Stem Cell Therapy in Neurological Disorders

3rd Edition

Dr. Alok Sharma

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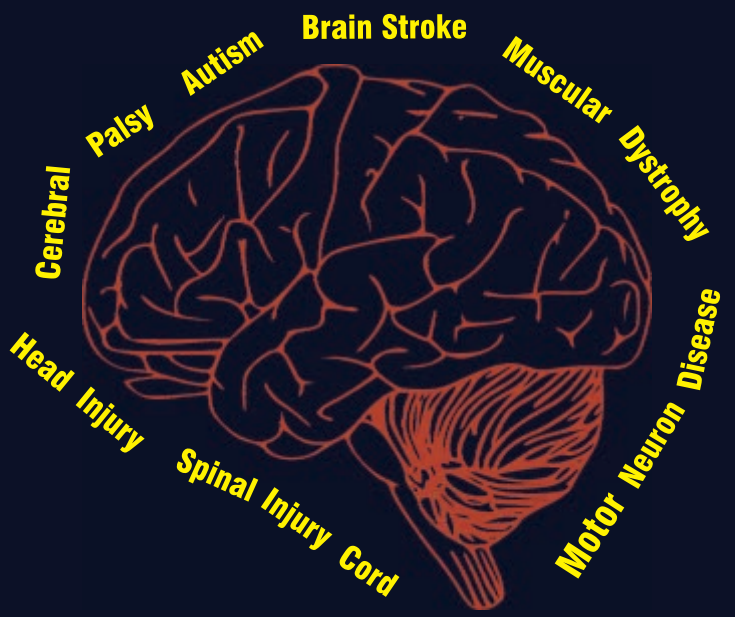

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Stem Cell Therapy
In Neurological Disorders

Third Edition

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Stem Cell Therapy in Neurological Disorders
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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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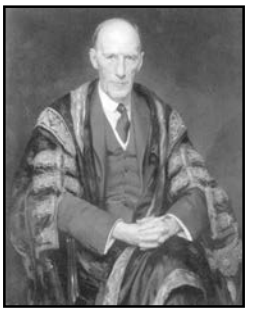
This Book is Dedicated to all our Patients



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 aliments 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 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 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 form 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 / practioners 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 practioners 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 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 aliments 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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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."

Contents

Scientific Publication by the Authors of the Book on Stem Cell Therapy

A) AUTISM:

1. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Anjana Nagrajan, Amruta Paranjape, Pooja Kulkarni, Akshata Shetty, Priti Mishra, Mrudula Kali, Hema Biju, Prerna Badhe. Autologous bone marrow mononuclear cell therapy for autism – an open label proof of concept study. Stem cell international. 2013 (2013), Article ID 623875, 13 pages.

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D) SPINAL CORD INJURY:

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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: Neurogeneration and Neurorestoratology

In 1926, prominent histologist and Nobel laureate Santiago Ramon y Cajal stated "Once the development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, ended, and immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree." (1) Until the 1990's, scientists and neurologists practiced their profession governed by this doctrine. This deep-routed and long-standing "dogma" of neuroscience has been overturned by the discovery of neurogenesis and neural stem cells (NSCs) in the adult central nervous system (CNS).

Regenerative medicine is an emerging field of modern medicine, focusing on restoration, repair and replacement of damaged tissues by a safe and effective administration of living cells in solitude or in combination with specially designed materials (2). It has opened up new avenues of therapeutic strategies for multiple disorders with no definitive treatment or cure available, such as neurological disorders (spinal cord injury, autism, cerebral palsy, brain stroke, muscular dystrophy, traumatic brain injury, motor neuron disease, etc.), 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.

Stem cells are the building blocks of this field. These cells have the capability to multiply manifolds and convert or differentiate into any specialized cell types of the body. A variety of stem cells are now being used from diverse sources for regeneration. The potency and plasticity of stem cells depends on the source or origin. Embryonic

stem cells are the most potent but are associated with ethical issues and side effects like formation of teratomas

In order to bypass the ethical and medical issues associated with embryonic and fetal stem cells, researchers and clinicians have researched and developed other sources of stem cells, such as haematopoietic and mesenchymal stem cells from the bone marrow and umbilical cord, stem cells from the adipose tissue, olfactory ensheathing, endometrium, neural stem cells, etc., which have varying potencies for differentiating into different cell types. The most popular cells are the adult stem cells which have a relatively better safety profile and sidesteps the ethical and moral issues. In principle, these cells can be procured from a patient and utilized for repair of damaged tissues.

The year 2006 marked a revolution in stem cell research, when Takahashi and Yamanaka demonstrated that it is possible to reprogram embryonic or adult mice skin cells by the use of Yamanakas factors, which can also be performed for human skin cells (3). Currently, efforts are being made towards the attempt of developing patient-specific induced pluripotent stem cells which will be free from any alterations or genomic instability (4).

Neurorestoration, as defined by International Association of Neurorestoration, is the concept which forms the basis for increased optimism in the medical community. It is a novel branch of neuroscience which studies and discusses the therapeutic strategies for neural regeneration, repair and replacement of the damaged elements of the central nervous system. The resultant processes like neuroplasticity, neuroprotection, neuromodulation, angiogenesis, immunomodulation are the principal components whose mechanisms are discussed in great depth (5).

The hope is that by using the plasticity of the nervous system and combining it with the regenerative potential of the stem cells it would be possible to evolve definitive treatments for degenerative and traumatic disorders of the nervous system.

This book is focused on recent advances in the field of neuroregeneration and neurorestoration. It is directed towards clinical applications of stem cell therapy for incurable neurological and neuromuscular disorders. It is an attempt to put forth information from various preclinical and clinical studies since stem cells have now reached from bench side to bed side.

Neuroprotection	A beneficial interaction that prevents or slows neurons from dying. A need for disease presymptomatic biomarker.
\Neurorestoration	A beneficial interaction that replaces dying or dead neuronal cells with viable cells. Acting during symptomatic phase.
Neurorescue	A beneficial interaction that rescues cells where neuronal cell death has already started. Acting during symptomatic phase

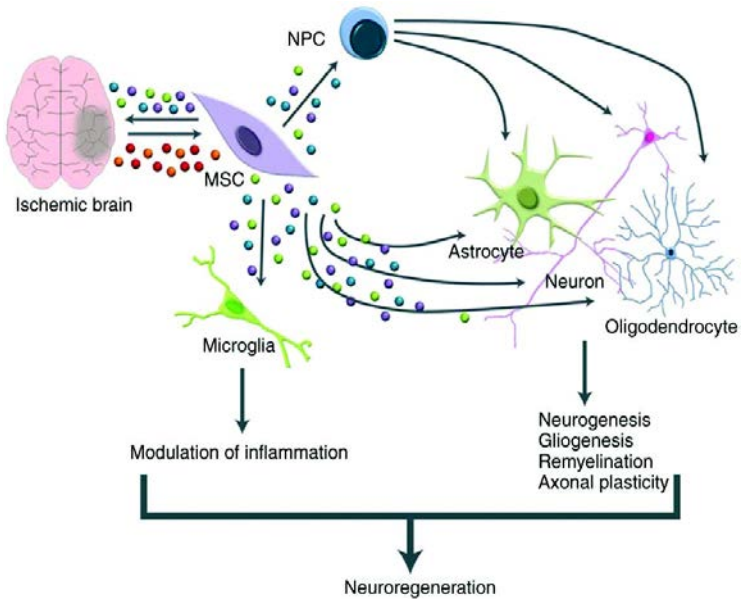

Figure 1: (Courtesy: van Velthoven, Cindy TJ, Annemieke Kavelaars, and Cobi J. Heijnen. "Mesenchymal stem cells as a treatment for neonatal ischemic brain damage." Pediatric research *71.4-2 (2012): 474-481.)*

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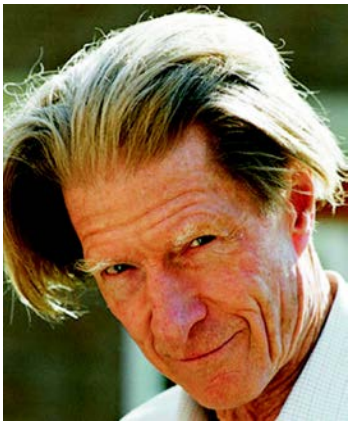
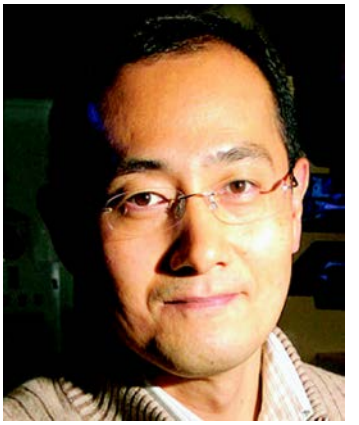
Nobel Prize Winners in Stem Cell Research

2012



John B. Gurdon

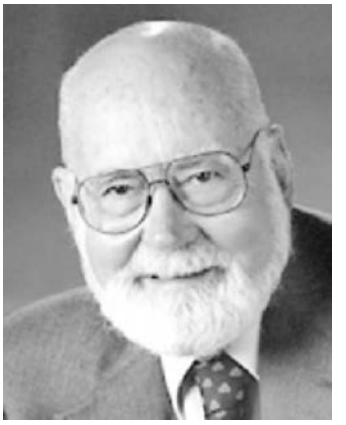


Shinya Yamanaka

2007

Sir Martin Evans

1990



Dr. E. Thomas

2

Historical Review : Evolution of Stem Cell Therapy

For centuries scientists have known that animals such as the starfish, newt, earthworm, various reptiles etc can regenerate missing parts of their bodies. 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 the 1950's, the identity of the powerful cells that allowed us to regenerate these tissues was first revealed when experiments with bone marrow established the existence of stem cells. This led to the use of bone marrow transplantation as a therapy which is now commonly used in medical practice. 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 create many of the bodies specialized cells. Once the medical potential of regeneration was recognized scientists turned to the embryo to identify similar cells since early human developmental studies had demonstrated that the cells of the embryo were capable of producing all the different types of calls in the body.

In the 1980's scientists began to extract embryonic cells from mice however it was in 1998 that scientists first isolated human embryonic cells. The demonstration and use of stem cells in the bone marrow in the 1950's and the isolation of embryonic stem cells in mice could well be considered pivotal moments in medical history and so very appropriately both were recognized with the prestigious Nobel prizes. (Dr. E. Thomas in 1990 and Sir Martin Evans in 2007).

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 modern developments 50 years ago with bone marrow transplantation to the recent development in the last 10 years when stem cells are being researched and used for treatment of many other diseases.

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. An interest in adult tissues fell, historically, within the realm of pathologists and thus tended to be considered in the context of disease, particularly cancer. 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 multipotent or unipotent.

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. Due to these widespread basic and clinical implications, it is of interest to put modern stem cell research into historical context.

