted in terms of her Cognition, behavior and physical activities. On MMSE, her scores improved from 13/30 to 16/30 at 6 months follow up and finally to 20/30 at the end of 2 years. Her FIM score improved from 75 to 80 in 2 years. On PET-CT scan there was increase FDG uptake noted in the regions of bilateral parietal, frontal and temporal lobes (Figure 23A and 23B).

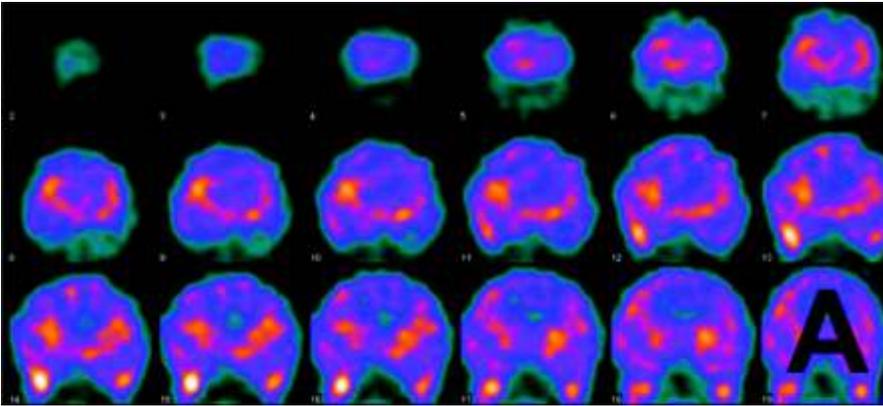


Fig 23A: Pre stem cell. There is overall hypometabolism seen in the brain, with purple areas depicting areas of hypometabolism and orange areas as areas of normal metabolism

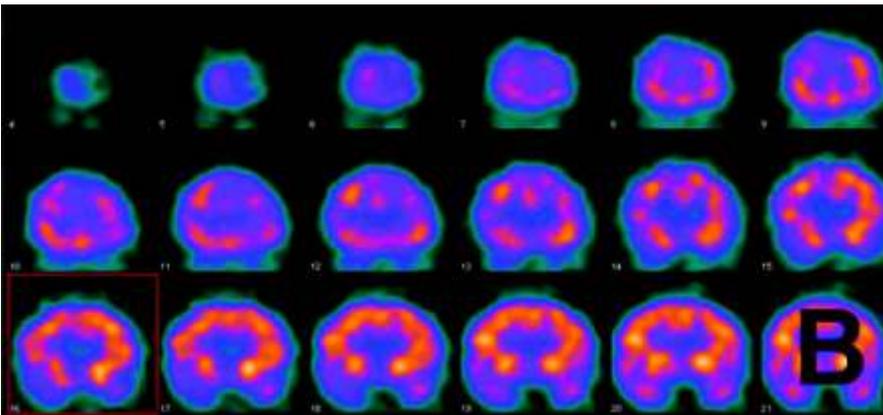


Fig 23B. Post stem cell. As compared to Fig A, there is increase in the orange area, which is the area of normal metabolism and reduction in the purple area. This suggests that after stem cell therapy, there is increased metabolic activity in the brain, thus improved neural activity.

Cerebellar Ataxia

1. An 18 year old girl with progressive cererbellar ataxia was treated with autologous BMMNCs intrathecal transplantation. The symptoms were progressive beginning at the age of 3 years of age. Although initially it started only with minor loss of balance while walking the symptoms progressed rapidly with inability to speak clearly, severe tremors in the arms, legs and trunk, continuous uncontrolled movement of head and visual focusing deficits. She slowly regressed in her physical abilities and was wheel chair bound since the age of 15. 6 months after 1st stem cell transplantation there was improvement seen in her symptoms and the progression of the disease had completely halted. She could walk with the help of a walker, her speech was much louder and clearer, the shivering of hands had reduced and

uncontrolled head movement had reduced. She was moderately dependent for daily activities but could initiate most of those activities. Her PET-CT scan evaluation had shown severely reduced metabolism in bilateral cerebellar lobes. 6 months after transplantation the metabolism in the bilateral cerebellar lobes had increased as depicted in Figure 24 A & B.

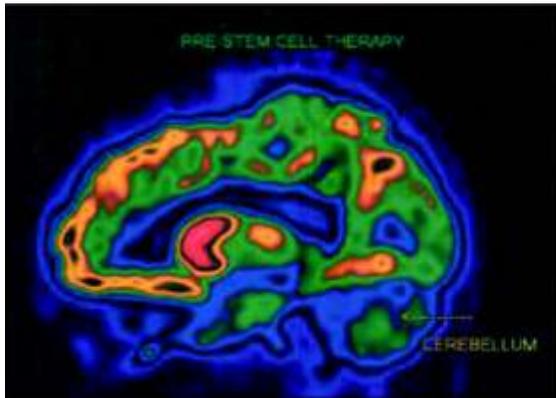


Fig 24A: Pre stem cell therapy there is hypometabolism seen in bilateral cerebellar lobes

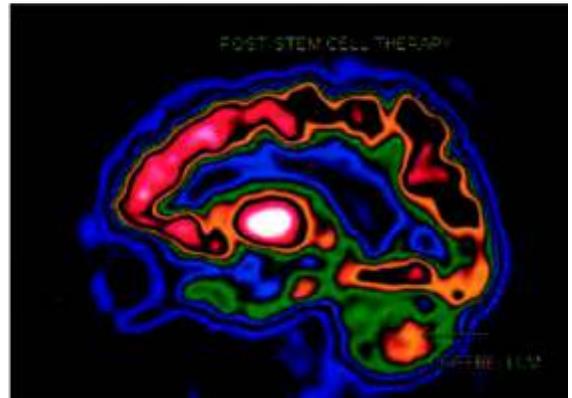


Fig 24B: Post stem cell therapy there is increased metabolism seen in bilateral cerebellar lobes

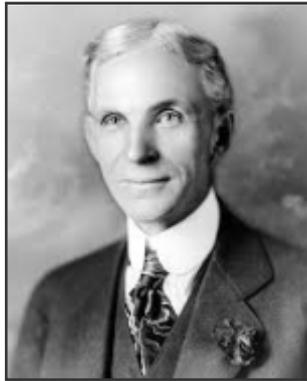
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"You can do anything if you have enthusiasm. Enthusiasm is the yeast that makes your hopes rise to the stars. Enthusiasm is the sparkle in the eyes, the swing in your gait, the grip of your hand, the irresistible surge of will and energy to execute your ideas. Enthusiasts are fighters. They have fortitude. They have staying qualities. Enthusiasm is the bottom of all progress. With it, there is accomplishment. Without it, there are only alibis."

- Henry Ford

18

Importance of Neurorehabilitation – Concept of NRRT

Introduction

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 being to make patients functionally independent, neurorehabilitation requires a team of rehabilitation specialists such as nurses, physical therapists, occupational therapists, speech therapists, psychologists and others (1).

Importance of Neurorehabilitation:

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 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 (2). 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.

Various studies have suggested that regular physical activity and exercise leads to neoangiogenesis [1], anti-inflammatory effect on the local [2] as well as global systemic environment [3], anti apoptotic effects [3,4] and effects modulating the immune system [4,5]. But the most important advantage of exercises is the mobilization of the local stem cells for repair and regeneration of the tissue [6].

Exercise also secretes various growth factors that stimulate the resident stem cells to grow into the tissue cells. It is therefore of utmost importance that stem cell therapy should be followed by the rigorous rehabilitation (3).

Concept of Neuro Regenerative Rehabilitation Therapy (NRRT):

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). 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.

Studies have shown that exercise enhances the effect of injected stem cells by inducing mobility in the injected stem cells, by activation and proliferation of the local stem cells, muscle angiogenesis and release of cytokines and nerve growth factors, thereby enhancing the achievable outcomes. Hence, neurorehabilitation appears to work complimentarily with stem cells therapy (3,4).

Multidisciplinary rehabilitation in Various Neurological Conditions

1. Autism

Autism is one of the most common conditions which remain undiagnosed until late into the developmental or formative years of a child. Diagnosing a child with autism becomes very difficult as they do not display any obvious physical problems and hence they require multiple evaluators like developmental pediatrician, child psychiatrist, clinical psychologist, speech and language pathologist, etc.

Management and treatment for a child with autism would call for a multidisciplinary holistic approach.

- Psychological Intervention: A clinical psychologist/ child psychologist plays an important role as they may look at what effect a behavior has on the child and

may introduce a behavior management plan accordingly.

- **Applied Behaviour Analysis:** An ABA analyst may use different strategies of ABA like discrete trial teaching, pivotal response therapy, etc to reduce the maladaptive behaviours and increase the appropriate ones.
- **Occupational Therapy:** This therapy focuses on teaching activities of daily living (ADL) like eating, bathing, grooming, etc and more specifically sensory/ motor integration training, would work on optimizing sensory processes and training for developing gross and fine motor skills.
- **Speech Therapy:** The speech therapist works with the child and his/her family to facilitate effective communication among them such that it benefits the overall development of the child. The therapist would conduct oro-motor exercises, deep breathing exercises or may teach the child to communicate through Picture Exchange Communication System (PECS).
- **Physiotherapy:** A Physiotherapist can help in optimal development of motor skills and addressing any underlying weakness in both sensory and muscular systems in children with autism.
- **Aquatic therapy:** An aquatic therapist makes use of properties of water and hydrodynamic principles to address the motor and sensory problems observed in these children. Aquatic therapy helps to reduce hyperactivity, improve attention and concentration, improved sleep patterns, improved tone and improved strength of the muscles.

There are many more effective therapies for children with autism like art therapy, play therapy, special education, dance therapy, aquatic therapy, etc

2. Spinal cord Injury

Regardless of the level of the lesion, the goal of rehabilitation for patients with SCI, include the following:

- Prevention of all secondary complications as a result of being bed ridden.
- Restoration of functional independence to the maximum possible.
- Psychological counseling
- Social and Vocational Rehabilitation
- Family Education and Home adaptation (5)

1. Education:

Patient and caregiver education plays an integral part of rehabilitation. This includes preventive skin care, bladder-bowel training, and safe ways to perform

ADL tasks, nutritional guidelines, thermoregulation precautions, pulmonary management, cardiopulmonary resuscitation, equipment management and maintenance, transfer techniques, wheelchair mobility and transfer techniques, and leisure skills.

2. Home programs to increase strength, endurance, ROM and function are taught. Components of an exercise program include flexibility, muscular strength and cardiovascular endurance.

Frequency ranges from 2-5 times per week with at least 1 day of rest between strengthening sessions. Duration of an exercise program could be as little as 20 minutes or as much as 90-120 minutes.

Intensity should range between 40% and 85% of maximal heart rate or within 13-15 on Borg Rate of Perceived Exertion Scale.

Injuries can be prevented or slowed if clients perform a proper warm up with stretching/flexibility exercises, wear protective equipment (i.e. helmet and padded gloves), and get proper rest between exercises sessions.

3. Preventing and Managing Pressure Ulcers and Skin compromise:

- Turning the positions at regular intervals, every 2-3 hrs.
- Pillows and rectangular foam pads to cover bony prominence should be used.
- Treatment like hydrotherapy, electromodalities and thermomodality to increase circulation can be given.
- Surgical intervention with skin flaps or muscle flaps can be used to close the wound if not healed.
- Patient should be educated to maintain skin integrity.

4. Prevention and Management of Joint Contracture:

- Daily ROM exercises and proper positioning will prevent contractures.
- Use of splints for proper joint alignment techniques like wt bearing, ADLS and functional exercises prevents contracture.
- Splinting to prevent Joint Deformity:
- Education regarding splint wearing schedule, skin checks and splint care should be emphasized. (6).

5. Sexual Issues:

- Altered sexual function result in impairment of erection, ejaculation, orgasm, male fertility and vaginal lubrication.

- Formal sexual counseling and education programs like group sessions to addresses general issues and individual sexual function evaluations should be addressed in areas of sexual dysfunction, alternative behaviours, precautions and other related areas.
- Coordinated effort between client, significant other, psychologist and urologist can help with treatment of sexual dysfunction.
- Options like surgical implantation of a penile prosthesis, vacuum erection devices, intracorporeal injection therapy and use of lubricants can be used to treat sexual dysfunction (7).

Psychological Aspect in Spinal Cord Injury

Spinal Cord Injury (SCI) leaves a major impression on the person's body and mind. A person who had been leading an independent satisfying life becomes immobilized, bowel and bladder incontinence, loss of sexual functioning and becomes dependent on others for every small necessity. The patient not only faces loss of body control but also experience changes in self worth, sense of independence, confidence, attractiveness, sexuality, and relationship with family and friends.

There are various stages that one goes through post spinal cord injury: 1) shock and denial 2) grieving followed by depression or vice versa 3) anxiety / frustration 4) anger / aggression 5) trying to adapt to the situation.

Psychological treatment of SCI often includes group psychotherapy, which is an excellent method to both maximize patient learning and efficiently use therapist time.

Patient groups can provide emotional support, peer role models; teach new coping skills, and decrease social discomfort. Likewise, multiple-family group psychotherapy is a powerful and effective tool for facilitating family adjustment to SCI. Family members experience similar emotional responses to the patient and similarly benefit from psychological intervention.

Role of Aquatic therapy is Spinal Cord injury

Aquatic therapy uses physical properties of water and hydrodynamic principles to facilitate movement and muscle contraction. In spinal cord injury it provides great advantage as it negates the effect of gravity and therefore the patients can experience higher degree of freedom of movement. Aquatic therapy helps to reduce spasticity, improve strength, improve standing and walking balance and promotes faster progression of Ambulation in Spinal Cord Injury.

3. Cerebral palsy:

Aims of Rehabilitation:

- a. Improve performance components (postural management and hand functions) e.g. improve accuracy when reaching for a toy.
- b. Enhance performance of functional activities (performance areas), e.g. eating a wafer biscuit independently.
- c. Support the overall motor program through complementing therapy aims using the appropriate selection of equipment solutions, e.g. apply active seating principles to selection of toilet seat and transfer/facilitation techniques.
- d. Minimize restriction on participation and social role function.
- e. Increase self-esteem and self - actualization.
- f. Promote positive interactions and relationships.