Historical Review And Evolution of Stem Cell Therapy

Early history : Coining of the Term "Stem Cell"

''Stammzelle'' and Germline Development

The term stem cell appears in the scientific literature as early as 1868 in the works of the eminent German biologist Ernst Haeckel. Haeckel, a major supporter of Darwin's theory of evolution, drew a number of phylogenetic trees to represent the evolution of organisms by descent from common ancestors and called these trees "Stammbäume" (German for family trees or "stem trees"). In this context, Haeckel used the term "Stammzelle" (German for stem cell) to describe the ancestor unicellular organism from which he presumed, all multicellular organisms evolved and thereby, he also proposed that the fertilized egg also be called stem cell. Uses of the term stem cell referring to a distinct cell in the embryo capable of giving rise to more specialized cells can be found later in that century. (1)

As embryology evolved in the 19th century along with August Weismann's theory of the continuity of the germplasm (germ cells being different than somatic cells) became the focus of research and debate. Theodor Boveri while tracing the ascaris embryo concluded that the early germline cells maintained the full complement of chromatin so as to transmit the intact genetic material to the next generation, in support of

Weissman's theory. In 1892, Boveri explicitly took Haeckel's definition of stem cell as the fertilized egg one step further and proposed that cells along the germline lineage between the fertilized egg and committed germ cells be called stem cells. (2, 3)

In Hacker's early studies(in Crustacean Cyclops), the term stem cell referred to what we today call the germline lineage, primordial germ cells, and germline stem cells. Four years later, Edmund B. Wilson popularized the term stem cell in the English language by reviewing Häcker's and Boveri's work in his book 'The Cell in Development and Inheritance'. (4) Wilson's book was inspirational to a generation of turn-of-the century embryologists and geneticists, particularly in the United States. Given the wide readership and influence of Wilson's book, he is generally credited as having coined the term stem cell. (5) However, Wilson used the term stem cell in the same sense as in the earlier studies of Boveri and Häcker, that is, it referred to the unspecialized mother cell of the germline.

"Stammzelle" and Hematopoiesis

The term stem cell can be also be traced to very early publications of the hematopoietic field. As early as 1896, Pappenheim used stem cell to describe a precursor cell capable of giving rise to both red and white blood cells.

But the subject became hot, only around the time hematopoietic transplantation was getting popular, since research on the development and regeneration of the hematopoietic system raised the question of whether a common precursor of the various cell types of the blood existed. Due to limitations of the experimental methods available at the time, the debate about the existence of a common hematopoietic stem cell continued for several decades. Paul Elhrich (using staining techniques) was able to identify different white blood cell lineages, splitting investigators of hematopoiesis into two camps, one(dualists) who did not believe in the existence of a stem cell common to all hematopoietic lineages and the other (Unitarians) according to whom a cell existed that represented the common origin of erythrocytes, granulocytes, and lymphocytes. Various terms were used to describe the common precursor of the hematopoietic system, Alexander Maximow, Wera Dantschakoff, Ernst Neumann and others began to use the term stem cell to refer to the common precursor of the blood system after the turn of the century. However, definitive evidence was provided by the work of James Till, Ernest McCulloch, and others in the 1960s. (6-9)

However, still Maximow is often credited with coining the term way back in 1909

Modern history:

Hematopoietic Stem Cell Transplantation:

In the early 1900's, the first real stem cells were discovered when it was found that some cells generate blood cells. In the early 1900's physicians administered bone marrow by mouth to patients with anemia and leukemia. Although such therapy was unsuccessful, laboratory experiments eventually demonstrated that mice with defective

marrow could be restored to health with infusions into the blood stream of marrow taken from other mice. This caused physicians to speculate whether it was feasible to transplant bone marrow from one human to another (allogenic transplant). Among early attempts to do this, were several transplants carried out in France following a radiation accident in the late 1950's.

The use of stem cell medicine was first used in 1956 by Dr. E. Donnall Thomas, a bone marrow transplant specialist. He administered donor adult stem cells to a leukemia patient who went into complete remission. Dr. Thomas and Joseph E. Murray are co-winners of the 1990 Nobel Prize in Physiology of Medicine for their contribution to discoveries concerning cell and organ transplantation in the treatment of human diseases. Performing marrow transplants in humans was not attempted on a larger scale until a French medical researcher made a critical medical discovery about the human immune system. In 1958 Jean Dausset identified the first of many human histocompatibility antigens. A bone marrow transplant between identical twins guarantees complete HLA compatibility between donor and recipient. These were the first kinds of transplants in humans. It was not until the 1960's that physicians knew enough about HLA compatibility to perform transplants between siblings who were not identical twins. (13)

In the early 1960s, McCulloch and Till started a series of experiments that involved injecting bone marrow cells into irradiated mice. They cemented their stem cell theory and in 1963 published their results in Nature. Forty years later, they were honored with 2005 Albert Lasker Award for Basic Medical Research an award often referred to as America's Nobel.

In 1973, a team of physicians performed the first unrelated bone marrow transplant. It required 7 transplants to be successful. In 1984, Congress passed the National Organ Transplant Act, which among other things, included language to evaluate unrelated marrow transplantation and the feasibility of establishing a national donor registry. This led ultimately to National Marrow Donor Program (NDWP), a separate non-profit organization that took over the administration of the database needed for donors in 1990. (14) The 1990's saw rapid expansion and success of the bone marrow program with more than 16,000 transplants to date for the treatment of immunodeficiencies and leukemia. Adult stem cells also have shown great promise in other areas. These cells have shown the potential to form many different kinds of cell types and tissues, including functional hepatocyte-like (liver) cells. Such cells might be useful in repairing organs ravaged by diseases.

Cord blood stem cells have been used in the treatment of blood cancers and/or blood diseases since 1988. That same year, Elaine Gluckman replaced allogenic cord blood for a bone marrow transplant in order to treat Fanconi Anemia, a rare recessive blood disorder. The child remained completely disease free. In 2001, treatment protocols were developed which permitted the removal of white blood cells from the umbilical cord, making the treatment safe with no risk of Graft-Versus-Host disease.

Recent history

The discovery of embryonic stem cells opened up a new era in the use of stem cells. Basic and experimental work showing that these cells could be useful in the possible treatment of many incurable conditions resulted in researchers and clinicians now looking at stem cells in completely new way. 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.

Embryonic Stem Cells:

In 1964, researchers isolated a single type of cell from a teratocarcinoma, a tumor now known to be derived from a germ cell. These cells isolated from the teratocarcinoma replicated and grew in cell culture as a stem cell and are now known as embryonic carcinoma (EC) cells. Although similarities in morphology and differentiating potential (pluripotency) led to the use of EC cells as the in vitro model for early mouse development, EC cells harbor genetic mutations and often abnormal karyotypes that accumulated during the development of the teratocarcinoma. These genetic aberrations further emphasized the need to be able to culture pluripotent cells directly from the inner cell mass.

In 1981, embryonic stem cells (ES cells) were independently first derived from mouse embryos by two groups, Martin Evans and Matthew Kaufman from the Department of Genetics, University of Cambridge published first in July, revealing a new technique for culturing the mouse embryos in the uterus to allow for an increase in cell number, allowing for the derivation of ES cells from these embryos. Gail R. Martin, from the Department of Anatomy, University of California, San Francisco, published her paper in December and coined the term "Embryonic Stem Cell". She showed that embryos could be cultured in vitro and that ES cells could be derived from these embryos.

In 1998, at the University of Wisconsin, James Thompson isolated the first embryonic stem cells from a blastocyst of a five day old in vitro fertilized egg. This discovery provoked a multitude of scientific studies, research documents, and heated debates over the ethical issues surrounding embryo destruction for medical purposes. In the same year, John Gearhart, Johns Hopkins University, derived germ cells from cells in fetal gonadal tissue (primordial germ cells). Pluripotent stem cell "lines" were developed from both sources. The blastocysts used for human stem cell research typically came from in vitro fertilization (IVF) procedures. (10-12).

McDonald J W et al. in a seminal paper showed that transplanted neural differentiated mouse embryonic stem cells into a injured rat spinal cord after traumatic injury home onto the site and differentiate into astrocytes, oligodendrocytes and neurons, and migrated as far as 8 mm away from the lesion edge. (13) This lead to an explosion of new thoughts and avenues for research into possible application of this newfound development, especially into treatment of spinal cord injury and other neurological disorders and papers.

However, thereafter, the course of embryonic stem cell research has been greatly influenced by the political decision of President George W. Bush 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, putting a virtual halt on any further derivation of human stem cell lines and research. This ruling has lead to a setback of almost a decade in the field of stem cell research and therapy. Hence, is construed to be a historical decision in the field of regenerative medicine. Following this landmark, stem cell research in the US and UK slowed down considerably. President B. Obama in 2009 reversed this decision, clearing the way again for the stem cell research to progress again in the US.

The onus of taking this ahead was shouldered by other European nations, such as Russia, Germany, Portugal, Spain, to name a few, where laws are less strict and the general opinion is in favour of stem cell research.

More interestingly, the scenario shifted to the Asian nations, especially China, Korea and India, since public as well private support in terms of funding also seems to be growing along with a economic shift toward globalization.

In fact, China is one country which is pursuing the field most aggressively. In China, research on both ESCs and adult stem cells is supported by governmental funds. Stem cell research fits the Chinese Ministry of Science and Technology's ambitious plans to vault the country to the top of the research ranks. China has pumped money into this area through multiple sources: cities, provincial governments and two special national research initiatives (863 and 973 plans). Though, The Chinese government allows research on human embryos and cloning to continue for therapeutic purposes but reproductive cloning is strictly not allowed, as per Ethical Guidelines for Research on Human Embryonic Stem Cells were enacted by the Ministry of Science and Technology and the Ministry of Health of China.

The beginnings of stem cell research in China may be traced back to 1963, 34 years before Dolly the sheep was introduced to the world, when the late embryologist Dizhou Tong transferred the DNA from a cell of a male Asian carp to an egg of a female Asian carp, and produced the world's first cloned fish (Tong et al.1963). Tong's achievements were not acknowledged, partly because his work was published in a Chinese journal, Acta Zoologica Sinica, which did not have an English-language abstract, a common problem in non-Western scientific periodicals.

The first human embryonic stem cell line was established in China, way back in 2002 and researchers in Sheng of the Shanghai Second Medical University had reprogrammed human cells by fusing them with rabbit eggs emptied of their genetic material in 2003. A lot of work on derivation and differentiation of hESCs has happened in the ongoing years.

However, keeping in sync with the global reservations on ethical issues of these cells, China also has taken a lead in exploring various sources of adult pluripotent stem cells. Researchers led by Zhao at the Chinese Academy of Medical Sciences

reported that a cell population derived from human foetal bone marrow which not only had osteogenic, adipogenic and endothelial lineages, but also hepatocyte-like, neural and erythroid cells at the single-cell level . The most significant achievements made in China can be recognised by the quick transfer of the basic research to clinical application. Lot of work on use of bone marrow stem cells in myocardial infarction, liver failure, diabetes, spinal cord injury is being actively pursued in China. Institutes taking a lead are the Chinese Academy of Medical Sciences and Peking Union Medical College. (14)

Similarly, In India, the political and legal guidelines in India have always favoured research on stem cells - whether using embryonic or adult stem cells. Keeping in mind the potential therapeutic applications, both basic and translational research are being promoted by the various government departments, ministries, private research institutions and R&D companies in various public research institutions, hospitals and private industry.

An increasing number of publications on stem cell research and therapy (from 2003 till 2015) along with increasing private companies, non-profit organizations and government funded hospitals and institutes participation in this field (mainly focused on adult stem cells, mesenchymal stem cells and cord blood banking) shows the shifting of the stem cell hub to the Indian subcontinent.(15)

Inspite of the controversy associated with Woo-Suk Hwang, Korea continues to concentrate on human embryonic stem cell research and somatic cell nuclear transfer technologies. Before this incidence, Korea was almost on the verge of becoming the "world stem cell hub" under the leadership of Woo-Suk Hwang. Though a setback in the respect has been suffered, however, government policies continue to favour this research and technology.

Japan, too, has a long tradition of stem cell research, with many of the important discoveries in the study of hematopoietic stem cells being made by Japanese researchers (16)

With the background of stem cell research and a strong developmental biology capability, the Japanese government had started to invest a substantial amount of money to research on regenerative medicine, which includes stem cell research, in the beginning of the 21st century. One notable result is the establishment of the Riken Center for Developmental Biology (CDB) in Kobe.

Currently, the focus is primarily on human iPS (induced pluripotent stem cells), especially following the publication of the human iPS cell paper in 2007 by ShinyaYamanaka and his team at Kyoto University. (15)

As the field evolved, with ethical issues being raised regarding the morality of embryonic stem cells source, researchers began to explore other sources of pluripotent stem cells. The potency of other adult stem cells, especially hematopoietic stem cells began to be understood. In 2002, Catherine Verfaillie at the University of Minnesota proved that CD34+ stem cells from bone marrow could repopulate every single cell

in a developing mouse. This study prompted more studies using adult stem cells to generate far more than just blood cells. It was proven that there are great potentials for adult stem cells to treat a wide range of blood diseases, cancers, degenerative diseases, and injuries.

In 2004, Duke University published data from a human study confirming the Verfaillie study. The study featured the heart treatment of a boy who received CD34+ stem cells derived from donated umbilical cord blood. Not only did the investigation show differentiation to neurons and other cell types, but also proved that cord blood stem cells:

- Migrate to the site of disease,
- Have the ability to differentiate into specialized heart cells,
- Engraft yielding clinical benefits. (17)

The field of stem cell research and therapy, thereby, has evolved and come a long way since 1868, when the term "stem cells" was coined. We are now looking toward using various different kinds of stem cells 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.

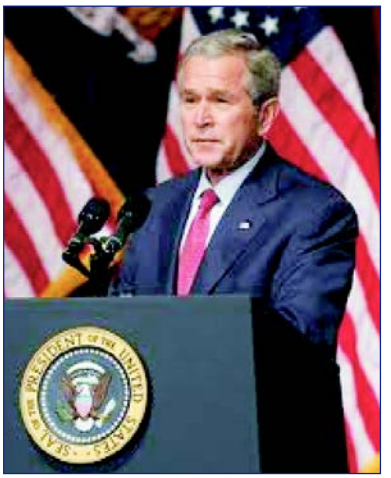
Dear Mr. President,

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 hadent continued, I wouldn't be here today. Once again, thank you very much Mr. President!

Sincerely,
Gavin Nore

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 lead 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 that 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."

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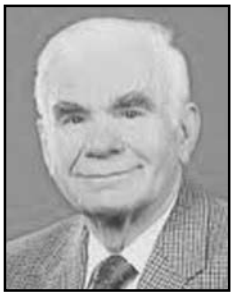
"Our enduring hope is invested in Biological research"



M. Gazi Yasargil

(Neurosurgeon of The Millenium)

3

Basics of Stem Cells : Types and Sources

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 clonote 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)

Though the above classification has evolved over decades, understanding of the potency of these cells are ever-changing. Many of these cells, which were earlier considered to be multipotent, have shown limited pluripotent properties Also, the external stimulation or manipulation can transdifferentiate monopotent/unipotent cells have shown that these classifications are fast becoming redundant.

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) Developmental studies in mouse revealed that the fertilized oocyte, the zygote, has the capacity to form the whole embryo which further divides progressively to give rise to an 8 cell

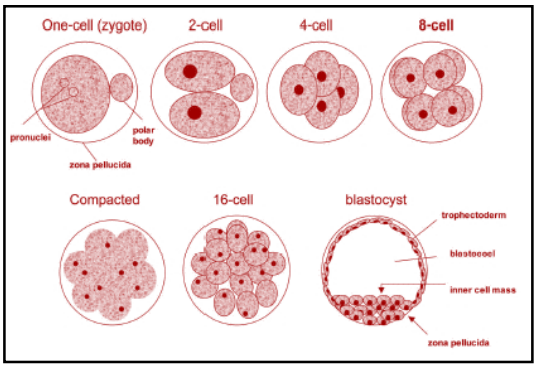


Figure 1: Development of a zygote to a blastocyst (from where embryonic stem cells are derived)

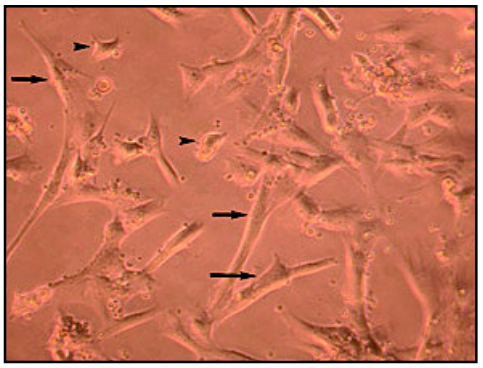


Figure 2 : Mesenchymal stem cells

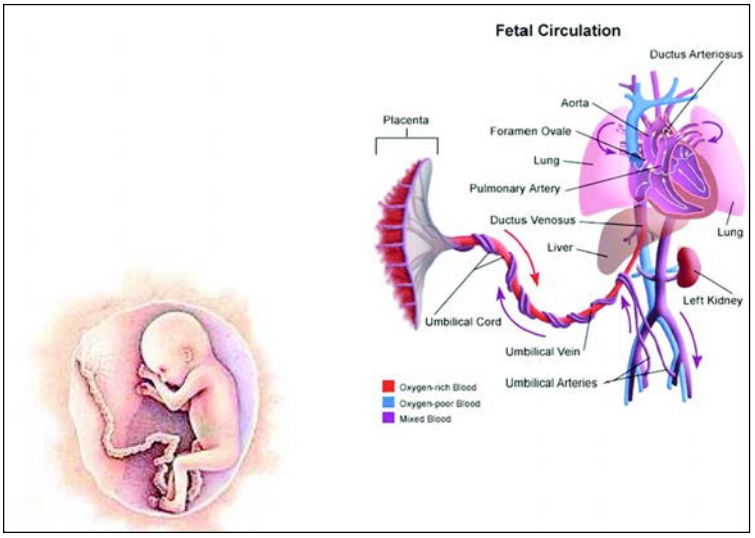


Figure 3 : The umbilical cord and placenta : a rich source of stem cells.

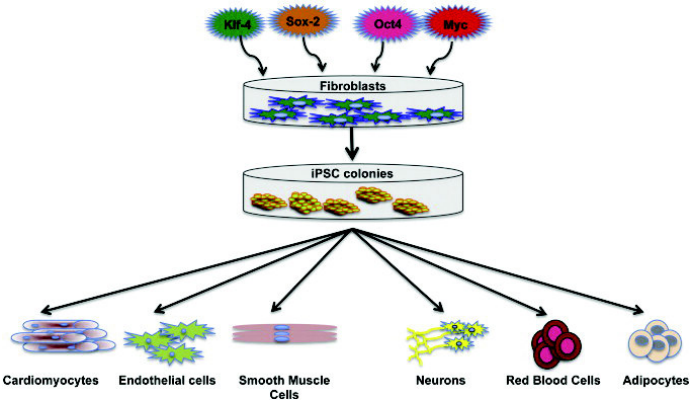


Figure 4: Induced Pluripotent Stem Cells
(Courtesy: Nsair, Ali, and W. Robb MacLellan. "Induced pluripotent stem cells for regenerative cardiovascular therapies and biomedical discovery." Advanced drug delivery reviews 63.4 (2011): 324-330.)

staged, 16 celled, 32 celled blastomere and then finally the blastocyst. The blastocyst is demarcated into the outer transparent trophoblast and the Inner cell mass (ICM) which is a 30-34 celled clump. (Figure 1) The ICM ultimately gives rise to the three germ layers and subsequently the whole embryo. Hence, the embryonic stem cells are derived from inner cell mass which has lost the "totipotency" of the zygote, but is now "pluripotent".

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.

However, even before the first embryonic stem cell line was derived in 1981, embryonal carcinoma cells derived from germline tumors called "teratocarcinomas" were widely studied(4) .

Embryonic Stem cell lines could be maintained in vitro without any apparent loss of differentiation potential. The "pluripotency" of these cells was demonstrated in vivo by the introduction of ES cells into blastocysts. The resulting mouse chimeras demonstrated that ES cells could contribute to all cell lineages including the germ line.

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.

Uses of Embryonic Stem Cells:

1. Embryonic stem cells as cellular models

   Gene-targeting techniques, along with transgenic mice have proven critical to the creation and evaluation of some models of human disease. Embryonic stem cell lines are useful mediums for genetic manipulation, understanding developmental processes and correction of genetic defects. (11)

2. Embryonic stem cells in pharmacology

   Stem cells also represent a dynamic system suitable to the identification of new molecular targets and the development of novel drugs, which can be tested in vitro for safety or to predict or anticipate potential toxicity in humans. (12) Human ES cell lines may, therefore, prove clinically relevant to the development of safer and more effective drugs for human diseases.

   The application of hES cells in pharmacology and embryotoxicology could have a direct impact on medical research, but to date, such an approach has primarily been used with mouse ES cells.

3. In stem cell based therapies:

   The in vitro developmental potential and the success of ES cells in animal models

demonstrate the principle of using hES-derived cells as a regenerative source for transplantation therapies of human diseases. Before transfer of ES-derived cells to humans can proceed, a number of experimental obstacles must be overcome. These include efficient derivation of human ES cells in the absence of mouse feeder cells, and an understanding of genetic and epigenetic changes that occur with in vitro cultivation. It will be necessary to purify defined cell lineages, perhaps following genetic manipulation, that are suitable for cell-based therapies. If manipulated, then it will be important to guard against karyotypic changes during passaging and preparation of genetically modified ES-derived cells. Once introduced into the tissue, the cells must function in a normal physiological way. Finally, assurances against the formation of ES cell-derived tumors and donor/recipient immunocompatibility are additional requirements of stem cell-based therapies. As pointed out, significant progress has been made in the isolation of defined cell lineages in mouse, and important advances have already been seen with hES cells. Before therapeutic application, any ES-based treatment must overcome obstacles of toxicity, immunological rejection, or tumor formation. (13, 14)

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 hemopoetic stem cells (HSCs). These cells exhibit rapid proliferative rate than those present in cord blood or adult bone marrow. As these cells share similar growth kinetics and expressing 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. Populations of non-hematopoetic stem cells (MSCs), present in the first trimester fetal blood, support hemopoesis and possess the ability to differentiate along multiple lineage. Both fetal HSCs and MSCs possess the properties of better homing and engraftment, with greater multipotentiality and better immunologic compliance. Fetal stem cells are less ethically litigious than embryonic stem cells, as it can be argued that FSCs are currently been obtained from terminated fetuses, thus using the tissue that would be discarded otherwise. Hemopoetic stem cells, mesenchymal stem cells, endothelial stem cells, epithelial stem cells and neural stem cells are different types of stem cells (18).