Principles of Treatment:

1. Repetition and reinforcement are essential for learning and establishing of modified motor pattern.
2. Maximize sensory motor experiences.
3. Adequate consideration for developmental training and facilitation of purposeful activities: Therapist incorporates the principles of the neuro-developmental concept

Integrated approach for CP:

1. Developing rapport with parents and patients:
2. Normalising tone of muscles
3. Stretching and Mobility
4. Developing Postural Reaction: Postural reactions consist of righting reactions, protective extension and equilibrium reactions. These reactions are best developed by various exercises on vestibular ball and tilt board.

5. Sensory integration Therapy:

This therapy helps to overcome problems experienced by many young children in absorbing and processing sensory information. Encouraging these abilities ultimately improves balance and steady movement. Therapies include stimulating touch sensations and pressures on different parts of the body. This therapy can also motivate children to learn sequences of movements.

6. Oromotor control training (depends on good head control):

Common oromotor problems are drooling, problems in sucking, swallowing, inadequate tongue movements and speech. Hence, therapy consists of good neck control, use of brush to decrease drooling and speech therapy.

Psychotherapy

Mental Retardation: Because cerebral palsy and mental retardation can exist at the same time in an individual, they can contribute to emotional stresses as well. Learning disabilities may be present, depending on the area of the brain that was damaged (8).

Behavioral Problems seen in Cerebral Palsy: Behavioral problems and cerebral palsy usually correlate, depending on the degree of mental retardation. The child may have behavioral problems or emotional issues that in turn, may affect psychological development and their ability to have social interaction (9).

1. Frustration:

2. Communication difficulties:

3. Attention Deficit Disorder:

Treatment: Education and vocational preparation come into the foreground by school age. **Neuro-cognitive therapy:** A new approach to treating cerebral palsy from Snowdrop. It is based upon two proven principles. (1) Neural Plasticity. (2) Learning can lead to development.

Counseling and behaviour therapy, for emotional and psychological challenges may be needed at any age, but is often most critical during adolescence. Behaviour therapy is often used to increase a child's ability and discourage destructive behaviors. Aversion therapy i.e. to reward rather than punish on negative consequences can help enhance self-esteem. Expressive therapies are usually used with people who have difficulty verbalizing their feelings such as art, music, poetry, etc which could help freeing and empowering oneself.

Role of Aquatic therapy is Cerebral Palsy

Cerebral palsy is primarily a movement disorder. Children with CP develop abnormal movement patterns due to cortical damage, spasticity, dystonia, muscle weakness and inability to fight the effect of gravity. Water provides them a unique environment to explore, experience and retrain the movement patterns. Aquatic therapy uses the hydrodynamic principles of water and physical properties of water to achieve these goals. Aquatic therapy can reduce spasticity, improve voluntary control, improve breathing, improve oromotor control, improve posture, improve strength of the muscles and facilitate faster ambulation.

4. Muscular Dystrophy:

In Muscular Dystrophy patients, due to lack of mature dystrophin, the muscle membrane is very fragile, so some forms of exercises are more likely to cause muscle fibre damage by breaking the muscle membrane integrity, especially activities involving high load eccentric exercise.

Eg: lot of running, walking on stairs etc.

Conversely, concentric activities where muscle fibre shorten when they fire, stress on muscles is reduced significantly and are thus advised.

Eg: water exercises, where gravity is eliminated. (10)

Aims of Physical Rehabilitation

1. Maintain / Improve muscle strength.
2. Prevent Deformity from Contractures.
3. Maintain Function and Mobility for as long as possible.
4. Prevent Respiratory Complications.
5. Prevent Pressure sores.

Aims of Functional Rehabilitation

1. Self-Care activities such as

- i) Eating
- ii) Grooming
- iii) Bathing
- iv) Dressing

2. Mobility training:

Transfers in and out of bed/ chairs/ car transfers etc.

Use of aquatic therapy is also advised as many experts agree that water exercises and swimming help to tone and strengthen muscles and joints without putting stress on those parts of the body that are already weakened or weakening. Hot baths during hydrotherapy sessions also help to keep tendon and joints loose and flexible, thereby avoiding contractures.

Role of Aquatic therapy in Muscular Dystrophy

MD is a neuromuscular disorder with progressive muscle weakness due to contractile damage to the exoskeleton of myocytes. Therefore day to day activities can also damage the muscle cells. There are different types of muscles contractions, out of which, eccentric contraction is the most detrimental to the muscles. Patients with muscular dystrophy need to use these harmful contractions several times a day to fight the gravity and maintain upright posture. In water because of increased buoyancy and reduced gravity these contractions are reduced, which means the

same movement on land causes more harm to the muscles than in water. Therefore aquatic therapy can provide greater benefits to these patients, immersion in water is responsible for secreting neuroprotective and anti-inflammatory cytokines which help to maintain the muscle strength further. Aquatic therapy can improve strength of the muscles, provides higher degree of freedom of movement, helps to reduce contractures and fibrosis, promotes sitting and standing balance, facilitates ambulation even after they are unable to walk on land, improves respiratory muscle strength and cardiovascular endurance.

5. Stroke:

Specific Measures for Stroke:

Rehabilitation approaches for stroke patients include Neuro-developmental Treatment (NDT), Movement Therapy in Hemiplegia. -Brunnstorm Approach,

Proprioceptive Neuromuscular Facilitation (PNF) and Sensory stimulation techniques. Currently, there is increased emphasis on functional/task specific training using intense practice on functional tasks along with behavioral shaping and environmental enrichment e.g. Constraint-induced movement therapy (CIMT) for paretic UE or locomotor training using body weight support and treadmill training (BWSTT). Compensatory training strategies are also used to restore resumption of function using the less involved extremities. These are indicated for patients who demonstrate severe motor impairment and limited recovery. Early emphasis on improving functional independence provides an important source of motivation for patient and family.

Thus the strategies used are as follows:

1. Strategies to improve Sensory function:

Sensory stimulation is important for recovery by focusing on restoring sensitivity of more affected extremities, with sufficient intensity to engage the system.

2. Strategies to improve Motor Function:

- i) Strategies to improve Flexibility and Joint Integrity
- ii) Strategies to improve Strength
- iii) Strategies to manage Spasticity
- iv) Strategies to improve Initial Movement Control
- v) Strategies to improve Motor Learning
- vi) Strategies to improve Postural Control and Functional Mobility
- vii) Strategies to improve Upper Extremity Function
- viii) Strategies to improve Lower Extremity Function
- ix) Strategies to improve Balance
- x) Strategies to improve Locomotion

3. Strategies to improve Aerobic Function

Endurance training has shown to yield significant improvements in physical fitness, functional status, psychological outlook and self-esteem.

4. Strategies to Improve Feeding and Swallowing:

Positioning of head, Oral exercises, Food preparation and verbal cues helps to improve feeding and swallowing.

Psychological Rehabilitation:

The psychological reaction to having a stroke can cause feelings of frustration, anxiety, apathy, anger or depression. Depression can seriously hinder an individual's willingness and ability to participate in rehabilitation. Social isolation, or lack of access to social contact or resources, can be a consequence of difficulties in cognitive and emotional functions that influence interpersonal relationships, changes in social roles, communication difficulties, and challenges in transportation and employment. Social stigma and marginalization also contribute to isolation.

Attention training helped people with acquired brain injury and seemed to work best with younger patients less than a year after injury. Visuo-spatial training helped stroke patients with visuo-spatial neglect, the inability to respond or orient to something shown on the side opposite to the site of the injury. Visuo-spatial training also tended to improve performance in other cognitive domains. Family counseling is a major factor for psychological rehabilitation in stroke.

Role of Aquatic therapy in Stroke

Aquatic therapy helps to reduce tone, improve posture, improve voluntary control, retrain movement to break synergy patterns in stroke. It is a tool for faster recovery of the patients. Immersion in water also releases certain neurotrophic factors and anti-inflammatory cytokines that can help aid the neural recovery.

6. Motor neuron disease:

Specific Measures for MND:

The efficacy of therapeutic interventions is related to:

1. Timing of interventions,
2. Motivation and persistence of patient in carrying out the program.
3. Support from family members.

Rehabilitation intervention plan depends on the following:

1. The rate of progression of the disease.

2. Presence of spasticity, bulbar involvement, respiratory involvement causing hypoxia and fatigue.
3. Phase of Disease. Exercises are to be prescribed according to level of impairment, functional limitation and level of disability (11).

Phase I (Independent)

Stage 1: In case of mild weakness advice is to continue normal activities. In case of clumsiness, stretching exercises like Yoga. In case of ambulatory patients, gentle resisted exercises without fatigue.

Stage 2: In case of moderate selective weakness, stretching exercises to avoid contractures.



Figure 1: Exercises to improve grip strength



Figure2: Gait Training



Figure 3: Over head activity while standing with walker.



Figure 4: Standing on standing board with bilateral push knee splints and high boots



Figure 5: Quadruped for trunk balance



Figure 6: Strengthening of scapular muscles



Figure 7: Strengthening of back extensors



Figure 8: Strengthening of lower abdominals



Figure 9: Strengthening of neck muscles



Figure 10: Strengthening of upper abdominals



Figure 11: Stretching of dorso lumbar fascia



Figure 12: Trunk Strengthening act.

In case of difficulty in ADLs like climbing, overhead activities and difficulty in buttoning etc, strengthening exercises should be prescribed avoiding fatigue. In case of difficulty in ambulation, orthotic devices like AFO, hand splints should be considered.

Stage 3: In case of fatigability in long distance ambulation, deep breathing exercises should be added.

In case of non-ambulatory cases, consider wheelchair; standard or motorized.

Phase 2 - (Partially Independent)

Stage 4: In case of pain and edema in hand and feet, consider modalities like massage, elevation and active exercises. In case of severe weakness in extremities, caution is to be taken to support the joints while doing rotations. In case of fatigability in ADLS, encourage isometric exercises to the level of tolerance and to consider slings or arm support, motorized chairs etc.

Stage 5: In case of severe lower extremity weakness, teach family members proper techniques of transfer and positioning of patients limbs and modifications at home.

Phase 3 (Dependent)

Stage 6: In case of totally bedridden patients with dysphagia, consider suction, soft diet, tube feeding, PEG feeding etc.

In case of severe breathing difficulty, frequent clearing of airways, tracheostomy and respiratory support if needed.

Studies with other neuromuscular diseases (NMD) such as poliomyelitis, Duchene's muscular dystrophy, myotonic dystrophy, hereditary motor and sensory neuropathy, spinal muscular atrophy and limb-girdle, Becker and fascioscapulohumeral dystrophy have found that exercises programs are beneficial and do not produce overuse weakness.

Role of Aquatic therapy in Motor Neuron Disease

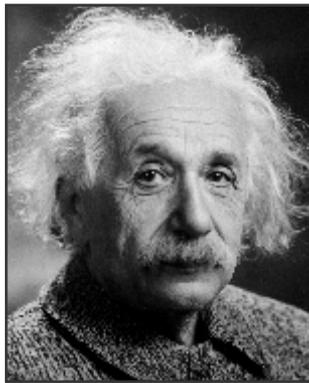
Aquatic therapy benefits the patients of Motor neuron disease in multiple ways. It provides higher degree of freedom of movement. It helps to reduce spasticity, improves posture, improves balance, improves voluntary control and improves respiratory as well as cardiovascular endurance.

Process of neurorehabilitation is to reintegrate the patient in community. Therefore, the most important aim of the neurorehabilitation is to facilitate maximum neurological recovery and functional independence. However, some of the other important aspects of neurorehabilitation are to prevent secondary complications, deformity prevention and crisis management. The process of neurorehabilitation educates the patient about the prognosis of the condition, makes them aware of the possible complications and helps them to manage the complications in the most effective way. Neurorehabilitation process also includes compassionate therapies and palliative care provided at the terminal stages of the progressive disease. Rehabilitation process is essential after stem cell therapy to exploit maximum benefits of stem cell therapy.

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Every error is an opportunity to learn, just don't commit the same mistake again. That is stupidity. But commit as many new mistakes as you are capable of. Don't be afraid, because its the only way nature allows you to learn."

-Albert Einstein

19

Complications

Cell replacement 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. The field was first brought alive by blooming of the differentiation potential of the embryonic stem cells (McDonald et al). 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 and Fetal 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 and fetal stem cells.

(1) Tumorigenicity/Teratomas

Embryonic and Fetal 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 allogeneic fetuses, the cells may, however, form a tumor if they "leak" into an improper space (1).

In 2007, Amariglio et al reported the first case of a human brain tumor complication in human fetal neural stem cell therapy (2). Their findings suggest that fetal neuronal stem/progenitor cells may be involved in formation of gliomas. This provides the first example of a donor-derived brain tumor. Further work is urgently required to assess the safety of fetal stem cell therapies.

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.

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.

Ablating the immune cell niche with immunosuppressive drugs to prevent immune rejection following allogeneic transplantation, has the potential to create a tumorigenic microenvironment. A recent paper demonstrated tumor formation from the donated cells following transplantation of donor derived fetal neural tissue into an ataxia telangiectasia patient [3].

So far, we have not come across any reported complication, such as tumorigenicity, for treatment of neurological diseases using autologous adult stem cells.

(2) Seizures

Seizure is one of the possible adverse events of stem cell therapy. This side effect can

be seen with any type of stem cell transplantation. Earlier bone marrow transplantation in children with leukemia has exhibited epilepsy as an adverse event post transplantation [1]. A case series of autologous BMMNCs transplantation in stroke also reported one patient who developed seizures post transplantation [5]. Seizure is considered to be an adverse event in case of development of new seizures post transplantation and increase in the intensity or frequency of pre-existing seizures. In our experience we observed that children with neurological disorders like cerebral palsy and autism developed seizures as an adverse event post autologous BMMNCs transplantation [6].

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 [7-9]. Also these disorders present with seizures as a co-morbidity [10,11]. The percentage of children that developed seizures as an adverse event was very small (Table 1). However, this adverse event is preventable by using an antiepileptic prophylactic regimen (Table 1). After the use of antiepileptic prophylactic regimen (Table 1) the percentage of seizures as an adverse event reduced significantly.

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%)

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

Published data

We analyzed the incidence of seizures as an adverse event in 131 children (Mean age 9 years) with incurable neurological diseases, treated with autologous bone marrow mononuclear cell (BMMNCs) intrathecal transplantation. Seizures occurred as an adverse event in 8 (6.10%) of the children. There was correlation between the electroencephalograph (EEG) examination and the occurrence of seizures with seven children showing a pre-existing epileptogenic. The history of seizures was not so strongly correlates ad only four children had history of seizures and 3 children did not have any history of seizures. Seizures was considered as an adverse event in an event of new onset of the seizures or increased intensity or

frequency of preexisting seizures.

A prophylactic antiepileptic regimen was designed based on these findings and 67 patients were analyzed for the incidence of seizures. The antiepileptic regimen was implemented based on following factors, EEG examination findings, the history of seizures and the current medical treatment for the seizures. The antiepileptic regimen is given in detail in Table 2.

Criteria		Patients with pre-existing epileptogenic focus on EEG (Abnormal EEG)	Patients without pre-existing epileptogenic focus on EEG (Normal EEG)
Patients with history of seizures	Patients already taking antiepileptic medication	<p><u>On the day of transplantation</u> Previous medication with same dosage, IV, twice a day at an interval of 12 hours</p> <p><u>From the next day of transplantation</u> Previous medication with the same dosage as before, oral</p>	<p><u>On the day of transplantation</u> Previous medication with same dosage, IV, twice a day at an interval of 12 hours</p> <p><u>From the next day of transplantation</u> Previous medication with the same dosage as before, oral</p>
	Patients not taking any antiepileptic medication	<p><u>On the day of transplantation</u> Levetiracetam 10mg/kg body weight, IV, twice a day at an interval of 12 hours</p> <p><u>From the next day of transplantation</u> Levetiracetam 10mg/kg body weight, oral for 3 months</p>	<p><u>On the day of transplantation</u> Levetiracetam 10mg/kg body weight, IV, twice a day at an interval of 12 hours</p> <p><u>From the next day of transplantation</u> Levetiracetam 10mg/kg body weight, oral for 3 months</p>
Patients without history of seizures	Patients not taking any antiepileptic medication	<p><u>On the day of transplantation</u> Levetiracetam 10 mg/kg body weight, IV, twice a day at an interval of 12 hours</p> <p><u>From the next day of transplantation</u> Levetiracetam 10mg/kg body weight, oral for 3 months</p>	Antiepileptic prophylaxis was not given to any patient

Table 2: Prophylactic antiepileptic regimen

Out of the 67 patients only 2 patients showed seizures as an adverse event after implementation of the antiepileptic regimen. The percentage of patients with seizures was therefore reduced significantly from 6% to 2.98%. Both these patients showed increase in the frequency and severity of the pre-existing seizures. Therefore, the percentage of new onset seizures reduced to 0% from 2.29%.

(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 [12,13]. When allogenic stem cells are used there is a risk of stem cell-tissue rejection which may be partially overcome by donor-patient matching, by immunological sequestration or using immune suppressants, all of which have their own drawbacks [14]. Use of histocompatibility leukocyte antigen (HLA) matching of the donor to the recipient to prevent immune rejection is often not readily achievable [14]. Immune suppression may put the patient at risk of infection. The risk of donor-to-recipient transmission of bacterial, viral, fungal or prion pathogens may lead to life-threatening and even fatal reactions. Disease transmission has been reported after allograft transplantation [15,16].

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 400 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 morbidity 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.

(3) 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 intraspinal, 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.

Unpublished data for regarding adverse events associated with Autologous bone marrow mononuclear cells

We analyzed 1001 patients over an average follow up period of 18 months. All the patients were diagnosed with an incurable neurological disorder and underwent autologous BMMNCs intrathecal transplantation followed by rigorous rehabilitation. Figure 1 shows the percentage of minor and major adverse effects noted after transplantation.

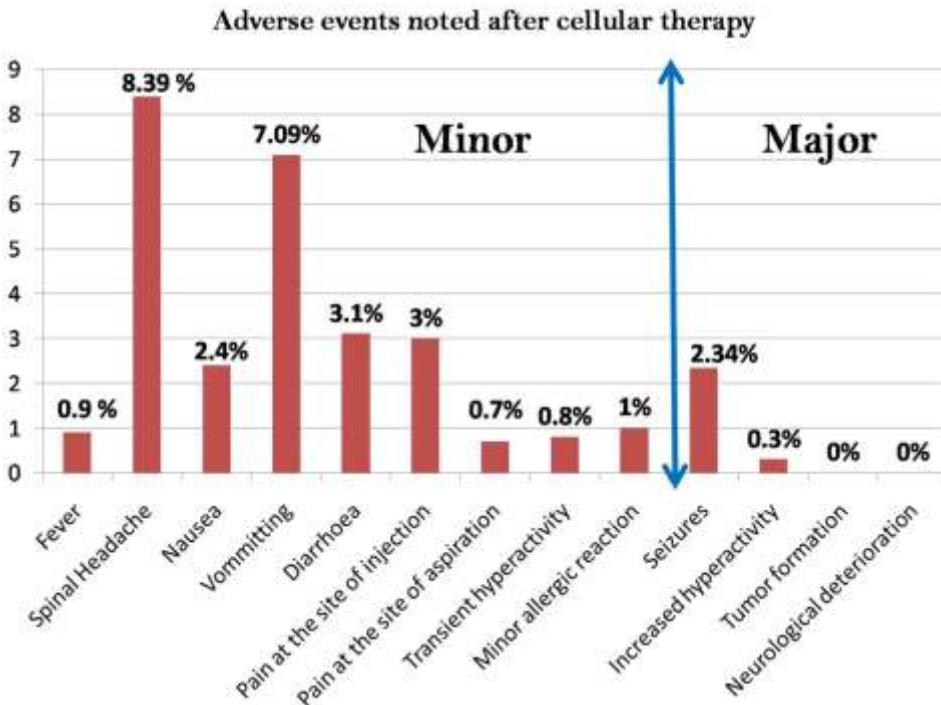


Figure 1. Percentage of adverse events observed post transplantation

Minor adverse events:

Fever was noted in 0.90% of the patients which resolved during the hospital stay by administration of antipyretic medication.

Spinal headache was observed in 8.39% of the patients during hospitalization. Patients with mild headache were advised to lay flat in supine position for as long as possible and to increase the oral fluid intake to 4 to 5 liters. In patients where the pain did not resolve or the patients who had severe headache or the patients who had headache along with nausea or vomiting or patients who had poor command following and cognition, I.V fluids were given. It was noted that the spinal subsided within 72 hours of administration of I.V fluids.

Nausea was observed in 2.4% and vomiting was observed in 7.09% patients and subsided within 48 hours of administration of antiemetic medication.

Diarrhea was observed in 3.10% of the patients which resolved with antidiarrheal medical management.

Pain at the site of injection was noted in 3% of the patients. Pain at the site of aspiration was noted in 0.7%. Analgesic medication was given to all the patients prophylactically immediately after the procedure. Dosage of the same was altered in case pain persisted.

Transient increase in hyperactivity was noted in 0.8% which subsided with rehabilitative therapies without any medical intervention during the hospital stay.

Minor allergic reactions were noted in 1% of the patients and presented as mild generalized itching and localized rashes which resolved with antiallergic medical management

Major adverse events:

None of the patients exhibited major adverse events like persistent increase in hyperactivity till six months, neurological deficits, nerve root damage, parasthesia in lower limb, loss of sensation in lower limb, loss of motor function in the lower limbs, hematoma at the site of injection, hematoma at the site of aspiration, bleeding at the site of injection or aspiration, local infection at the site of injection or aspiration, meningismus or meningitis, brain infection, bowel or bladder incontinence, respiratory distress, cardiac failure, major allergic reaction and tumors.

Seizures as an adverse event, was noted in 2.34% of the patients. All of these patients had either history of seizures or abnormal EEG. Another cell transplantation related adverse event was persistent increase in hyperactivity over 6 months post transplantation which was noted in 0.30% of the patients which subsided upon rehabilitative management with occupational therapy in combination with medical management.

Literature Review:

7 studies [16-23] were published including a total of 175 patients with ataxia that were treated with stem cell therapy. In one single case report in 2007, Amariglio et al reported the first case of a human brain tumor complication in human fetal neural stem cell therapy [24]. Their findings suggest that fetal neuronal stem/progenitor cells may be involved in formation of gliomas. This provides the first example of a donor-derived brain tumor. Further work is urgently required to assess the safety of fetal stem cell therapies. One study [25] reported minor adverse effects with 4 patients developing dizziness, 2 patients developing back pain and 1 patient developing headache all of which were self-limiting and resolved within 3 days.

16 studies [16-39] have been published (160 patients) that studied efficacy and safety of stem cell therapy in patients diagnosed with autism. Headache, nausea, vomiting, pain at injection site and pain at aspiration site were the encountered minor procedure related adverse events which resolved within a week in a study [24]. In another study involving 23 patients, 12 events of allergic reaction manifested as urticaria and/or cough were related to the procedure with 2 requiring an additional dose of IV Benadryl.

In the 5 studies [40-44], involving 220 patients with cerebral palsy, two studies [41,43] reported vomiting and headaches, in the participants, which were self-limiting.

28 studies including 426 patients with MD were published [45-75]. A study involving 12 DMD patients, reported transient soreness in injected muscles which self-resolved in 3-4 days. 1 patient developed cellulitis which was treated with antibiotics and drainage [37]. In another study including 10 DMD patients, fever and cough in 1 patient, diarrhea, nausea, vomiting and abdominal pain in another, hirsutism in 1, and perioral rash in 1 patient were reported [38]. Mild graft versus host disease was reported in another study [42]. Minor procedure related events including nausea, headache and backache was reported in a study [45] and; pain at aspiration site, pain at the injection site, fatigue, pain in upper and lower limbs, neck pain which were self-limiting and resolved in a week, were reported in another study [50].

Park and his colleagues, investigated on the role of autologous bone marrow cell transplantation (BMC) for six complete spinal cord injury (SCI) patients. During six to eighteen months follow up, fever and myalgia were noted [76]. No other complications were reported. Samuil et al 2003, injected stem cells from fetal nervous and hemopoietic tissues in 15 patients. During 1 month to 6 years, no serious complications were noted [77]. Olfactory mucosa autografts were injected into seven SCI patients, and noticed decrease in the sensory in one patient suggestive of difficulty in locating lesion. Moreover, transient pain was notice in patients, and was relieved by medication [78].

Four studies used autologous BMC for 195 SCI patients and found no adverse effects in all the patients during follow-up [79-82]. Moreover, autologous mesenchymal stem cells were transplanted into 10 SCI patients. No adverse effects were noted during the long-term follow-up [8].

Adipose tissue-derived mesenchymal stem cells were administered intravenously in 8 patients with SCI and showed no severe adverse events during the three-month follow-up [9]. Hematopoietic stem cells (HSCs) and progenitor cells (PCs) were administered in 202 cases of SCI patients, and have shown no adverse events [83]. Thirty-nine patients with complete cervical and thoracic SCI were injected with autogenous peripheral blood stem cells. During the two and half year follow-up no complications were reported [84].

Six patients with SCI were selected for the grafting autologous activated Schwann cells, and during follow-up of five years no complications were noted [85]. Saberi and his team investigated on intramedullary Schwann cell transplantation on 356 patients with SCI, and during 2 years of follow-up found no infection, or worsening or tumor formation [86].

Transplantation of peripheral nerve tissue were grafted into 12 SCI patients. During 2-year follow-up patients were noted with certain complications which includes 1 case of transient increased spasm, one case of transient cystitis, 3 patients with transient increased neuropathic pain and 1 case with transient episode of autonomic dysreflexia. Moreover, no donor site infections were observed. The authors, support that the above complications were transient as they responded to temporary medical treatment [87].

Olfactory unsheathing cells, were transplanted into 108 patients with complete chronic SCI patients. During the follow-up of 3 years, none of the patients had complication involving neoplasm, bleeding, swelling, cysts, neural tissue destruction or infection (abscess) or any other pathological changes in or around OEC transplant sites [88]. Autologous bone marrow cells were injected into 4 patients with SCI. During 1 year of follow-up, no adverse events were recorded in patients [89]. Moreover, Bone marrow (BM) mesenchymal stromal cells (MSC) were injected into 30 patients, with complete SCI patients. During 1 year of follow-up, none of the patients have reported any adverse events associated with BM MSC transplantation [17]. Further, autologous bone marrow stem cell was injected in 20 patients with transversal spinal cord injury (SCI). No adverse events were noted [90-94].

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"Stem cell research, with appropriate oversight, should be directed by scientists, not politicians."

- **Dr. E Thomas,**
Winner of the Nobel prize in Medicine, 1990

20

Regulations of Stem Cell Therapy

Regenerative medicine is a rapidly evolving field of medicine, possibly the most promising and most challenging to regulate. There has been a colossal increase in the information regarding regenerative medicine in the past decade. Stem cell science has moved from laboratory to clinic with published evidence, exploring possible treatment and cure of numerous incurable diseases. Unlike pharmacological science the science of stem cell therapy is very complex due to availability of multiple types of cells, multiple routes of administration and multiple possibilities for dosage and unlimited combinations of these. This is what makes it very challenging to regulate on the basis of current evidence based medical system. If the same regulations as that of drugs are used for cellular therapy and cellular products it will be another decade before we can use cellular therapy as a treatment modality. Therefore various regulatory medical bodies have felt an urgent need to monitor and regulate the research in the field of stem cell therapy. An overview of the regulatory procedures in the global players shows that these vary from nonexistent to extremely stifling. Both ends of the spectrum are not conducive for the healthy progress of this highly promising area and we feel there needs to be a discussion so that a middle ground can be reached.