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 pediatric 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.

Umbilical cord blood contains circulating stem cells and the cellular contents of umbilical cord blood appear to be quite distinct from those of bone marrow and adult peripheral blood. The frequency of umbilical cord blood hematopoietic stem cells equalsor exceeds that of bone marrow and they are known to produce large colonies in vitro, have different growth factor requirements, have long telomeres and can be expanded in long term culture. Cord blood shows decreased graft versus host reaction compared with bone marrow, possibly due to high interleukin-10 levels produced by the cells and/or decreased expression of the beta-2-microglobulin. Cord blood stem cells have been shown to be multipotent by being able to differentiate into neurons and liver cells.

While most of the attention has been on cord blood stem cells and more specifically their storage for later use, there have also been reports that matrix cells (wharton's jelly) from the umbilical cord 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; have been propagated for long population doubling times; 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 bone marrow grafts. A 4 out of 6 match is sufficient for clinical use.
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 cropped up 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 it resident environment and also, may have transdifferentiating abilities.

Adult stem cell niches have been found in most organs of the human body, eg. bone marrow, adipose tissue, heart, liver, brain, muscles etc. The primary role of these adult stem cells is initiation of repair process in the organ following an injury. 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 to markers for characterization and isolation of the "stem cell population" from various organs (21).

The field of Regenerative medicine, which has opened up widely following the discovery of the embryonic stem cells, is now in search of the "almighty" pluripotent stem cell, following ethical, legal and medical questions raised against the ES cell research and therapeutic use.

The search has now been directed towards adult stem cell niches, which pose a non controversial and safe option for use in human subjects. However, the debate over its pluripotency is ongoing and the fields as well as the concept of adult stem cell plasticity have been extremely dynamic.

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. The excitement regarding HSCs diminished after it was found to have limited potency. However, increasingly, evidence is pouring in regarding the heterogenous population of cells having varying plasticity.

Potential Pluripotent Stem Cells candidates identified in adult tissues (especially, bone marrow)

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 "transdedifferentiation" 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 immunhistochemistry) 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.

Other Organs Where Potential Stem Cell Population Exists:

1. Neural stem cells:

Currently, neural stem cells are being explored as potential candidate for treating incurable neurological disorders.Neural stem cells (NSCs) have been isolated and characterized from various areas such as the adult CNS including the spinal cord. Adult-derived neural progenitor and stem cells have been transplanted in animal models, and shown functional engraftment, supporting their potential use for therapy. (29)

Site/origin of neural stem cells:

In the mammalian adult brain, the genesis of new neurons continues throughout life within two 3-layered cortical regions, the hippocampus and olfactory bulb (OB), where it is sustained by endogenous stem cells. 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 which represents a remnant of the embryonic germinal neuroepithelium, and persists throughout life as an active mitotic layer in the wall of the telencephalic lateral ventricles and along its rostral extension toward the olfactory bulb.(30) A complete turnover of the resident proliferating cell population occurs every 12 to 28 days in the SVZ; about 30,000 new neuronal precursors (neuroblasts) being produced every day and migrating to the OB. Two main cell types are found in the SVZ: migratory, proliferating neuroblasts and astrocytes. These cells reach the more superficial OB layers and terminally differentiate into granule and periglomerular neurons. Glial tubes are composed of a special type of astroglia that expresses the marker of mature

CNS astrocytes [glial fibrillary acidic protein (GFAP)] and also contain the cytoskeletal proteins vimentin and nestin. (31)

Astroglial tubes and NSCs do not coexist solely within the periventricular aspect of the SVZ but also within the rostral migratory stream that extends into the OB, with the former perhaps contributing to create an appropriate stem cell "niche" for the maintenance of NSCs all along the pathway. In recent years, neurogenesis was reported to occur in other regions of the adult brain under normal conditions, such as neocortex, amygdala, and substantia nigra. (32)

Alternative sources of neural stem cells/progenitor cells for cell therapy

(i) *Olfactory ensheathed cells (OECs) / Olfactory mucosa cells:* The nose contains neurons that send signals to the brain when triggered by odour molecules. The axons of these neurons are enveloped by OECs, a special type of neuronal support cells (glial cells) that guide the axons and support their elongation. The bundles travel from the nose to the brain's olfactory bulb, where these make connections with other neurons. Because olfactory tissue is exposed to the external environment (i.e., the air), it contains cells with considerable regeneration potential, including renewable neurons, progenitor/ stem cells, and OECs. OECs theoretically 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)

(ii) *Skin :* The skin contains a precursor capable of generating neural cell types was indicated by the finding that Merkel cells, neural sensory receptors found in the dermis, can be generated in adult skin. Skin derived Skin stem cells (SKPs) can generate both neural and mesodermal cell types and that most of the neural cells generated by SKPs have characteristics of peripheral neurons and Schwann cells (35)

(iii) *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)

(iv) *Schwann cells (SCs):* 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)

2) 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)

3) 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).

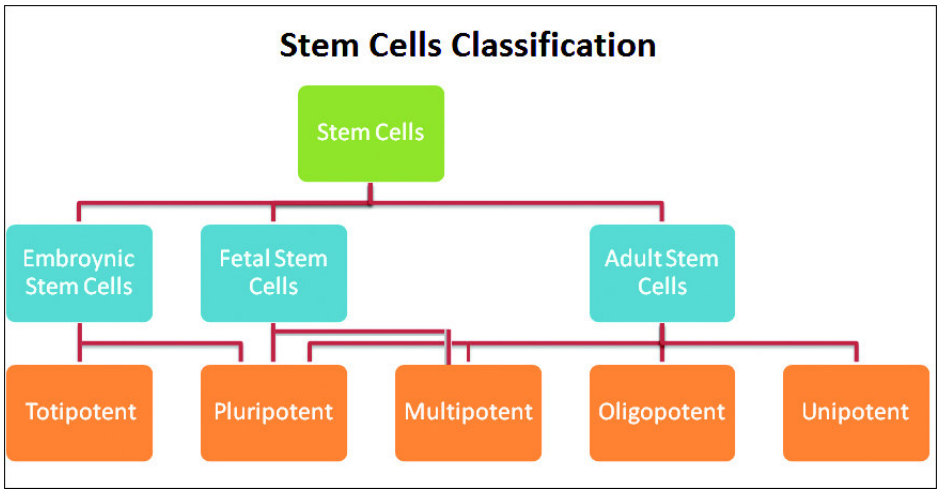
4) Muscular Stem Cells:

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).

The progression of Adult Stem Cells to Induced Pluripotent Stem Cells (IPSCs) is already a dynamic area of research in stem cell therapy. However, there 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 a good amount of 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).



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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

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. The different chemotactic factors direct the stem cells to the injured or damaged site through signaling pathways. Cell-based therapy could therefore potentially be used to treat a wide array of clinical conditions where cellular damage is the underlying pathology.

Plasticity, Pluripotency And Production

While pluripotency and plasticity are considered properties of early ESC, remarkable plasticity in differentiation potential of stem cells derived from adult tissues has been seen. (2).

In 1998, Ferrari et al. first reported that mouse bone-marrow-derived cells give rise to skeletal muscle cells when transplanted into damaged mouse muscle. (3)Thereafter, transplanted bone marrow cells were reported to generate a wide spectrum of different cell types, including hepatocytes, endothelial, myocardial , neuronal, and glial cells. HSC can produce cardiac myocytes and endothelial cells, functional hepatocytes and epithelial cells of the liver, gut, lung, and skin. (4-10) 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. (11-13)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.

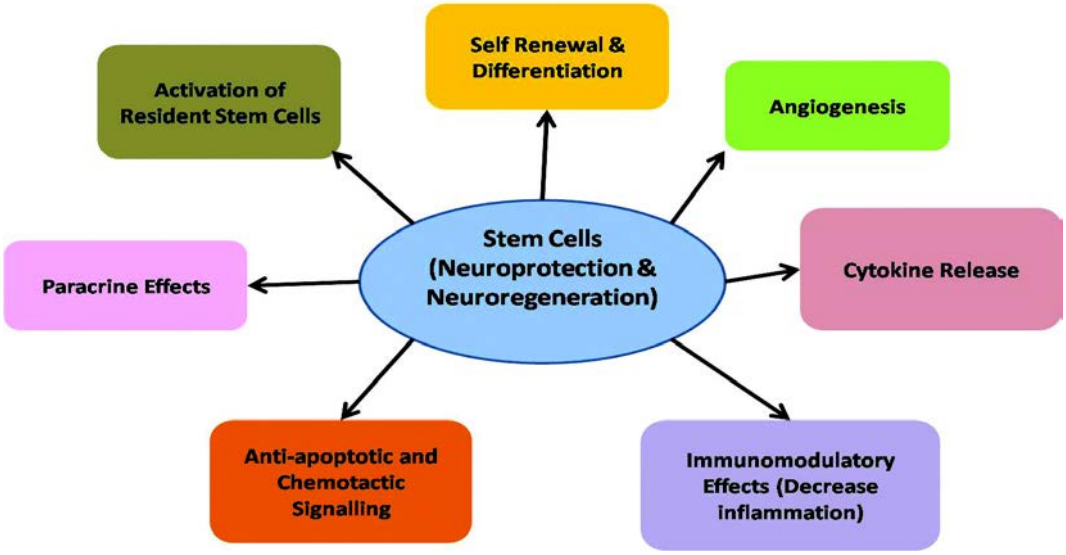

Figure 1: Mechanism of Action of Stem Cells

A critical observation of adult stem cell plasticity is that in order for plasticity to occur, cell injury is necessary(14), thus micro-environmental exposure to the products of injured cells may play a key role in determining the differentiated expression of marrow stem cells. (15)

The events underlying stem cells plasticity could relate to a multiple mechanisms such as dedifferentiation, trans-differentiation, epigenetic changes, and/or cell fusion. Rerouting of cell may result from dedifferentiation where cells revert to an earlier, more primitive phenotype characterized by alterations in gene expression pattern which confer an extended differentiation potential.. In trans-differentiation cells may differentiate from one cell type into another within the same tissue or develop into a completely different tissue without acquiring an intermediate recognizable, undifferentiated progenitor state. (18)

Recent studies clearly show that bone-marrow-derived cells can colonize a wide variety of tissues in the body of a host. (19, 20) Although derived from the embryonic mesoderm, bone marrow cells have also been shown to populate tissues of ectodermal and endodermal origin.(21) Both mesenchymal stem cells and bone marrow- derived cells can give rise to a wide array of non-hematopoietic cell types such as astrocytes and neurons in the brain, cardiac myocytes in models of infarction, skeletal muscle, and hepatocytes (22).