Global trends in the regulation of stem cell therapy

Majority of the countries have very cautious and skeptical approach towards regulating stem cell therapy and have very stringent regulations but there are some countries like Japan, Korea and USA that have quiet progressive approach of regulating stem cell therapy. Some of the other countries like Australia, Canada,

European countries have strict regulations but have provisions for clinical use for terminally ill patients suffering from incurable diseases to protect patients' rights for using unproven treatments. However, the regulations in India are restrictive without any provision for clinical use even for terminally ill patients suffering from incurable diseases.

Permissive regulations in other countries

Korea [5]

Korean guidelines make a clear distinction between the levels of manipulation of the cells very clear. The guidelines state, 'Cell therapy product' means a medicinal product manufactured through physical, chemical, and/or biological manipulation, such as in vitro culture of autologous, allogeneic, or xenogeneic cells. However, this definition does not apply to the case where a medical doctor performs minimal manipulation which does not cause safety problems of autologous or allogeneic cells in the course of surgical operation or treatment at a medical center (simple separation, washing, freezing, thawing, and other manipulations, while maintaining biological properties).'

Regulations should be more permissive for cells that are autologous, of adult origin and minimally manipulated than the cells that are allogenic, of embryonic origin or are significantly manipulated.

Korean Food and Drug Association (FKDA) Regulation on review and authorization of biological products, Article 41 not only excludes the minimally manipulated cells from the definition of cell therapy product, but has a fast track review process for the use of cell therapy in life threatening, serious diseases and conditions for which treatment is not possible with existing therapy [5].

The article 41 states, "(Fast Track Review Process) For the following medicinal products, the Commissioner of the KFDA may allow post-marketing submission of some documents required under this Regulation or apply the fast track review process. Medicinal products that may have therapeutic effects against AIDS, cancers, or other life-threatening or serious diseases. 2. Medicinal products of which fast introduction is deemed necessary because treatment is not possible with existing therapies (due to development of resistance or other reasons) 3. Medicinal products that may have preventive or therapeutic effects against bioterror diseases and other pandemic infections."

The Korean setup is much more permissive for stem cell research. The government allows and funds work on human embryonic stem cells. The Bioethics and Safety act lays down the legal boundaries for permissible area for stem cell research. The early guidelines made by the Ethics Committee of the Stem Cell Research Center in 2003 permitted the use of only spare embryos for hES cell line derivation. They

prohibited cloning, inter-species transplantation of reproductive cells that might lead to chimeras, production of embryos for research purposes, and somatic cell nuclear transfer to prevent attempts to engage in reproductive cloning. A further advanced version of the Bioethics and Safety Act enacted in January 2004, and enforced since 2005 as a penal law identifies criminal offenses pertaining to stem cell research. It prohibits human reproductive cloning. The transfer of embryos between two different species, embryo production other than for the purpose of pregnancy and also disallows research on spare embryos that have the embryological primitive streaks appearing in their developmental process. It only allows research on spare embryos for research aimed at curing rare or incurable diseases. The though on surface it appears prohibitive, but in practicality provides a legal platform to allow legitimate researchers to conduct research on human embryonic stem cells, including somatic cell nuclear transfer for the purpose of conducting research aimed at curing currently incurable diseases., if they adhere to the procedures laid down by the act.

In 2006, Dr. Hwang Woo-suk scandal, raised not only ethical issues regarding procurement of the eggs, but also questions regarding scientific ethics & falsifying results brought disrepute to the stem cell " hub" which was to be lead by him. This also, lead to enactments of stricter rules regarding embryo donor for research, which came in the form of Bioethics and safety act 2008. Nevertheless, South Korea continues to pursue research for the purposes of therapeutic cloning, with complete financial and legal backing from the government [6].

The Korean guidelines have taken into consideration the need for different regulations for minimally manipulated cells and the need for more efficient pathways for the approval of the same. Other regulatory bodies need to keep these two important points in consideration whilst framing their regulations.

Japan [7,8,9]

Evolution of regulatory framework in Japan

Till 2014 Japan had no statutory body for regulating regenerative medicine separately. All the drugs and regenerative medicine products were regulated under same regulations. In 2014 Japanese Health authorities realized the need for separate regulations for regenerative medicine and implemented the changes in their current regulatory system. Japanese Diet passed 'Regenerative medicine promotion act' which made it necessary to amend existing laws to promote growth and safe implementation of generative medicine. In view of this Pharmaceuticals Affairs Law (PAL) was amended Pharmaceuticals and Medical Devices Act (PMD Act). This amendment made a provision for separate regulation of regenerative medicine products by companies and set standards and regulatory criteria for the manufacturing and sell of the same. It was very progressive of Japanese regulatory

evolution to recognize difference between drugs and regenerative medicine as well as difference between regenerative medicine product and therapies for treatment of diseases. While PMD Act regulated the products, Act on Safety of Regenerative Medicine (ASRM) was designed to promote safe regenerative medicine therapies. ASRM safeguarded the patients from unsafe cellular therapies.

In these two laws they have made a clear distinction between the companies that make stem cell products, institutes that offer medical services and medical research. The laws introduce the concept of conditional marketing for medical products, separate approval systems based on the risk stratification for the medical services, presumed efficacy of the treatments and post hoc efficacy analysis.

Highlights of the Japanese regulations

(A) Regulations for the product

(B) Regulations for the medical services and research (stem cell services)

(A) Regulations for the product:

Pharmaceuticals, Medical Devices, and Other Therapeutic Products Act (PMD Act)

The partial amendment in this law created a separate approval channel for the cell based therapies and products. This amendment recommended that the cell based products may not need to use the phased clinical trials to establish efficacy for marketing approval. The provision was made for a conditional approval for the marketing of these products once the safety and presumed efficacy was established. Investigators could demonstrate efficacy in pilot studies of as few as 10 patients in one study if the change was dramatic enough or a few hundred when the improvement was marginal. At the provisional approval stage the treatment could be approved for commercial use as well as national insurance coverage.

(B) Regulations for the medical services and research (stem cell services): (Figure 1)

Act on safety of regenerative medicine (ASRM)

While PMD act regulated the products, ASRM regulated the therapies to make sure the safety of the treatments provided and to ensure that the efficacy was established in the due course. Regenerative medicine treatments were categorized as regenerative medicine I (High risk), Regenerative medicine II (Medium risk) and Regenerative medicine III (Low risk) (Figure 1). Each of these classes had a separate approval channel and different approval procedure.

Low risk regenerative medicine therapies (Class III):

The approval process is by a committee within the institute and by submitting the provisional plans to the department of health and welfare.

The institutional committee is called as, “Certified Committee for Regenerative Medicine” includes experts in the regenerative medicine technologies as well as legal experts and is approved by the ministry of health, labor and welfare.

Medium risk regenerative medicine therapies (Class II):

The approval process is by a committee outside of the institute and by submitting the provisional plans to the department of health, labour and welfare.

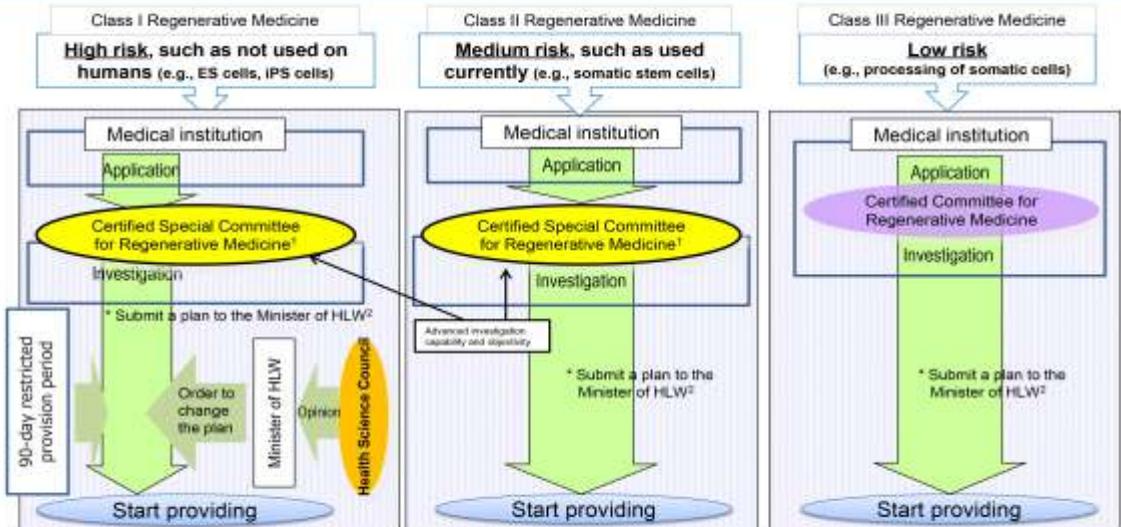
The institutional committee is called as, “Certified Special Committee for Regenerative Medicine” approved by the ministry of health, labor and welfare; which includes experts in the regenerative medicine technologies as well as legal experts with capabilities for specialized investigation and objectivity.

The Japanese guidelines have made a provision for a middle level regulatory body for faster approval process. This committee has an authority to conditionally approve the treatment and marketing using the cell based products; but the provisional plans are required to be submitted to department of health, Labour and welfare. Once the conditional approval is granted the institute must conduct.

High risk regenerative medicine therapies (Class I):

The approval is through the “Certified Special Committee for Regenerative Medicine” which is from outside of the institute as that in Class II but the Ministry of health, labor and welfare (MHLW) will impose a certain period of restricted implementation.

1-3. Risk-Dependent Procedure under the Act on the Safety of Regenerative Medicine



Note 1: "Certified Committee for Regenerative Medicine" is a council-type committee consisting of knowledgeable persons including experts on the technologies of regenerative medicine or legal matters, which is approved by the Minister of HLW through certain formalities. "Certified Special Committee for Regenerative Medicine" is the Certified Committee for Regenerative Medicine with specifically advanced investigation capability and objectivity.

Note 2: The procedure of submitting a provision plan will be obligated. Penalties will be imposed if regenerative medicine is provided without submitting a provision plan.

Figure 1: Categorization of regenerative medicine

*Diagram available online at <http://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/150407-01.pdf>

During this period the MHLW will confirm the safety by hearing opinions of the Health Science Council. The Ministry can order change of the plan if there is nonconformity to the standards of safety and the institute will have to adhere to these changes for the conditional market approval.

Thus the Japanese government has been very permissive in promoting the regenerative medicine. The classifications that are made are based on the safety of the cell products and not the efficacy. The approval is granted with proven safety and presumed efficacy, imposing further testing to establish safety satisfying the standards of evidence based medicine. Regulatory bodies from other countries should consider following the Japanese model of regulations for regenerative therapies.

USA

Evolution of regulatory framework in USA

The original guidance regarding use of tissue products was drafted and approved in 1996. In the subsequent year (1997) a separate code of federal regulation (CFR) 1271 was drafted to regulate these products. (3) These were classified under Human cells and tissue and cellular and tissue based products HCT/Ps in this CFR which made a clear distinction between a 'drug' which is a chemical molecule from these biological products. The products were further classified as biological products or medical devices based on difference criteria and a separated set of regulations was drafted for both. These guidelines took into consideration the differences not only between the type of cells but also between the procurement procedures and routes of administration that may significantly alter the safety and efficacy profile of the cells. Although the classification was primitive and inadequate, it was based on the available body of evidence and existing trends and concepts for monitoring development of new therapeutic drugs. The products were classified into; minimally manipulated cells, defined as, cells that do not alter their relevant biological characteristics (due to the technique and/or chemicals used to procure them) and more than minimally manipulated cells. The regulations also differentiated between the route of administration as homologous and non-homologous use. Homologous use was defined as the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor.

These products were regulated by 2 governing laws, first, Public health services act (PHS) which mandated that any new biological product for licensing will be required to produce the data from clinical study or studies that demonstrate the safety, purity and potency of the cells. (4) However, the guidelines did not define

what type of studies or number of patients will be considered appropriate for demonstrating this.

The 21CFR 1271.15 clearly stated that minimally manipulated cells used for non-homologous use will be exempted from the regulatory requirements of FDA for marketing approval if the cells are procured and transplanted in the same surgical procedure. Another provision in the 21CFR 601.40 allowed for the accelerated approval for serious or life threatening illness. This was applicable to certain biological products that had been studied for safety, efficacy and provided meaningful therapeutic benefit to the patients over existing treatment. In accordance with these guidelines various autologous regenerative medicine products that were used for homologous use were approved and licensed in USA between 1997 to 2011.

Between the years 2011 and 2015 in the view of growing clinical evidence for stem cell therapy, Right to try act was designed. Although the regulations prevented generalized marketing of regenerative medicine products up till 2015, after the introduction of Right to try act marketing of experimental drugs for the terminally ill patients was allowed on a case by case review basis.(5)

The act quoted, "Notwithstanding the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), the Controlled Substances Act (21 U.S.C. 801 et seq.), and any other provision of Federal law, the Federal Government shall not take any action to prohibit or restrict the production, manufacture, distribution, prescribing, dispensing, possession, or use of an experimental drug, biological product, or device that (1) is intended to treat a patient who has been diagnosed with a terminal illness; and (2) is authorized by, and in accordance with, State law."

Recently based on the growing clinical evidence for the use of cellular therapy for the treatment of various incurable disorders, Senator Mark Kirk introduced a bill to amend the Federal food, drug and cosmetic act; in front of committee on Health, Education, Labor and Pensions, in 114th congress of the American senate. The amendment was named Reliable and Effective Growth for Regenerative Health Options that Improve Wellness act, REGROW Act.

Highlights of the US regulatory system

REGROW act alters the current regulatory framework to provide conditional marketing approval to minimally manipulated cells for non-homologous use and more than minimally manipulated cells without going through the formal procedure of approval as per section 351 (A) of part F of Title III of PHS law. Subsequently it is required that the licensing application be filed in the next 5 years, based on the post marketing research, as per section 351 (A) of part F of Title III of PHS law. The cells and products exempted under these conditions are described in detail in the REGROW act as,"

- (1) Such cells or tissues are adult human cells or tissues.
- (2) Such cells or tissues have been evaluated to examine immunogenicity and do not provoke a significant unintended immune response in the recipient.
- (3) Such cells or tissues are –
 - a. minimally manipulated for a non-homologous use; or
 - b. more-than-minimally manipulated for a homologous or non-homologous use, but are not genetically modified.
- (4) Such cells or tissues are produced for a specific indication.
- (5) Such cells or tissues are produced exclusively for a use that performs, or helps achieve or re-store, the same, or similar, function in the recipient as in the donor.
- (6) Within 5 years of the safety and effectiveness determination described in this section, the sponsor of the conditionally approved new product prepares and submits an application for approval of a biological product under section 351(a), demonstrating potency, purity, safety, and efficacy of the use. The Secretary may permit continued use of such product until the Secretary completes the review of the application and makes a determination. Upon a determination by the Secretary not to approve the application, use of the cellular therapeutic shall not be permitted.
- (7) During the conditional approval period and before approval of an application under section 351(a), the sponsor shall prepare and submit annual reports and adverse event reports to the Secretary containing all the information required for approved biological products.
- (8) The sponsor has submitted an application under section 505(I) of the Federal Food, Drug, and Cosmetic Act for the treatment of the patients during the 5-year conditional use period.
- (9) The sponsor has not previously received conditional approval for such product for the same indication.”

Although REGROW Act is uniformly applicable to stem cell products as well as stem cell therapies the highlight of this act is A] Conditional marketing approval and B] Provision for post-hoc efficacy analysis. (2) This relieves the burden of the evidence from newer upcoming cellular therapies and products. It allows medical innovators and practitioners to develop promising therapies without having to go through phased approval process as before. The proposed law has created criteria to protect patients from unsafe therapies. This ensures easy and faster availability of promising cell therapies to patients that can benefit from them without any risk of adverse effects.

Once this law is implemented the principles of Conditional marketing approval and Provision for post-hoc efficacy analysis will become part of the regulations.

Another law passed by the state of Texas in USA named as Charlie's Law. Charlie's law legalizes provision of investigational (unproven, but safe medical therapies) stem cell treatment in certain patients with chronic diseases or terminal illness.

The law categorically defines investigational stem cell treatment as adult stem cell treatment, that is being used in clinical trials but is unavailable for use in general.

This is a very progressive step by the state of Texas which has definitely brought back hope for patients suffering from incurable illnesses. USA as well as other countries should follow in these directions to bring hope to the patients who can't wait for the conventional evidence based medicine to provide answers and who are suffering from years in absence of any cure or treatment to alleviate their symptoms provided by modern medicine.

Although president George bush had banned the federal funding for the research on embryonic stem cells and by using embryonic cell lines in 2001, President Barack Obama subsequently lifted this ban. Currently embryonic stem cell research is eligible for federal funding. To obtain federal funding to conduct research using stem cells, a sponsor must submit its application to the NIH. Guidelines for applying to the NIH can be found on the Federal Register (Vol 65, No 166/Friday, August 25, 2000/Notices). Under the auspices of the Obama administration, the National Institutes of Health plans to expand federal funding for stem cell lines that meet following ethical requirements: the embryo used is discarded after IVF; informed consent is obtained from the donors; the couple must not receive compensation (neither financial nor medical benefits) or be coerced or threatened. Older stem cell lines created in the spirit of the new regulations will be considered for federal funding, whereas embryos created solely for research purposes will be excluded [12].

Regulatory framework in Europe:

European medical agency (EMA) has drafted a separate legislation for regenerative medicine products which is known as act on advanced therapy medicinal product (ATMP).(9) Various regenerative medicine products are put into this newly designed category for such products. This legislation recognizes the difference between the drugs and stem cell products. Another law formulated by EMA, called Hospital Exemptions act (HE) allows a practitioner or an institute to offer stem cell therapy as a form of treatment for terminally ill patients. (10) The law states that,

“Advanced therapy medicinal products which are prepared on a non-routine basis according to specific quality standards, and used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner, in

order to comply with an individual medical prescription for a custom-made product for an individual patient, should be excluded from the scope of this Regulation whilst at the same time ensuring that relevant Community rules related to quality and safety are not undermined.”

Recently in the year 2016 EMA also formulated a PRIOrity Medicines (PRIME) program to support development of medicines for unmet medical needs. (11) Under this scheme promising therapies and medicines that are important for public health will be given additional support and accelerated regulatory approval. The products that are in the stage before Phase II as well as Phase III trials can be a part of this scheme.

These schemes highlight patient’s right to seek treatment for a disease that has no cure. If there is no treatment available, then regulatory bodies should not prevent patients from taking benefit of safe but unproven therapies. This concept was known as compassionate use in Europe earlier which has now evolved in the legislation explained above. In Australia and Canada as well such laws exists which allow marketing and provision of safe but unproven therapies and drugs to patients that suffer from incurable disorders after taking their informed consent and by reporting any possible adverse events noted. In Australia this is known as Special access scheme and in Canada it is known as special access program. (12,13)

Some common themes and concepts emerge from these new and more permissive regulations. It is important to implement these in the guidelines and regulations of countries that have less permissive regulations.

New concepts that have emerged from the recent regulations (Figure. 2)

1. Conditional marketing approval
2. Risk Stratification
3. Post-Hoc efficacy analysis
4. Presumed efficacy
5. Patients right to seek treatment
6. Distinction between cellular therapies
7. Distinction between a stem cell product and medical service
8. Non-homologous use

Conditional approval

Conditional approval first introduced by Japan and later also implemented by USA is revolutionary concept that allows for faster marketing of promising stem cell therapy products. In the last century the most promising medical research was done

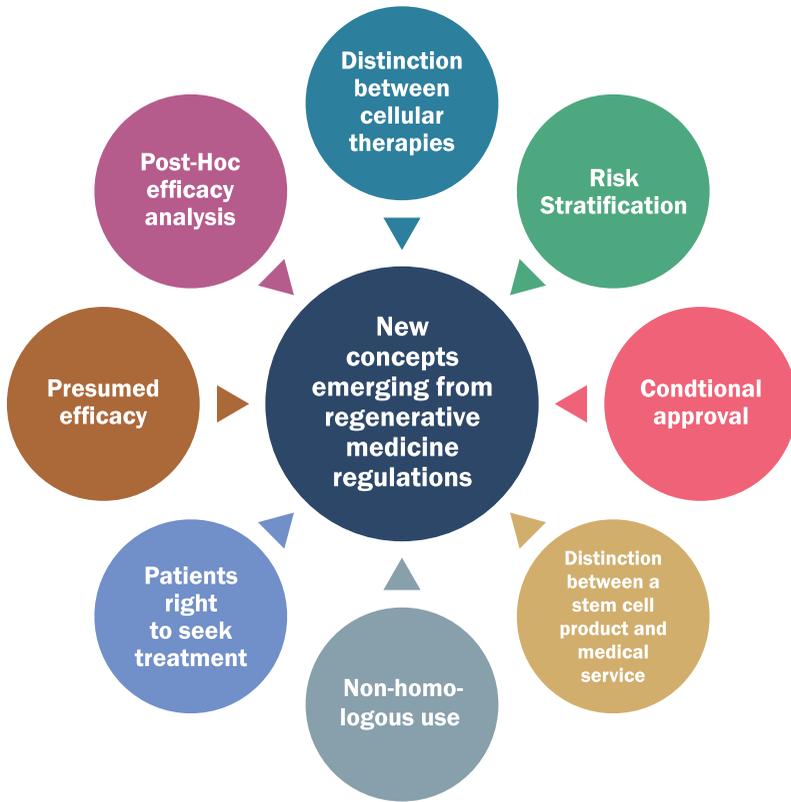


Figure 2: New concepts emerging from the recent regulations

by individual doctors in their field of practice who kept patient care at the center of their research. But industrialization of pharmaceutical sciences and stricter regulations implemented for getting marketing approval made it impossible for individual practitioners to develop promising therapies and medicines. Conditional approval allows for promising therapies to be marketed for a stipulated time at an earlier stage of Phase I or pilot trials which are sufficient to prove the safety of the therapy and suggest efficacy of the same.

The concept of conditional approval has shifted the control of medical innovation back in the hands of individual doctors practicing and researching to provide better patient care.

Risk Stratification

Risk stratification means grouping the stem cell therapies and products based on their risk to human life and health. Such stratification helps to differentiate between less harmful and more harmful cellular therapy products.

A] Using this principle Korea has excluded the safer forms of therapies from their regulatory framework.

B] Japanese guidelines have based their regulatory requirements on risk

stratification. With low risk needing only institutional clearance, medium risk needing outside institutional clearance and high risk requiring clearance from MHLW.

C] The new proposed American law REGROW Act, using this principle has proposed more permissive regulatory pathway for safe cell therapies such as cells or tissues that are minimally manipulated for a non-homologous use; or more-than-minimally manipulated for a homologous or non-homologous use, but are not genetically modified.

Post-Hoc efficacy analysis

Concept of Post-Hoc efficacy analysis means that true efficacy of the product or therapy can be determined post marketing. This is the most dramatic shift in the current medical regulations that do not permit marketing of unproven drugs and therapies. However based on this principle therapy or product can be permitted for marketing based on studies showing definite safety but preliminary efficacy analysis. The roots of this concept are in the basic principle of compassionate use, facilitating early availability of potentially lifesaving experimental medication which are safe but unproven.

This is a revolutionary concept that has already been implemented in Japan since November 2014 and 2 products have already received approval under this legislation. Recently, based on this concept REGROW Act has also been put forth in the USA.

The basis of post – hoc efficacy analysis lies in the concept of Practice based evidence which allows for gathering information regarding efficacy of a particular therapy after using it clinically as a form of treatment and recording the clinical outcomes in the patients treated. Unlike evidence based medicine, the concept of practice based evidence gives the flexibility to offer a treatment after the safety is established and offer it as a treatment while simultaneously studying the effects on clinical outcome.

Presumed efficacy

It has been debated earlier that the modern standards for efficacy testing are too idealistic and may in turn slow down the progress of medical science. Although the regulations are for safe guarding the patients they fail to determine when a therapy will be considered as proven. The current regulations ask for Phased clinical trials that take up to 6 to 8 years before a new product can come in the market and have a cost estimate of about 5 million dollars. Current research and statistical methods are more suited for a drug or a molecule that has finite chemical reactions in the body, however in biological products there are infinite possibilities for interactions and therefore it may take decades before a conclusive efficacy analysis can be done.

Japan in their regenerative medicine regulations for the first time proposed a

concept of 'Presumed efficacy'. This means that the preliminary trials that lack statistical rigor but are suggestive of beneficial clinical outcome can be considered as the evidence for efficacy of the treatment. Simply put, it means that it can be reasonably assumed that therapy will be effective in larger population based on a finding with a smaller population.

It was earlier considered unethical to charge for therapies that have shown efficacy in smaller populations. Japan in their recent regulations allowed for marketing of such therapies under a conditional approval and these therapies were also covered under Japan's national health insurance schemes. In the recently proposed REGROW act, USA; similar suggestions have been made for allowing safe therapies to be marketed based on their presumed efficacy.

Patient's right to seek treatment

Up till the last century availability of the clinical treatments was solely based on decisions of regulatory bodies. If a treatment did not fit the criteria laid out in the regulations then it was not allowed in the market, thereby denied to the patients. Although this was to safeguard patients from adverse effects of under investigated therapies, terminally ill patients were losing out on promising therapies due to strict demands for proving efficacy.

Most of the patients with progressive fatal disorders do not have enough time for an experimental drug which has proven safety and has shown efficacy in smaller trials to be tested in the statistical rigor of bigger trials. These drugs could be potentially lifesaving for these patients. There were many efforts lead by patients and non-profit organizations, which demanded access to such experimental drugs for patients with terminal illnesses.

The origin of compassionate use is in the World Medical Association's Declaration of Helsinki on ethical principles for medical research involving human subjects. The declaration in their clause on unproven intervention in clinical practice states that, 'In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available'. (14)

The concept of patient's right to seek treatment is highlighted in the White paper published by the International society of Cellular Therapy which states that "Patients seeking medical treatment for cellular therapies have the following rights that must be respected by healthcare providers and all associated with their care.

The right to seek treatment: patients and their families/partners have the right to seek treatments for their diseases. No entity should withhold this fundamental right unless there is a high probability of harm to the patients.”(15)

Efforts made by the patients in accordance with this ethical principle led to changes in the legislation for USA, Europe and several other countries in the world. In USA this was implemented as Treatment/Emergency IND initially and later as Right to try Act in 2015. (5) In Europe this was implemented as Compassionate use Act and recently a program was launched to support to development of priority medicines for unmet medical needs, PRIME. In addition other Acts like Hospital Exemption Act in Europe (10), Special access program in Australia (12) and special access scheme in Canada (13) are based on this principle.

These compassionate use programs highlight patient’s right for seeking unproven but safe experimental drugs and allows access to such medicines and therapies at the personal recommendation and responsibility of the treating physician. Such use is deemed ethical and can be charged for after receiving an informed consent from the patient, explaining the possible adverse effects if any and informing the patient about the experimental nature of the therapy.

Unfortunately, in India there are no laws or regulations for compassionate use. Indian regulators and guideline formulators have not taken into consideration the right’s of these patients to seek treatments that may potentially save their lives.

Distinction between different types of cellular therapies

Earlier the guidelines did not make distinction between different types of cells, processes of procurement and routes of administration. However the recent guidelines have made various distinctions and have made separate regulations and guidelines accordingly.

In USA the REGROW Act makes distinction between minimally manipulated cells and more than minimally manipulated cells.(2) Minimally manipulated cells are defined as, “cells procured using technologies when there is no intended alteration in the biological characteristics of the cell population relevant to its claimed utility, performed by a medical doctor at a medical center during the same surgical procedure without compromising the safety of the cells; this may include separation of mononuclear cells, washing, centrifugation and suspension in acceptable medium.” All the other cell types are characterized as more than minimally manipulated cells.

In Japan, there is a separate law designed only for the classification of the regenerative medicine products based on their safety profile.(7) These products are divided into 3 separate classes as, class I – High risk, Class II – Medium risk and Class III – Low risk products (Figure).