Non-hematopoietic cell populations from bone marrow and umbilical cord blood were enriched by in vitro culture and demonstrated to have the potential to differentiate into derivatives of all three germline layers with meso-, endo-, and ectodermal characteristics. (26,27) Known as multipotent adult progenitor cells

(MAPC), these cells contribute to most, if not all, somatic cell lineages, including brain, when injected into a mouse blastocyst . (28) Interestingly, while MAPC express Oct4, a transcription factor required for undifferentiated embryonic stem cells maintenance at levels approaching those of ESC, MAPC do not express two other transcription factors known to play a major role in ESC pluripotency, Nanog and Sox2. (29) This particular expression profile may contribute to the fact that the use of ESC, but not MAPC, carries the risk of generating tumors. Thus, MAPC are a promising source of autologous stem cells in regenerative medicine. Their low tumorigenicity, high regenerative plasticity, and optimal immunological compatibility are essential assets for the successful transplantation of MAPC-derived tissue-committed cells without immune-mediated rejection. (30)

The Paracrine Effect

Exploration of the various cellular processes occurring (both during normal physiology as well as after tissue injury) in the process of stem cell renewal and differentiation, suggests that transplantation of stem cells remodels and regenerates injured tissue, improves function, and protects tissue from further insult. These have also led to phase I and II clinical trials regarding stem cell treatment for a variety of surgical diseases. Despite these encouraging advances, the mechanism of this protection is still not well-characterized. As discussed earlier, it was initially hypothesized that immature stem cells differentiated into the phenotype of injured tissue, repopulated the diseased organ with healthy cells, and subsequently improved function. But, recent evidence suggest that stem cells may mediate their beneficial effects, at least in part, by paracrine mechanisms. (31)

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

Increased Angiogenesis

Stem cells produce local signaling molecules 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.(32, 33)

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

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.

Decreased Inflammation

Stem cells appear to attenuate infarct size and injury by modulating local inflammation. When transplanted into injured tissue, the stem cell faces a hostile, nutrient-deficient, inflammatory environment and may release substances which limit local inflammation in order to enhance its survival. Modulation of local tissue levels of pro-inflammatory cytokines by anti-inflammatory paracrine factors released by stem cells (such as IL-10 and TGF-?) is important in conferring improved outcome after stem cell therapy. (34)

Anti-Apoptotic and Chemotactic Signaling

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

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

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. (35)

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

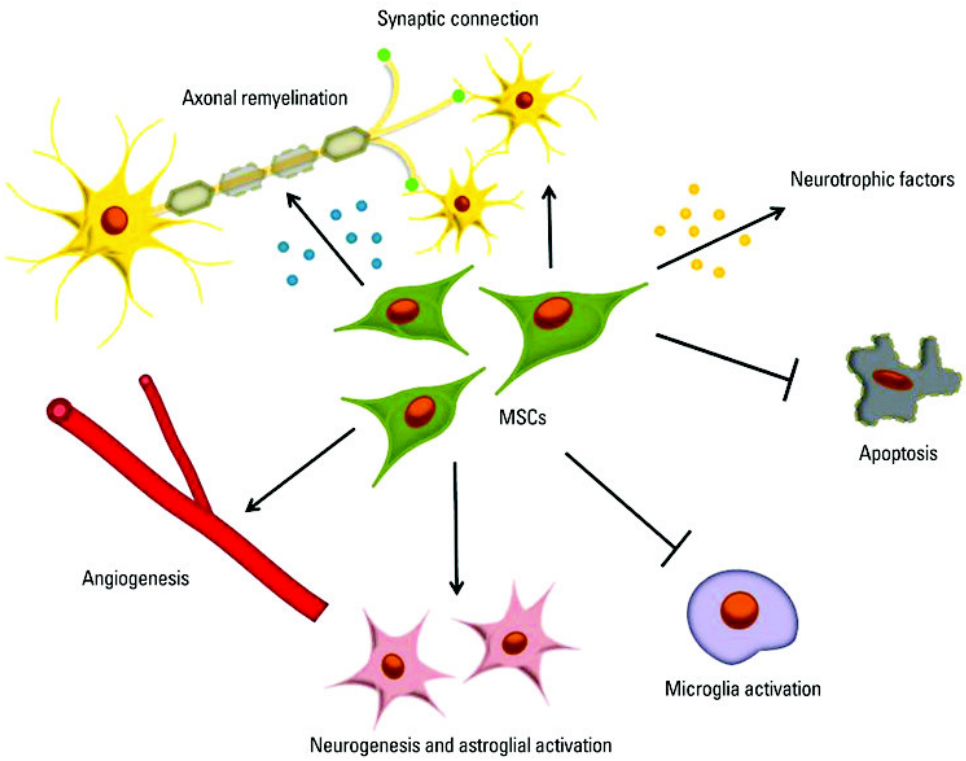

Figure 2: (Courtesy: Seo, Jung Hwa, and Sung-Rae Cho. "Neurorestoration induced by mesenchymal stem cells: potential therapeutic mechanisms for clinical trials."Yonsei medical journal 53.6 (2012): 1059-1067.)

cells, the extracellular matrix, and the infarct zone. Improved understanding of these paracrine mechanisms may allow earlier and more effective clinical therapies

Remyelination

Remyelination involves reinvesting demyelinated axons with new myelin sheaths. Previous attempts aimed at regenerating myelin-forming cells have been successful

but limited by the multifocal nature of the lesions and the inability to produce large numbers of myelin- producing cells in culture. Stem cell-based therapy can overcome these limitations to some extent and may prove useful in the future treatment of demyelinating diseases.

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.(36,37)

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

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 heteregenous 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 implantation. 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. Stem cells have been procured for therapeutic application primarily from haematopoietic sources such as the bone marrow, peripheral blood and umbilical cord, 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

Basically, the cells procured from any source are a mixture of various progenitor cells. The cells of interest for clinical application are separated from this mixture. Then either they are cultured before use or introduced in their original form without culturing. There are multiple methods of culturing using various growth factors, cytokines or biotechnologies which are specific to the cell type. This is a very diverse and vast area. Therefore, we have focused only on separation of commonly used cells.

Bone marrow harvesting

Open Method

Bone marrow blood (100-150 mL) aspirated from the iliac bone(generally either

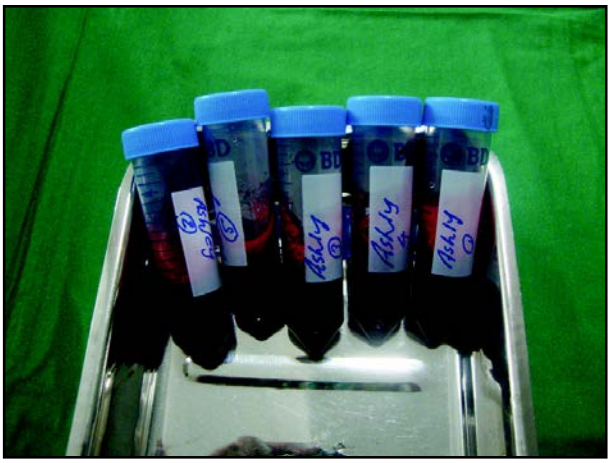

Figure 1: Aspirated bone marrow in tubes. Each tube contains about 20 ml bone marrow mixed with heparin.

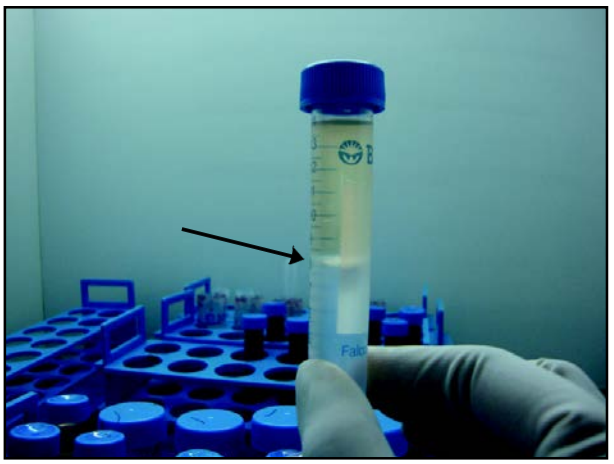

Figure 2: Buffy coat containing separated fraction of mononuclear concentrate (arrow indicating)

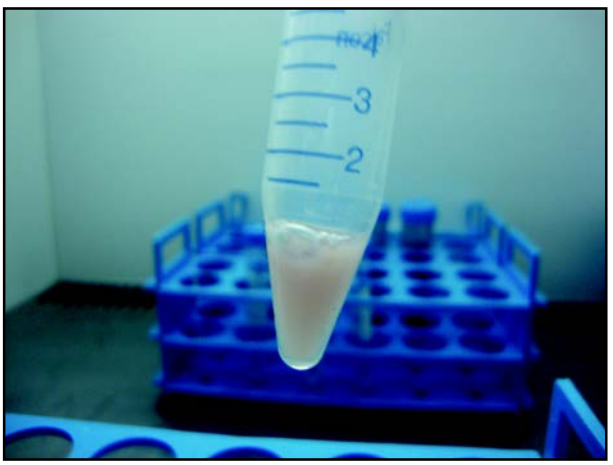

Figure 3 : Purified concentrate of mononuclear cells in solution (heterogenous mixture of stem cells - mainly hematopoietic)

anterior or posterior superior iliac spine) and is diluted in Hanks' balanced salt solution (HBSS) at a ratio of 1:1. After centrifugation of samples at 1000 x g for 30 min through a density gradient method (Ficoll-Paque Plus, 1.077 g/L; Amersham Biosciences,Piscataway, NJ), the mononuclear cell layer is recovered from the gradient interface and washed with HBSS. The cells are centrifuged at 900 xg for 15 min and resuspended in 1.8 mL of phosphate buffered saline (PBS) at a density of 1.1 x 106cells/ L. (1) (For further detailed methods please refer these references (2-4)

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. (5)

Peripheral blood

A short prototype is as follows:

Mobilization and harvesting of peripheral and bone marrow stem cells for AHSCT:

The most common method of collecting HSCs is by mobilization from the peripheral blood. Since negligible HSCs are detectable in the peripheral blood during the steady state, either a hematopoietic growth factor such as granulocyte colony-stimulating factor or chemotherapy (usually cyclophosphamide) with or without granulocyte colony-stimulating factor is necessary to mobilize HSCs into and subsequently collect HSCs from the blood. Hematopoietic growth factors used to mobilize HSCs also have immune-modulating effects and unlike malignancies may exacerbate disease depending on the growth factor.

Ex vivo hSC selection

Most mononuclear cells collected by peripheral blood apheresis/ leukaphereses by means of a Fenwall CS3000-Plus cell separator (Baxter, Fenwal Division, Deerfield, IL, USA) are immune cells such as lymphocytes and monocytes not HSCs. While the true identity of human HSCs remains elusive, either purified CD34 or CD133.