In European guidelines, the products are divided into minimally and more than manipulated as well. Minimal manipulation is defined as cells procured through simple technologies like cutting, grinding, shaping, centrifugation, soaking in antibiotics or antibiotic solutions, sterilization, irradiation, cell separation, concentration or purification, filtering, lyophilization, freezing, cryopreservation and vitrification. However there are no separate guidelines for the use of these products as allowed in Japanese and USA laws.

Distinction between a cellular therapy product and cellular therapy medical service

Advent of cellular therapy has given rise to a huge dilemma for regulators whether to regulate these as a product or a medical service. Therefore most of the guidelines are too restrictive where it is considered as a product or too liberal where it is considered a therapy. Although burden of evidence lies on both therapy and product; the criteria for marketing approval have been traditionally very different for both. Every new product is regulated separately and the evidence for one is usually not applicable to the other therefore companies designing different products need to seek different approvals. The guidelines for these are also very strict. However a therapy once proven safe and effective can be used by multiple practitioners and they individually do not need to seek approval for the same. This is a basic distinction in the product and therapy which most of the guidelines in the world including Indian guidelines fail to understand.

Japanese guidelines however have been very progressive and they have designed 2 separate laws for products and therapies. These two laws have also been very progressive in their field of application allowing fast track conditional approval for products and mandatory approval from MHLW only for high risk therapies. USA has taken a step ahead in not only allowing a fast track conditional approval for products but also allowing different companies to get faster marketing approval based on exhibited biosimilarity with an already existing approved product.

Non-homologous use

The proposed REGROW Act 2016 has for the first time made a provision for conditional approval of therapies and products using minimally and more than minimally manipulated cells for non-homologous use i.e. not in the same body system as that of the source of the cells. (2)

Current regulatory system in India [1,2,3,4]

The present situation in India with regards to guidelines for stem cell therapy

- 1) The National guidelines for stem cell research have been formulated by the Indian Council of Medical Research and the Department of Biotechnology in 2013 [1]. These guidelines have retained the 2007 classification of stem cell research into 3 categories namely Permissive, Restrictive and Prohibitive

research. Human embryonic stem cell derivation and differentiation falls in "restrictive" category, whereby, these cells can only be used for research purposes. "The prohibitive research" includes any research related to germ line genetic engineering or reproductive cloning of any in vitro culture of the intact human embryo, regardless of the method of its derivation, beyond fourteen 14 days or the formation of the primitive streak, whichever is earlier; transfer of human blastocysts generated by SCNT; or the breeding of parthenogenetic animals, in which human stem cells have been introduced at any stage of development. Adult and umbilical cord blood cells are clubbed under the "permissive" group [2]. It has introduced an additional layer of oversight besides the institutional ethics committee (IEC) in the form of Institutional Committee for Stem Cell Research (IC-SCR) and the National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT). A major recommendation has been to omit the word therapy from the title of the guidelines as compared to the guidelines in 2007. As per National guidelines, every organization (academic or otherwise) interested in working on stem cells, must formulate an Institutional Committee for Stem Cell Research and Therapy (IC-SCR). Members of the Committee must include people with appropriate expertise (representatives of the public and persons with expertise in clinical medicine, developmental biology, stem cell research, molecular biology, assisted reproduction technology, and ethical and legal issues in stem cell research) and this Committee must function at the institutional level. Projects will be approved on the basis of scientific evaluation and ethical conduct. The IC-SCR must also be registered with an NAC-SCRT. The NAC-SCRT is constituted by the Government of India. NAC would be comprised of experts from various fields, who would be responsible for examining the scientific, technical, ethical, legal and social issues in the area of stem cell based research and therapy. It will have around 10 members. A chairman, a deputy chairman, member secretary and nominees from DBT, DST, CSIR, ICMR, DCGI, DAE, and biomedical experts from pharmacology, immunology, cell biology, hematology, genetics, developmental biology, clinical medicine and nursing. Legal expert, social scientist, and a women's representative will also be part of NAC. NAC could also consult outside experts on a case to case basis. Institutions involved in stem cell research and therapy will have to be registered with the NAC through Institutional Committee for Stem Cell Research and Therapy (IC-SCR).

- 2) The Ministry of Health and Family Welfare, Government of India, established a High Powered Committee in June 2013 to suggest a road map for regulation of stem cells and other cell based therapies being practiced in India. Under the chairmanship of Professor Lalji Singh it submitted a Guidance Document for Regulatory Approvals of Stem Cell and Cell Based Products (SCCPs) in

December 2013. This Guidance Document is based on the recommendations of that committee and it is subsidiary to the amendments made in 2013 to the Drugs and Cosmetics Act (DCA), 1940 and the new rules proscribed there under. As per these amendments it has been decided that Government of India, through the DCG (I) and CDSCO, shall regulate all practices related to the use of stem cells, and other cells, for therapeutic purposes in India. The amendment in DCA also mandates that all stem cells and cell based products that can be used for therapeutic purposes shall be referred as Stem Cell and Cell Based Products (SCCPs) and all activities related to their usage i.e. manufacture/isolation/collection, storage and transplantation into patients must be done only under a license or permission that would be granted by the DCG(I)/CDSCO [3].

- 3) Another important and major development has been the proposal of the Drug Controller General of India DCG(I) to include "stem cells" in the definition of new drugs in the proposed bill titled "Drugs and Cosmetics (Amendment) Bill 2015" [4]. The revision in this was proposed earlier in this year which exclude autologous and minimally manipulated type of cells from this law.

The Gazette of India published in April 2018 states that,

In sharp contrast to the regulatory developments in the world, the latest Indian guidelines made by Indian council of Medical Research (ICMR) in 2013 are moving backward and are in the process of trying to implement policies that will completely destroy the stem cell therapy field in India. Indian regulators fail to understand the 1] distinction between drug and stem cell therapy 2] distinction between stem cell therapy product and stem cell therapy. The current Indian guidelines do not incorporate any of the new concepts that have emerged in the recent progressive guidelines of other countries.

Our recommendations for designing the guidelines

Based on various international guidelines, white papers and declarations from world medical association we would like to recommend some guiding principles while designing the guidelines in our country for approval and monitoring of stem cell based research as well as therapy.

The recommendations are based on the following documents

- 1) The regulatory guidelines from different countries like Japan, Korea and United States of America [5-12]
- 2) Opinions from white paper of the International society of cellular therapy (ISCT)[13]
- 3) Helsinki declaration of World Medical Association that guides the ethical principles of human research [14]

and

- 4) Beijing declaration of the International Association of the Neurorestoratology [15]

Recommendations

1. Acceptance of unproven cellular therapies for the treatment of incurable conditions, based on the World Medical Association' declaration of Helsinki.
2. Distinction between legitimate cell therapy medical services and fraudulent services, based on the ISCT White paper.
3. Distinction between clinical trials and medical innovation, based on the ISCT white paper.
4. The basic right of a patient to seek treatment should be respected, based on the ISCT white paper.
5. Distinguishing various centers offering cellular therapy, based on the recommendation of the ISCT white paper.
6. Recognition of the importance of cellular therapy as part of neurorestorative therapies, based on Beijing declaration of the International Association of the Neurorestoratology (IANR).
7. Giving importance to Practice Based Evidence
8. Regulations need to make a distinction between different types of cellular therapies, based on the regulations in countries like Korea, Japan and USA
9. Adapting regulations from countries that have been progressive and more permissive of cellular therapies like Korea, Japan and USA.

Proposed changes in the Indian regulations:

We would like to propose a road map for regulating stem cell work in India in such a manner that the safer forms of therapies are easily available to patients with incurable diseases whereas less safer forms of therapies are regulated more strictly.

{A} For this we propose that there should be 3 different sets of guidelines for,

- 1) Researchers – Those who are doing basic laboratory research and clinical trials in patients.
- 2) Corporate Manufacturers – companies that are manufacturing stem cells and stem cell related products on a large scale
- 3) Clinical stem cell therapists – doctors and institutes that offer cellular therapy as a treatment.

Separate rules and regulations should be formulated for these. The researchers should follow ICMR guidelines. Corporate manufacturers should follow CDSCO / DCG(I) guidelines.

Clinical stem cell therapies should further be categorized into

Low risk: Therapies using autologous and minimally manipulated stem cells. These therapies could be permitted under the oversight from the IEC.

Medium risk: Therapies using more than minimally manipulated allogeneic cells of non-embryonic origin. These therapies would need oversight of IEC and approval from CDSCO/DCG(I).

High risk: Embryonic/ Fetal stem cells and iPSCs. Therapies using these cells would require oversight of IEC and approval from CDSCO and ICMR.

A key aspect of debate between clinicians and regulatory bodies is what new clinical indications should be considered as approved to offer stem cell therapy. We believe that if there are publications, that document safety and presumed efficacy of stem cell therapy in a particular indication from any part of the world, then this should be considered as an accepted indication.

{B} The membership of NAC-SCRT should be expanded to include more members from the clinical side having experience and expertise in Stem cell therapy so that a more balanced view is taken. The Chairmanship of NAC-SCRT should be changed by rotation every year so that fresh insights are available to the committee.

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Difficulty of Being Good:

"In order to preserve dharma in this imperfect world of Kali Yuga, he had to commit 'smaller wrongs' for the sake of a 'bigger right'."

From the book "The Difficulty of being Good. On the subtle art of Dharma" in the chapter "Krishna's Guile" by Gurcharan Das (Penguin Allen Lane)

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Ethics

Consensus on the potential of stem cell therapy to address various incurable, debilitating disorders is unanimous. Stem Cell research and therapy is the front line of the biomedical field. However, no other area of biomedical research has faced the quantum of ethical, moral and political controversies that surrounds stem cell research. Adult stem cells as an alternative source, other than embryos, have been spared of controversies and have been generally welcomed and encouraged for research and therapy.

Embryonic stem cell, on the other hand, has been hounded by objections and restrictions due to the source of its procurement, by various religious bodies of the world.

Ethical Issues Associated With Embryonic Stem Cell Research

Religion and embryonic stem cell:

The major dictum common to all religions is : 1) Human life is sacred and has to be guarded 2) Alleviation of human suffering should be strived for. Though, there is consensus among all religions regarding the potential of stem cell research being a means towards addressing the second dictum, the opinion on what construes a human life differs vastly.

Should the 5-7 day embryo be given the status of a person and hence have the right to life or is this stage too early to confer this right?

For some religions, ensoulment of the embryo would make it a person. But, then when does ensoulment take place?

These are just a few issues surrounding the embryonic stem cell field. Contrasting opinions among various religions and even within the religion exist. The following is a sample of this diversity among different faiths.

Greek Orthodox and Roman Catholic Churches

The official position of these churches is that a human person begins at conception and the human embryo has the same moral status as human persons. Consequently, research on human embryos, including hES derivation and subsequent use is unethical, and if it involves the willful destruction of embryos, it is homicide. The argument that the cell lines are derived from excess embryos, after the fertility needs are dealt with, is of no consequence, since the production of excess embryos itself is unacceptable to the church. The fact that these embryos and their products would be used for the alleviation of human suffering, does not justify the destruction of the embryos.

Since the underlying belief is in the embryo's right to life, any use of the embryo that is not for its own good is immoral and therefore, impermissible. There is no consequentialist or utilitarian approach that would make this act acceptable. The belief in the personhood of human embryos also means that it is not possible to use hES lines previously derived from human embryos or to use therapies derived from hES research. The idea is that these cell lines and therapies are tainted by the immoral act of killing the embryo. To use them would be to become complicit in the immoral act.

However, this rigid stance, especially of the Roman Church, is somewhat diluted by certain other catholic groups, who do not believe that the embryo is a human person, but believe that its ensoulment is the morally relevant time with regard to personhood.

Protestant Churches

Most protestant churches do not believe that embryos have personhood and are open to embryo research but consider that the goals of the research are of paramount importance. In addition, considerable emphasis is placed on the need for both public discussion and for oversight of the research rather than leaving it as an unregulated private enterprise. They believe that the benefits from this and other medical research be distributed evenly and justly to all those in need, regardless of resources or geography.

Official positions vary from country to country on the moral status of the embryo and therefore, on the morality of embryo research in general. These divisions show just how personal an issue stem cell research can be. For these

churches like for the lay public, weighing the moral status of the embryo and the need to help ailing and suffering people is not a simple arithmetic. (1)

Judaism

Orthodox Jews believe that embryos do not have the same moral status as human persons. In fact, gametes and embryos outside a human body do not have any legal status under Jewish law. The result therefore, is that embryos created by IVF have no special moral or legal status. Under Jewish law (Halcha) the fetus does not become a person (nefesh) until the head emerges from the womb.

They believe that when the embryo is implanted it is "as water" up to the fortieth day. After that time and before the fetus emerges from the woman's body it is a potential life and has great value. Ensoulment is generally thought to occur sometime after the fortieth day. It gains full human status, however, only once it emerges from the woman's body. Since embryos used in hES research are outside the body, according to the Jewish faith it is possible to use excess IVF embryos in research.

In addition to the Jewish views on the moral status of the human embryo, this religion places emphasis on preventing and alleviating suffering. This leads to a deep belief in the morality of and value in pursuing medical research. The commitment to preserving one's body and health is joined by a commitment to helping others and alleviating suffering. So there is a moral imperative to help those who are suffering from diseases and to explore the potential of all types of stem cell research. This belief leads Jews to have a generally favorable view of stem cell research including hES research. (2)

Islam

In Iran, Turkey, Singapore (with a majority of Muslims) and other Islamic countries, embryo research policies are influenced by the religious belief that full human life with its attendant rights begins only after the ensoulment of the fetus. This is generally believed by Muslim scholars to take place at 120 days after conception (although a minority belief indicates ensoulment takes place 40 days after conception). This fact, in conjunction with the importance articulated in the Qur'an of preventing human suffering and illness, means that the use of surplus IVF embryos for stem cell research is relatively uncontroversial. What remains controversial in the Muslim world is creating embryos for the purpose of research. As with other religions, Islam and its followers have differing points of views on these issues. For example, in Egypt, a conservative religious country, the Muslim head of the Egyptian Medical Syndicate stated that embryos are early human life and should never be used in research.

Hinduism and Buddhism

In traditional Hindu belief, conception is the beginning of a soul's rebirth from a previous life. Some Hindu traditions place the beginning of personhood between three and five months of gestation, while few believe that the soul's rebirth can occur as late as the seventh month. Most Buddhists have adopted the classical Hindu teaching that personhood begins at conception. Though Buddhist teachings do not directly address the issue, like Hinduism there are two main tenets - the prohibition against harming or destroying others (ahimsa), and the pursuit of knowledge (prajña) and compassion (karua) - that divide Buddhists. Some Buddhists argue that embryonic stem cell research is in accordance with the Buddhist tenet of seeking knowledge and ending human suffering, while others argue that it is a violation of the notion of not harming others.

A central belief of Hinduism and Buddhism is that an individual's soul or self is eternal. In Hinduism the soul is believed to be passed from one living being to another in a process called reincarnation. In Buddhism reincarnation is described differently as the rebirth of the self. These beliefs, that the soul or the self are reborn lead to a greater acceptance of cloning technology. Although the use of embryos in stem cell research remains a divisive issue in these religions, the use of cloning technology in stem cell research is less controversial. (3-5)

Medical And Other Ethical Issues And ES Cell Research:

Proponents of embryonic stem cell research advocate that obtaining human ES cells from the embryos left over after successful pregnancy in the course of IVF treatment for the goal of treating diseases and saving lives justifies the symbolic loss that arises from destroying embryos in the process. They emphasize on the significance of saving life of many patients who need cell replacement therapy, as an essential reason for permission of research on embryos and obtaining ES-cells from them. A different set of ethical issues arises once researchers have learnt safe and effective ways to direct human ES-cell to differentiate into specified cell or tissue types, and to transplant them for therapeutic effects in patients. An important clinical issue at this point will be whether ES-cell not derived from the patient, will be rejected by the patient's immune system. The strategy for dealing with this problem, would then be to use a patient's nuclear DNA to create an embryo from which ES-cells compatible with that patient could then be derived. This process, known as somatic cell nuclear transfer could prove to be a safe and effective use of ES-cell derived replacement therapies. However, this would raise more ethical issues beyond the destruction of leftover embryos to obtain human ES-cells. One issue would be ethical concerns about creating human embryos for the sole purpose of destroying them to obtain replacement cells for the patient who provided the nuclear DNA. Ethical debates about creating human embryos solely for research have existed since the inception of debates over embryo research. One can question; however, whether those concerns are even relevant to generating human ES-cells by

somatic cell nuclear transfer, for the haplogenomes of gametes are not combined through sexual fertilization to form the blastocyst that provides the ES-cells. In addition, there is no intention of culturing the embryo beyond the blastocysts stage, nor of implanting that blastocyst in a uterus for reproduction. Given the asexual means of creating the embryo and the lack of intent of implanting it in the uterus, the embryonic entity produced in these circumstances lacks the reproductive significance that some have argued is the moral basis for valuing early embryos. The other issue is of egg donation for therapeutic cloning and effective cell replacement therapy. The ability to meet the therapeutic demand for oocytes would present an important problem. The ability of live, unrelated donors to meet such a demand is highly unlikely for several reasons: the hormone treatments that stimulate the production of many oocytes impose a considerable burden on women; surgery is required to retrieve the oocytes; and ethical problems now surround such donations.

Fetal Stem Cells And Ethics:

Pluripotent stem cells can be derived from fetal tissue after abortion. However, use of fetal tissue is ethically controversial because it is associated with abortion, which many people object to. Under American federal regulations, research with fetal tissue is permitted provided that the donation of tissue for research is considered only after the decision to terminate pregnancy has been made. This requirement minimizes the possibility that a woman's decision to terminate pregnancy might be influenced by the prospect of contributing tissue to research. Currently there is a phase 1 clinical trial in Batten's disease, a lethal degenerative disease affecting children, using neural stem cells derived from fetal tissue . (6,7)

Induced Pluripotent Stem Cells (iPS Cells)- a safe and ethical alternative?

Somatic cells can be reprogrammed to form pluripotent stem cells, called induced pluripotential stem cells (iPS cells). These would match the donor cells. This was initially tried using viral vectors, followed by plasmids. Currently, the aim is to be able to induce pluripotency without genetic manipulation. Because of unresolved problems with iPS cells, which currently preclude their use for cell-based therapies, most scientists urge continued research with hESC. (8) iPS cells avoid the heated debates over the ethics of embryonic stem cell research because embryos or oocytes are not used. Furthermore, because a skin biopsy to obtain somatic cells is relatively noninvasive, there are fewer concerns about risks to donors compared with oocyte donation. The President's Council (USA) on Bioethics called iPS cells "ethically unproblematic and acceptable for use in humans" Neither the donation of materials to derive iPS cells nor their derivation raises special ethical issues.

Evolution Of Policies On The hES Cell Research In The US:

The most keenly followed and studied policy change regarding the human ES cell

research has been that of the United States. This has been mainly attributed to be influenced by the ethical, moral & religious stand of the catholic church. In 1973 a moratorium was placed on government funding for human embryo research. In 1988 a NIH panel voted 19 to 2 in favor of government funding. In 1990, Congress voted to override the moratorium on government funding of embryonic stem cell research, which was vetoed by President George Bush. President Clinton lifted the ban, but changed his mind the following year after public outcry. Congress banned federal funding in 1995. In 1998 DHHS Secretary Sullivan extended the moratorium. In 2000, President Bill Clinton allowed funding of research on cells derived from aborted human fetuses, but not from embryonic cells. On August 9, 2001, President George W. Bush announced his decision to allow Federal funding of research only on existing human embryonic stem cell lines created prior to his announcement. His concern was to not foster the continued destruction of living human embryos. In 2004, both houses of Congress asked President George W. Bush to review his policy on embryonic stem cell research. President George W. Bush released a statement reiterating his moral qualms about creating human embryos to destroy them, and refused to reverse the federal policy banning government funding of ESC research (other than for ESC lines established before the funding ban). In the November 2004 election, California had a Stem Cell Research Funding authorization initiative on the ballot that won by a 60% to 40% margin. It established the "California Institute for Regenerative Medicine" to regulate stem cell research and research facilities. It authorizes issuance of general obligation bonds to finance institute activities up to \$3 billion dollars subject to an annual limit of \$350 million. Under President Obama, it is expected that federal funding will be made available to carry out research with hESC lines not on the NIH list and to derive new hESC lines from frozen embryos donated for research after a woman or couple using invitro fertilization (IVF) has determined they are no longer needed for reproductive purposes. However, federal funding may not be permitted for creation of embryo expressly for research or for derivation of stem cell lines using somatic cell nuclear transfer (SCNT)

The Korean Stem Cell Controversy

The meteoric rise and equally sudden fall of Korean scientist Woo-Suk Hwang depicts all that can possibly go awry, ethically and scientifically, in the world of stem cell research. What would have been regarded as a seminal paper in SCNT technology and human ES therapeutics turned out to be complete fraud and hogwash. Not only were the results fabricated, but also, unethical practices were employed to procure oocytes for the research.

At the end of 2005, the scientific community was shocked by one of the greatest cases of misconduct in the history of science. Two breakthrough articles about stem cell technology from a Korean laboratory headed by Woo-Suk Hwang, published in

Science, appeared to be almost completely fabricated and were therefore retracted. The two fraudulent papers concentrated on the concept of therapeutic cloning in humans. In this somatic cell nuclear transfer (SCNT) technology, a nucleus from a patient's somatic cell is transplanted into an enucleated donor oocyte. The resulting blastocyst embryo is used for the isolation of embryonic stem cell (ESC) lines that possess virtually all the patient's characteristics and thus will minimize immune rejection upon transplantation. Until the publication of the fraudulent papers, therapeutic cloning was a cumbersome and inefficient technique and successful therapeutic cloning in humans had not been reported before. In their 2004 paper, Hwang and his associates claimed to have isolated the first human ESC line derived from SCNT and in their second paper they reported to have improved the efficiency to such an extent that clinical application became within reach. Two months following the first paper, criticism arose on the ethics of obtaining the human oocytes used in the study. After initial denial it became clear that egg donors had been paid and two lab members had provided oocytes. This forced Hwang to admit these unethical practices. Subsequently, the scientific content itself raised questions. Duplications of microscopic photographs in different panels, and designated as different ESC lines, in the publication of 2005 were uncovered, but these were parried as an accidental mistake by Hwang and the Science editorial board. Furthermore, DNA fingerprint comparison of presumed donor and derived ESC lines showed no inter-experimental variety and were in fact performed on the same fingerprint profile. Hwang agreed to an independent investigation by Seoul National University. His three most important recent works were investigated: the retracted 2004 and 2005 Science papers and a publication in Nature about a cloned dog. The conclusions were clear. The claim of being the first laboratory to create a pluripotent human ESC line through SCNT was reported to be false. Verification of the DNA fingerprints of cell lines, teratomas and donors showed that the NT-1 cell line was not derived from the designated donor. Second, no evidence was found to verify the conclusions of their report of the 11 ESC lines in the paper of 2005. The claims were based on material obtained from two ESC cell lines derived by IVF rather than SCNT. Displayed results of DNA fingerprinting, karyotyping, data of MHC-HLA isotyping and photographs of teratoma and embryoid bodies were all fabricated. (9)

Ethical Issues For Cord Blood Banking

The ethical implications of cord blood banking in the case of donated samples for the purposes of allogeneic transplantation or research are the same as for any tissue bank. This issue has been addressed 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 underlined in this opinion are the following: body integrity, respect of privacy and confidentiality of data, promotion of solidarity, fairness of access to healthcare and information and consent of the donors.

(10) Umbilical cord blood banking process should comprise of a detailed consent explained clearly to the woman or to the couple of the prospective new treatments, but stress that they are still very much at the experimental stage. Principally, tissue bank activities should be reserved to public health institutions or non-profit making organizations. All public and private banks tissue banks should be monitored for quality measures and standards. 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.

(11)

There are also some value conflicts regarding the Umbilical cord blood banking. The values of freedom and free enterprise can 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 vital step to any research project. It is the process in which a patient/participant consents to participate in a research project after being informed of its procedures, risks, and benefits (12) After fully comprehending the information about the project, the patient/participant gives full and conscious consent for the physician/scientist to continue with the procedure. The consent is obtained after giving 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 main aspects of the informed consent are information, voluntariness and capacity. In keeping 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 judgement to whether or not to be a part of the trial or treatment.

Ethics in medical practice

Greek word Ethos, meaning character, is the origin of the word ethics. Ethics is part of philosophy in modern world and explores the rights, wrongs and morality of human behavior. Ethical principles related to medical practices form bioethics. Bioethical principles guide the current regulatory systems and are the foundation of

modern research practices [13].

Four core principles of bioethics are Autonomy, Nonmaleficence, Beneficence and Justice.

Autonomy:

Principle of autonomy states that the patient should be considered capable of taking an informed decision about the treatment after understanding the benefits and adverse events of the treatment should be given the right to exercise his/her choice of the treatment without any external influence preventing voluntary action.

Nonmaleficence:

The second principle of Nonmaleficence suggests that there should be no harm caused to the patient by providing or denying a treatment.

Beneficence:

The third principle of Beneficence is self explanatory and suggests that the physician has a duty to benefit the patients and also to prevent them from any harm that may be caused by a medical treatment.

Justice:

The fourth and final of the basic principles, is the principle of Justice; meaning a physician should be fair in offering his services and there should not be any preferential attribution of services.

The principles have been intentionally or unintentionally violated in past while offering medical treatments and performing medical research. Medical experiments conducted on the prisoners of war in Germany were intentional examples and occurrence of phocomelic infants after thalidomide consumption was an unintentional example of this violation. Because of these the need to enforce strict adherence to the biomedical principle in medical research and practice was necessitated. In modern world the ethical principles are implemented through various regulatory bodies to protect the patients from being taken advantage of and to protect them from any harm caused by the treatment or medical incompetence. The guidelines formed by these regulatory bodies are based on Evidence Based Medicine (EBM).

Principles of Evidence based medicine

Evidence based medicine (EBM) is defined as, "The conscientious and judicious use of current best evidence from clinical care research in the management of individual patients." [14] To cope with the growing medical information and to determine the true efficacy of medical treatments, EBM was used. EBM helps to form most informed conclusions about the efficacy of existing treatment options [14]. Various

steps involved in coming to this conclusion are forming a relevant clinical question, searching for the evidence to answer that question, appraising the evidence, integrating that evidence into the clinical practice keeping in mind patient preferences, evaluating the outcome of such integration using standardized scientific tools and then documenting and disseminating these findings for others to appraise [15]. This makes the whole process combusive.

Information gathered clinical experience, expert opinions, individual cases or series of cases and research trials using cohort studies or randomized can be considered as evidence but there is a hierarchy. While the clinical experience and collective expert opinions are considered to have least generalizability, randomized controlled trials and systematic reviews of such trials are considered to be the most applicable evidence. The quality of the evidence is based mainly on the epidemiological principles [16].

The primary aim of using EBM model is to provide synthesis of the available information to empower the doctors to take most informed clinical decisions. Using this system has prevented the consumption of unsafe drugs, serious adverse effects and use of ineffective medical practices. However, most of the revolutionary concepts of modern medicine were developed in the late 19th and early and mid 20th century when the notion of EBM was not there. This period has witnessed some of the most extra-ordinary medical inventions and they happened in the absence of regulatory bodies.

Although EBM is the most scientific approach towards concluding the information gathered, the process of EBM is very time consuming and has its limitations in assessing the effectiveness. It is difficult to apply the evidence gathered collectively from patients to an individual patient with dissimilar characteristics [16]. EBM relies on the empirical evidence for informed conclusions and is unable to formulate clinically relevant conclusions in absence of such evidence. Therefore most common fallacy of EBM is that lack of evidence of efficacy is considered as lack of efficacy itself. [16]. Empiricism of EBM undermines the philosophical origin of the medical innovations that are based on the clinical expertise and pathophysiological knowledge of the disease [16].