Hematolymphopoietic progenitor cells are sufficient for hematopoietic and immune reconstitution. In general, a minimum number of 2x106 CD34 cells per kilogram of recipient weight with the viability count of 98% will ensure engraftment. Hematopoietic stem cells may be positively selected or enriched exvivo using antibodies to CD34 or CD133 or purified by negative selection by using antibodies to remove lymphocytes. In practice, the most common method of purging lymphocytes is via CD34-positive selection using either the Miltenyi Clinimacs (Bergish Gladbach, Germany) or the Baxter Isolex (Deerfield, Ill) cell separator device. Whether enriching the graft for

CD34 + HSC is necessary or even superior to infusion of an unmanipulated graft remains unclear. CD34+ selection by removing lymphocytes is perhaps best viewed as another method of immune suppression. For an intense conditioning regimen, CD34+ selection may be unnecessary or even detrimental by increasing the risk of treatment related infection.

Cord blood processing

Currently, there are two types of processing in the cord blood market, manual and automated. Some companies choose to use manual processing systems while others have moved to automated processing systems.

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

Automated processing avoids airborne contamination by using a completely closed system and, most importantly, allowing for up to 99% recovery of necessary cells for transplantation.

Cord blood companies who price their cord blood banking service very low generally use manual processing systems, while major cord blood companies have moved to automated processing and manycharge between $1,600 - $2,100. Automated processing insures the ability to recover and save more of the important cells that will be used for transplants or transfusions, as well as the ability to keep out potential airborne contaminants. In addition, the possibility of human error is reduced. Unfortunately, these advancements make automated processing costly, and those costs are passed on to customers. (6)

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. (7)

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. (8) 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. (8,9) 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.(10) 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. (11)

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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 Cells 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

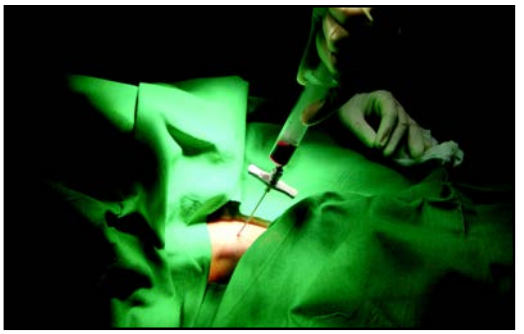


Figure 2: Bone marrow aspiration

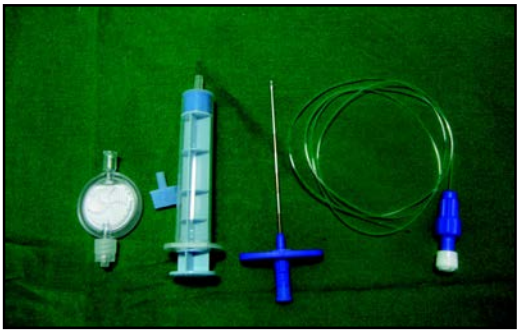


Figure 3: Epidural set (18 G) for intrathecal Inj.

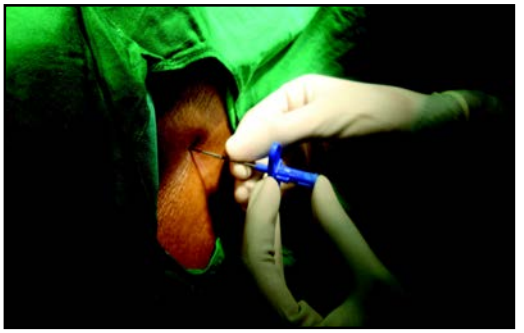


Figure 4: Intrathecal Injection step 1

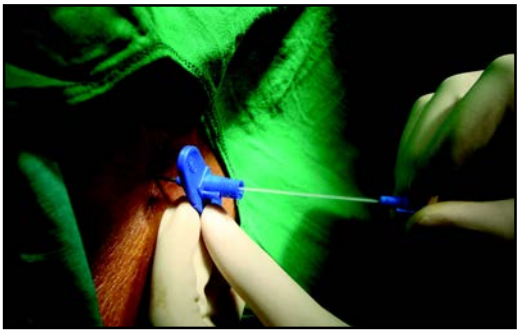


Figure 5: Intrathecal Injection step 2

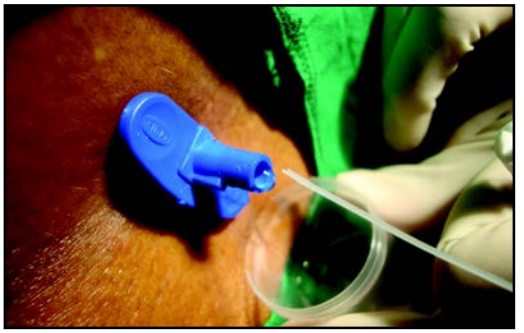


Figure 6 : Intrathecal Injection step 3

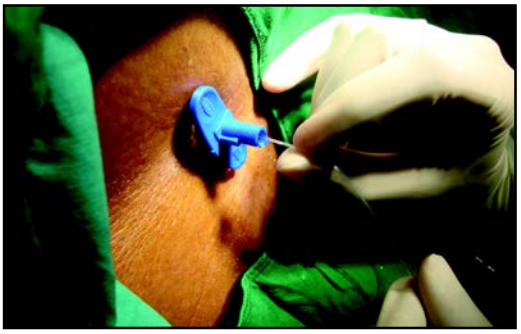


Figure 7: Intrathecal Injection step 4

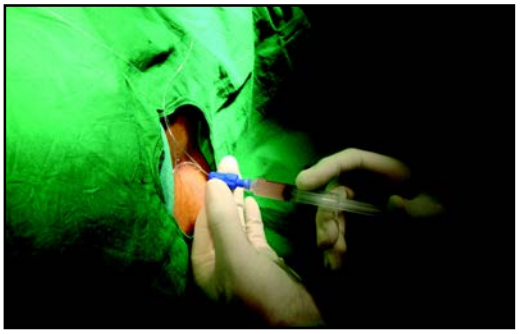
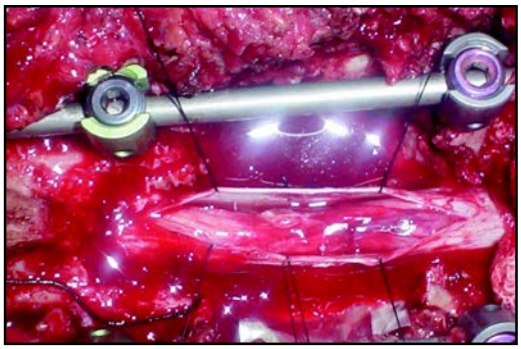


Figure 8: Intrathecal Injection - delivery of stem cells



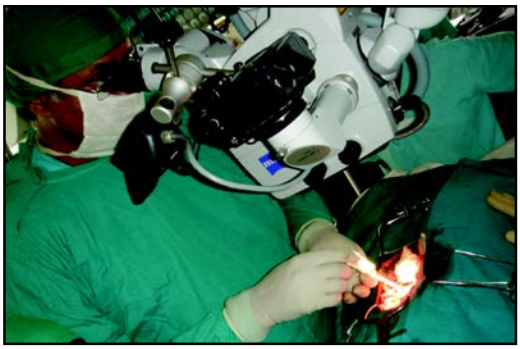


Figure 9 & 10 : Intraspinal transplantation of stem cells in a case of thoracic spinal cord injury.

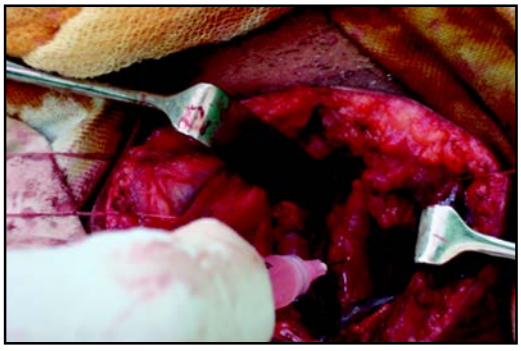


Figure 11: Intra-arterial direct injection of stem cells into the carotid artery following carotid endartrectomy

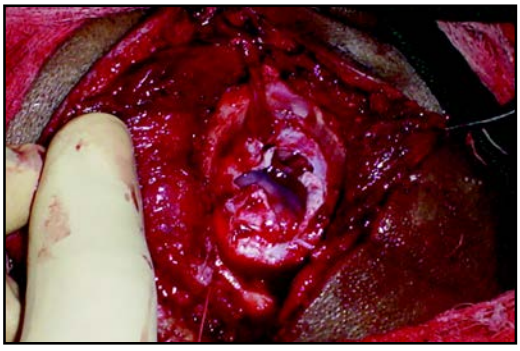


Figure 12: STA-MCA bypass

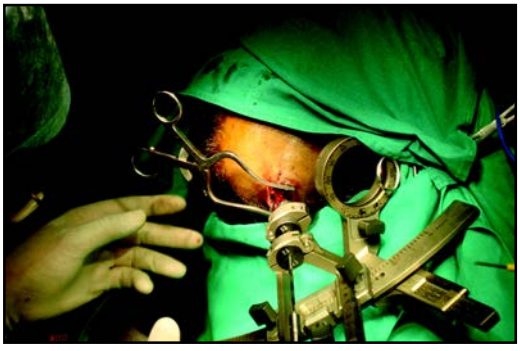


Figure 13: Leksell Stereotactic Frame for direct stem cell implantation into the brain.

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 µL.

Intra-arterial injection

Following revascularization surgery such as Carotid endartrectomy 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. Inspite of these

advantages, it is not the most effecient 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 delated cardiomyopathy. There may also be tachycardia, T-wave anomalies, ventricular arrythmias 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-convultants 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 genious, power and magic in it. Begin it now"