With the advent of evidence based medical practices, the development of new therapies and drugs has become very time consuming and the burden of evidence also increases the economical burden. Current process of developing new drugs includes preclinical laboratory & animal testing as well as multiple phases of human testing to first establish its safety and then efficacy first in a small group and then in larger groups [17]. It takes approximately 6 to 8 years and \$5,000,000,000 to develop a new marketable drug [18]. It is almost impossible for a single medical practitioner to spend the time and money required for this process. It can only be done collectively or by corporate. It is based on commercial interest and demand

from the consumers rather than medical expertise. Therefore the rare disease get neglected and development of drugs for rare diseases is limited.

So the debate is:

1. Are current medical practices for developing new treatments adhering to the principle of Justice?
2. Are we then really observing the principle of beneficence?[c] Is the system of EBM which was developed for making efficient, scientific and evidence informed clinical decisions, now perhaps slowing the pace of medical evolution. Therefore although very essential, practice of EBM needs to be relooked especially with reference to cell therapy.

Ethical Dilemmas of cellular therapy

The last decade has seen the evolution of cellular therapy. This is the field of regenerative medicine where healthy tissues could be used to replace or repair damaged tissues. Cell therapy holds a special place in the development of Neurorestorative treatments for otherwise incurable neurological conditions. Development of cellular therapy has also sprouted debates on various ethical grounds based on religious, social, political and capitalistic beliefs. It has been unanimously accepted that the science of regenerative medicine is vast and holds a tremendous potential of finding cure to various untreatable diseases. But in the past there has been a strong opposition to research associated with embryonic stem cell therapy. The genesis of this was the ban put by President George W Bush on the federal funding of embryology stem cell lines developed after 2001. This ban still remains in public memory with the result that subsequent clinical developments in the field have not received the recognition these should have. In any case, this ban was subsequently lifted by President Barak Obama. Cellular therapy consists of various other types of cells which are not of embryonic origin. The ethical dilemmas associated with embryonic stem cells need not be applied to the cells of non embryonic origin like umbilical cord stromal cells and adult stromal cells. The medical community, patients and regulatory authorities need to make this distinction and the objections for the use of one type of cells should not be applied to the other types.

Another facet of the ethical considerations is using cellular therapy as a form of treatment. Clinical practitioners in the field of cellular therapy face a moral and an ethical dilemma every single day of whether it is ethical to offer a treatment that has not yet been approved as a standard of care for that disease? But if they deny a safe and available treatment to the patients who have incurable diseases, just because there efficacy has not been established by the modern medical standards and wait for it to be established while the patients wither away, then Is that ethical? The basis of this dilemma lies in the conflict of the two fundamental principles of bio ethics,

Beneficence and Non-maleficence. On one hand one is expected to always benefit the patient on the other one may not offer the treatments to the patients unless those are approved as per the modern medical standards. By not offering such treatments one is actually violating the principle of non-maleficence through omission. EBM in modern medicine has become synonymous with ethical medical practice. But the unique challenges put forth by evolution of cellular therapies demands us to rethink about this. Cellular therapies are far more dynamic and diverse than drug therapies and therefore it may take decades or more to generate empirical evidence validating use of cellular therapy as a treatment form. The generation of such evidence will also be dependent on various socio-politico-economic variables and not on the need for medical innovation alone. Is it fair then, that on one hand we claim to protect patients from adverse effects and ineffectiveness of the therapy using EBM whereas on the other hand we let them die or suffer waiting for the treatment? It is time to revisit the concept of 'Practice Based Evidence' (PBE) and not rely solely on 'Evidence Based Medicine'. Whereas EBM may perhaps jeopardize the autonomy of the patients in choosing an unproven treatment for the lack of any other proven treatment, PBE protects such autonomy. PBE respects the evidence generated from a single practitioner whereas EBM will disregard it as the lowest form of the evidence. But this lowest form of the evidence is what has given medicine its most brilliant inventions. Medical field progressed when the individual clinical practitioners pioneered newer forms of therapy based on their clinical experience and expertise. Most of the surgical practice comes from individual surgical expertise and cannot be tested using multicenter randomized controlled trials. Day to day decisions made in an intensive care setups and operating rooms are primarily influenced by the clinical circumstances at hand and the clinicians own experiences in dealing with such situations. Not everything in the medicine can be measured on the yardsticks of EBM; neither can it all be tested using EBM. Further the question remains when can a particular form of treatment be considered to have conclusive evidence? How many clinical trials will it take for us to get that evidence? The more daunting question is whether we have the funds, the resources and the time to conduct multitude of trials. Should we accept to slow the pace of medical advancements for lack of funds and resources for formal clinical trials? PBE could therefore be a key to faster progress in medicine. Both the paradigms of evidence based medicine and practice based evidence need to co-exist. The science of cellular therapies provides a unique opportunity for this co-existence. This does not mean that the treatments should not be tested scientifically. Novel treatments must be very stringently monitored for its safety. However once the safety has been established, the use of such treatments may be permitted in case of the diseases where there are no other treatments available. Not only these treatments should be permitted but also more and more number of practitioners should be involved in providing these treatments so that a large body of evidence can be generated. Greater availability would also make such treatments easily

available and less expensive. Rather than relying on the corporates to generate evidence, individual practitioners and institutions should also be empowered and entrusted.

An ethical dilemma that often confronts practitioners of cellular therapy is whether it is appropriate to charge for treatments that are yet unproven. The answers to questions like this are complex however in general it could be said that what is important is whether a treatment method should or should not be offered to a patient. If it is ethical to offer a treatment to a patient that will benefit the patient then it there should not be any ethical issues about charging for the same.

Ethical Basis Of Stem Cell Therapy:

Ethical principles like autonomy, beneficence, non-maleficence and justice are expected to be adhered to in the medical research and treatment according to the modern regulatory practices. The primary intention of these is to safeguard the patients who are the consumers of medical services from any risk and exploitation during research and therapy. The evolution of cellular therapy as a new form of treatment has opened up a new debate about ethics of the stem cell practice.

American society of gene and cell therapy defines cell therapy as the, '*Administration of live whole cells or maturation of a specific cell population in a patient for the treatment of a disease by American society of gene and cell therapy.*' The primary aim of cellular therapy is to repair the damaged tissue by replacement or regeneration of new cells. Therefore cellular therapy is different from the drug therapies where a single molecule is investigated for a putative beneficial effect.

When considering whether stem cells can be offered as a form of treatment or not, there are two extreme views. There are those that strongly oppose the offering of cellular therapy as a treatment form till there is definitive evidence of its effectiveness. On the other hand there are the practitioners of these treatments who believe that patients suffering from many of the untreatable conditions should not be denied these treatments just because they are still unproven. Both sides are correct in their own ways and both these views are like two sides of a coin. The existence of one does not negate the other. Together they make a whole. It is therefore time to relook at the ethics, regulations and principles of evidence based medicine in a new light in connection with cellular therapy.

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. It 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 it 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." In accordance to the International policies as stated in the Helsinki Declaration, our centre NeuroGen Brain & Spine Institute follows the guidelines. There are in addition some other aspects of the Stem cell therapy debate that need further discussion.

These are:

- (1) That there is a need to make a clear cut distinction between embryonic stem cells and adult stem cells whilst strict regulations for embryonic stem cell work are completely justified the same are not needed for adult stem cell work.
- (2) That there is a need to look at the whole issue from the patients point of view 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 safety and efficacy of adult stem cells in neurological disorders and based on this evidence there is no need to keep on doing trials.

To elaborate on the above points:

(1) That there is a need to make a clear cut distinction between embryonic stem cells and adult stem cells whilst strict regulations for embryonic stem cell work are completely justified the same are not needed for adult stem cell work:-

It is clear from all the above that the entire ethical debate regarding stem cell therapy revolves around the use of embryonic stem cell and cloning. There are no ethical issues with the use of autologous stem cells derived from bone marrow, Yet there are various restrictions in place for the use of any types of stem cells in different countries. Until everyone concerned starts looking at stem cells of non-embryonic origin differently from embryonic stem cells we will continue to

involved in debating the issue and the price for these delays are paid for by the patients for no fault of theirs. Herein lies the tragedy. There is available a form of cellular replacement therapy that can give relief to millions of patients, for which there is enough published clinical evidence of safety and a satisfactory published evidence of efficacy yet this treatment cannot be freely used by one and all. It is our belief that by letting patient suffer and at the same time when there

are treatment options with stem cells that could possibly benefit them is unethical.

(2) That there is a need to look at the whole issue from the patients point of view respecting the

fact that even small functional improvements can mean a lot to a particular patient: We tend to judge improvements from normal peoples point of view. We don't realize that even small improvements, seemingly unimportant to us, can make a quantum difference in the lives of patients paralyzed with neurological problems. The Beijing Declaration of the International Association of Neurorestoratology (IANR) says it "recognizes the importance of small functional gains that have significant effects on quality of life". We need to stop being arm chair professors and talking only about evidence based medicine. We have to look at this from the point of view of the patients. To highlight this we highlight a case which shows us how improvements that may mean nothing to us can mean the world to suffering patients. This was one of the first cases of multiple sclerosis treated with stem cells. Patient had a lot of improvements including significant improvements in her speech, ability to use her hand to hold a cup and her mobility, ability to sit without support, ability to stand with support. All of these were not possible before the stem cell therapy treatment. Yet the improvement that mattered to her more than all of these was something very small. Earlier when lying in the prone position she could not turn in bed by herself. After the stem cell therapy she could do so. Prior to the treatment every night she would have to wake up her grandmother 3-4 times a night to help her turn her position in bed. This used to upset the patient since it used to emotionally hurt and pain her that she had to wake up her grandmother multiple times in the night just to turn her. And she needed to turn since sleeping in one position would make her very uncomfortable. So despite all her other improvements with her speech and hands what made her most happy and the improvements that mattered to her the most was after the treatment she could turn in bed by herself and did not have to wake up her grandmother every night. This has been highlighted just to make one very simple point. That we must look at this entire issue from the patients point of view. We must recognize that small improvements that do not mean anything to us can mean a lot to a patient with severe physical limitations. That at the end of the day all ethics, moral, values principles, laws and regulations have just one purpose. The well being of the common man.

What has unfortunately happened in the field of stem cell therapy is that the regulations we have made to protect ourselves are now limiting us and tying us up. These regulatory chains need to be unshackled. Physicians need to be free to use whatever modality of treatment they believe is in the patients best interests. However the other side of the argument is that these are helpless patients and they are likely to be exploited by physicians offering stem cell therapy. We must however note that there are black sheep in every profession. That those who don't have values and principles are doing all manner of unprincipled

and unethical practices with conventional treatments also. On the other hand there are researchers who have been working in this field for many years both in the laboratory as well as clinically. They should be permitted to offer treatments they believe are safe and will benefit patients. Unless more physicians offer

these treatments there will always be a supply demand gap with the result that fly by night operators will enter the field to make money. Therefore freeing up the field will bring more transparency and accountability to this aspect of medical treatment.

That there is an ethical ground for offering stem cell therapy as a treatment option based on the Helsinki declaration:-

The Helsinki Declaration that has been discussed earlier in this chapter makes one thing very clear that for diseases for which there are no cures or the cures have been ineffective the physician is justified in using an unproven treatment if the physician believes that it will benefit the patient. This is the ethical bedrock on which we offer stem cell therapy as a form of treatment for neurological disorders for which there are no other treatments.

That there is enough published clinical evidence about the safety and efficacy of adult stem cells in neurological disorders and based on this evidence there is no need to keep on doing trials. In the section on clinical aspects we have mentioned in this book numerous studies that have clearly shown the safety and efficacy of adult stem cells in various neurological disorders. A question that remains unanswered is when does a treatment that is "unproven or experimental" become a treatment that is "proven or established". How many publications documenting safety and efficacy will it take to make that shift? Is a single publication enough, or are 10, 50 or 100 ok, or are multicentric international trials the only basis to make any treatment option an accepted form of treatment. Is it necessary to go on reinventing the wheel just to satisfy our intellectual considerations whilst millions of patients continue to suffer?

Ethical issues surrounding the stem cell research and practice are complicated. There is growing published evidence about safety and efficacy of stem cell therapy explains the ethics of offering it to patients with incurable disorders who otherwise have no other option than to endure the suffering.

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NeuroGen Brain & Spine Institute



The NeuroGen Brain & Spine Institute is an International center of excellence for Neurological disorders. Founded by Dr. Alok Sharma it is India's First dedicated Hospital for Stem Cell Therapy and Comprehensive Neurorehabilitation. Located adjacent to the Arabian sea on the scenic Palm beach road in Navi Mumbai, this center has a multidisciplinary team of expert and experienced medical professionals that provide holistic care using the latest technological advances in the world. It has treated over 6500 patients from 60 different countries. The care offered here is very professional yet very caring.

A separate pediatric neurorehabilitation facility and other play areas makes it very child friendly. The institute is very scientific and academic in its approach and to date has published 80 scientific papers in international and national journals. 14 books have also been published and chapters contributed to several international textbooks. NeuroGen also has many international tie ups with leading organizations from America and other countries for research and treatment collaborations. The institute is very quality conscious and has several certifications (1. ISO 9001:2015, 2. GLP & 3. GMP certification). Despite all the international partnerships and treatments offered to patients from all over the world the institute is very socially conscious and through the Stemcare foundation financially supports patients from the lower socioeconomic strata to be able to avail of the treatments that are needed. Its a policy of the institute that no patient should be deprived of any treatment due to financial reasons. NeuroGen doctors conduct free medical camps all over the country. Conferences, workshops and CME's are regularly conducted to impart knowledge to doctors, therapists as well as patient families. Cutting edge research, pioneering new treatments, the best medical professionals, comprehensive treatment facilities all under one roof and a caring holistic approach and make the NeuroGen Brain and Spine institute a unique and special facility for patients with Neurological problems.

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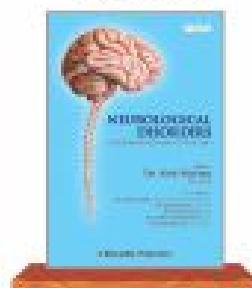
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