– Goethe

7

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

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

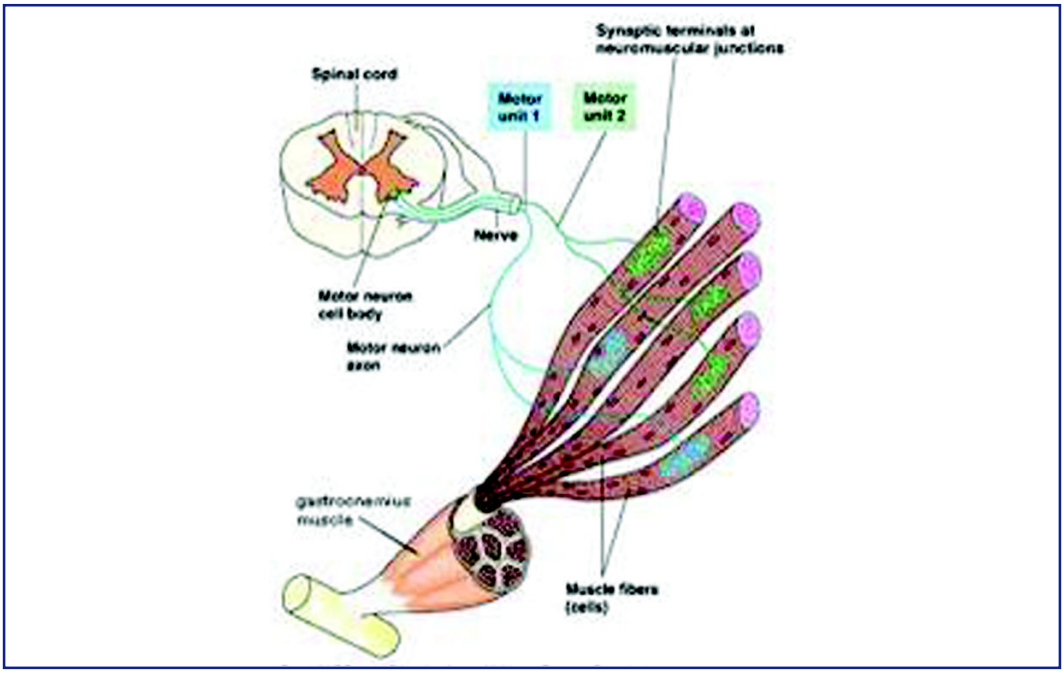


Figure 1: A Neuromuscular Junction

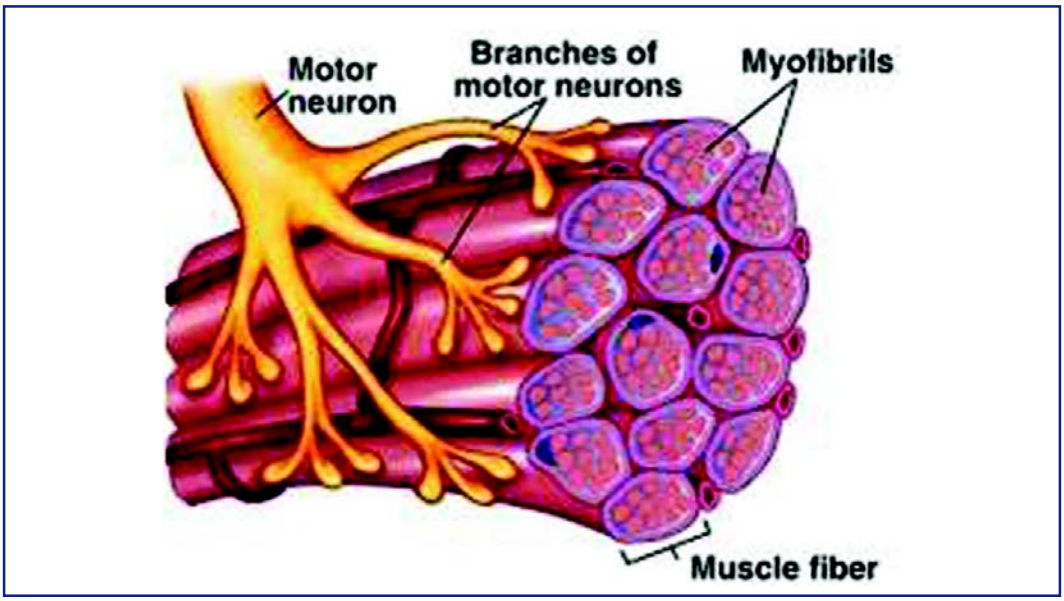


Figure 2 : The Motor Unit

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

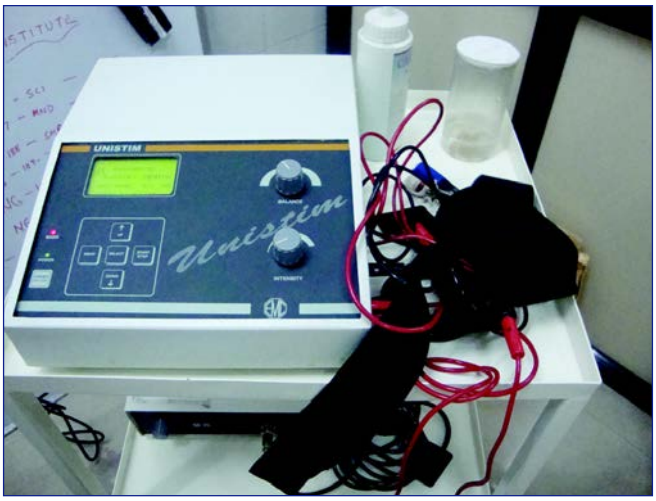


Figure 3 : Electrical stimulator used for stimulation and plotting of motor points.

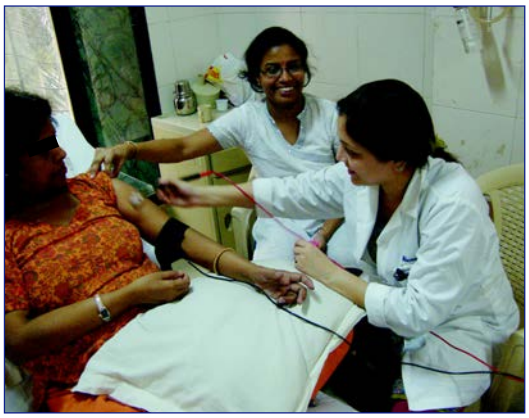


Figure 4 : Preparation of the patient for motor point plotting

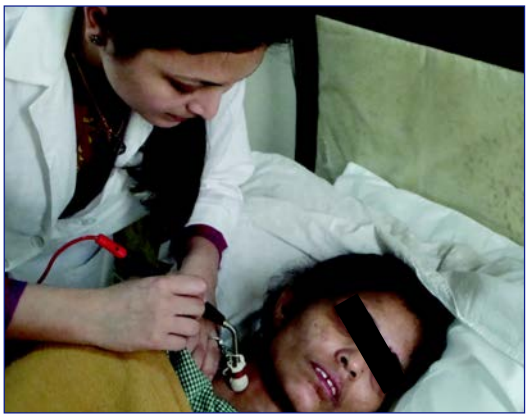


Figure 5 : Plotting of motor point (strenomastoid muscle)

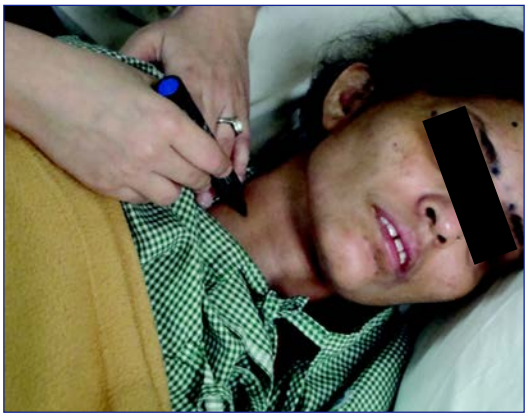


Figure 6 : Marking of sternomastoid muscle motor point.

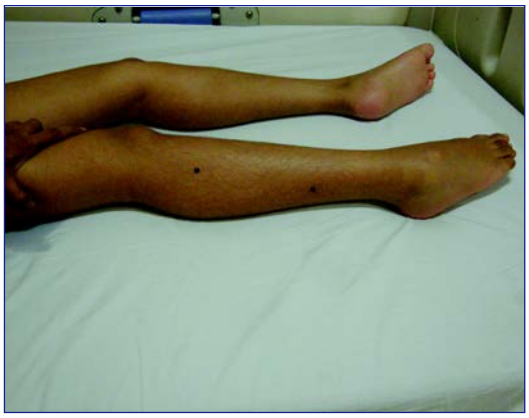


Figure 7 : Plotted motor points of tibialis anterior and peronei muscle

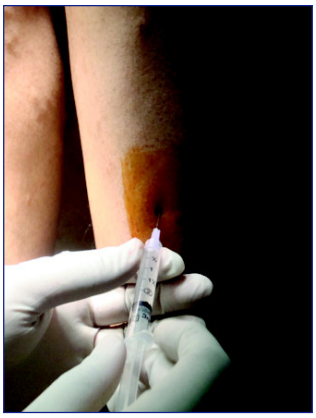


Figure 8 : Injection of stem cells in tibialis anterior muscle motor point.

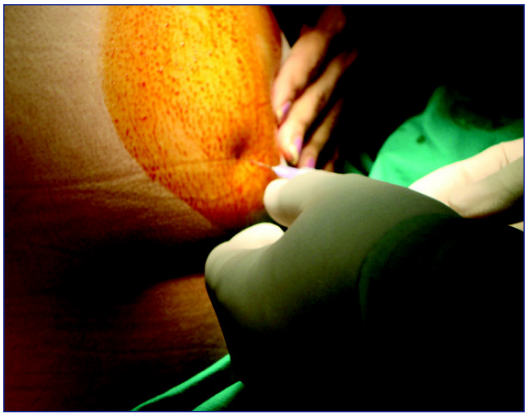


Figure 9 : Injection of stem cells in the glutei muscle motor point.

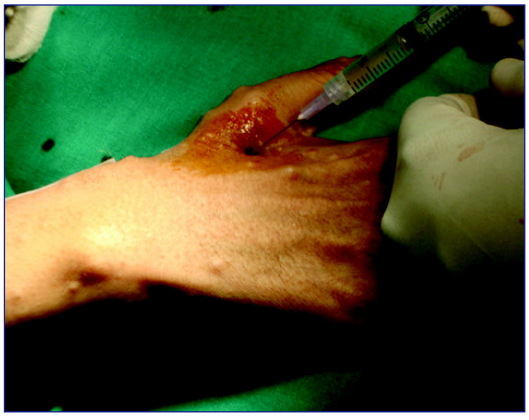


Figure 10 : Injection of stem cell injection in the adductor pollicis muscle motor point.

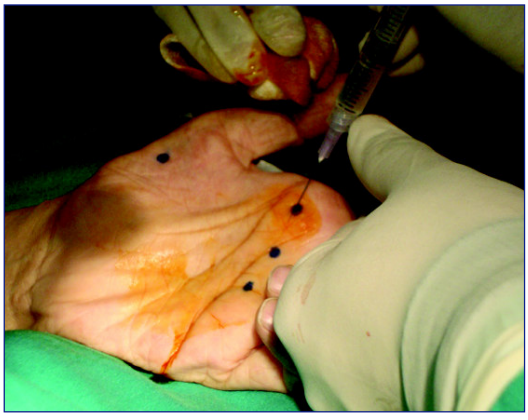


Figure 11 : Injection of stem cells in the lumbrical muscle motor points

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.

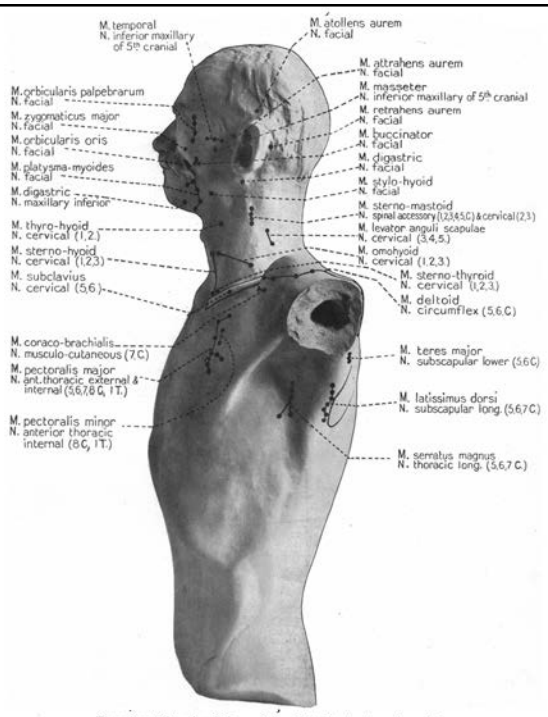
C) In trunk:

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

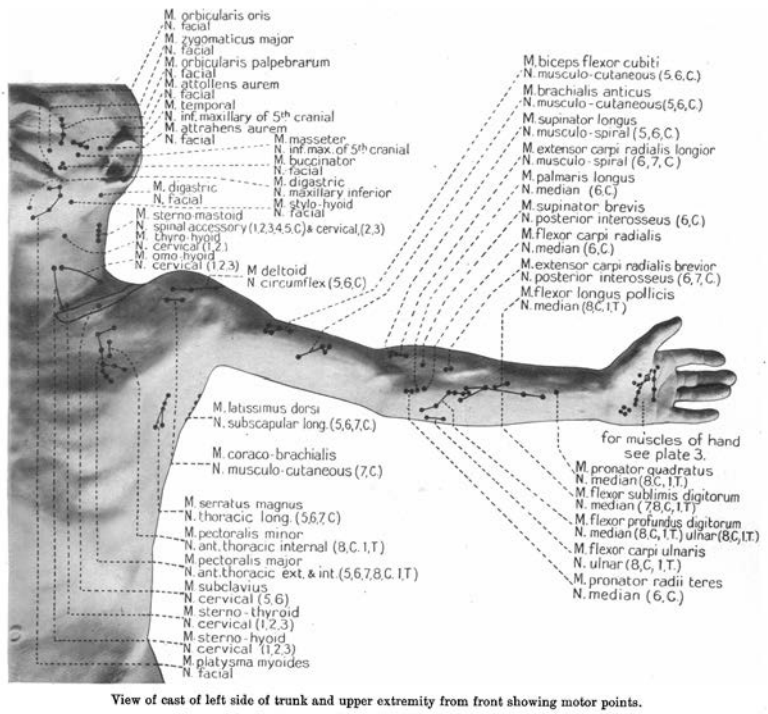
D) Facial Muscles:

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.

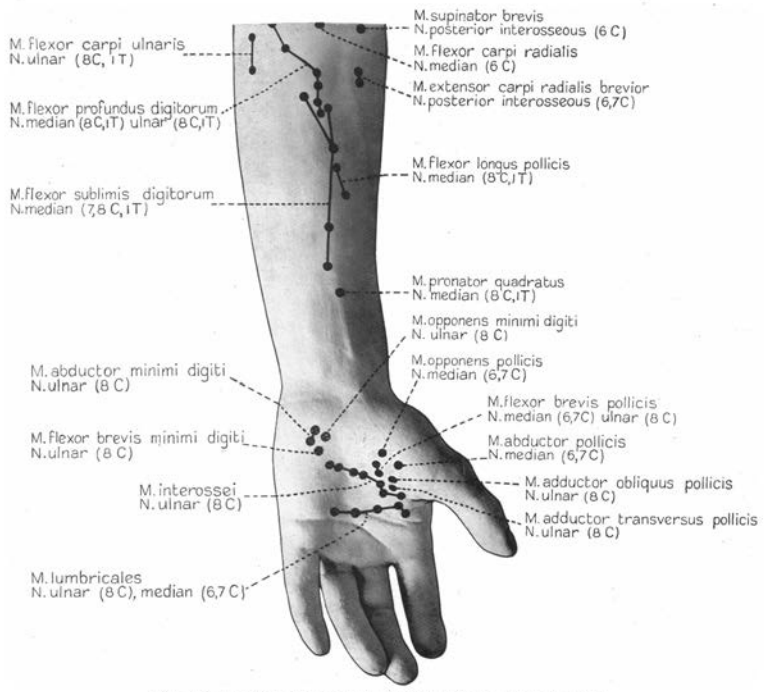
Intramuscular stem cells injection in motor points within the muscle is very specific transplantation. Also multiple motor points in chosen muscle group allows for a graded response. It facilitates increment in muscle strength depending on specific training & strengthening of individual injected muscles. An injection of stem cell in the motor end plate, can be identified in the neuromuscular system within few hours, although the onset of clinical effects is noticed as early as 72 hours post transplant, which varies from patient to patient.



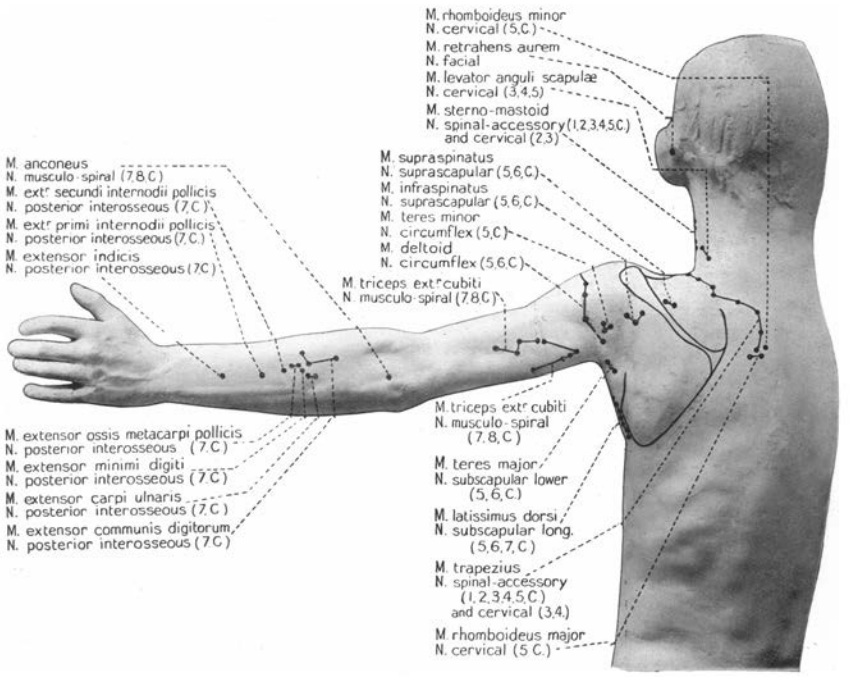
View of cast of trunk of living male from left side showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



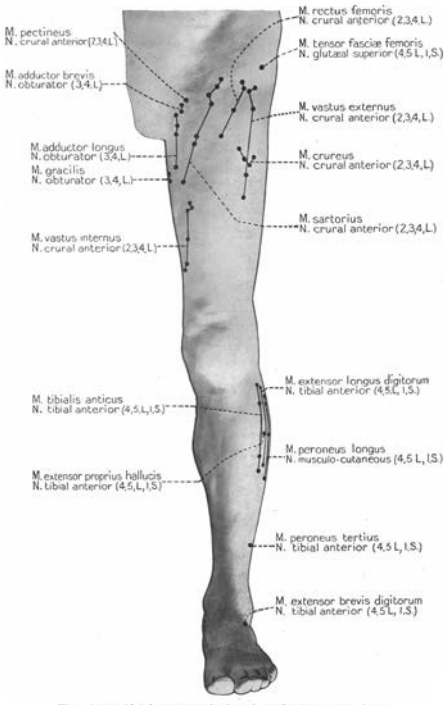
View of cast of left side of trunk and upper extremity from front showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



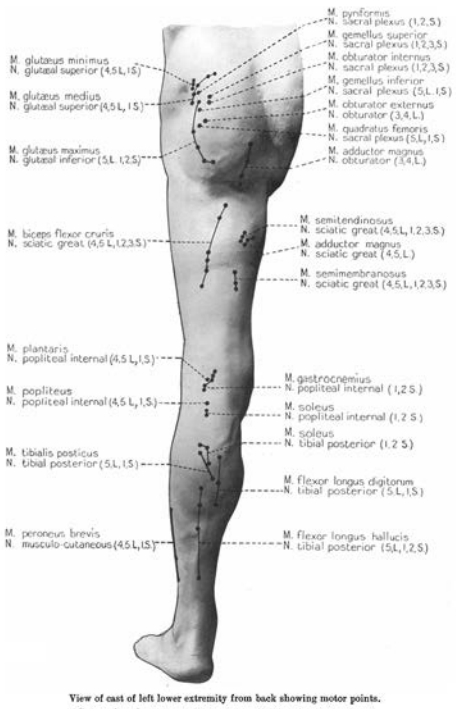
View of cast of left fore-arm and hand from front showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



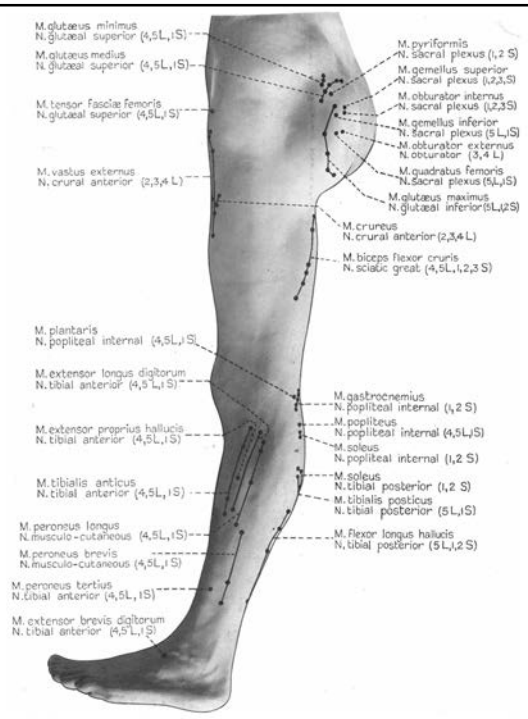
View of cast of left side of trunk and upper extremity from behind showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



View of cast of left lower extremity from front showing motor points.
(Prepared in the Anatomy Department of the University of Aberdeen.)



View of cast of left lower extremity from back showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



View of cast of left lower extremity from outside showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)

REFERENCE

1. Clayton'S Electrotherapy, Theory & Practice, Ninth edition 2004.Angela Forstet & Nigel Palatanga.
2. R.W Reid,M.D, Prof of Anatomy, University of Abeerdeen, Journal Of Anatomy, Vol LIV, part 4.

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

Autism spectrum disorders (ASD) are a range of neurodevelopmental disorders characterized by persistent deficits in social interaction, communication, language and behavior. The term "Spectrum" suggests a wide range of symptoms, skills, and levels of impairment or disability that children with ASD can have. These symptoms are usually showcased in the early developmental period of the child. Some children are mildly impaired, while others are severely disabled. A model of autism spectrum disorders (ASD) presented by Kevin et al suggests an early failure to develop the specialized functions of the social brain involved in processing of social information (1). They state that due to this early disruption, "an individual with autism must develop in a highly social world without the specialized neural systems that would ordinarily allow him or her to partake in the fabric of social life, which is woven from the thread of opportunity for social reciprocity and the tools of social engagement".

A report by the Surgeon General, states that autism has roots in both structural brain abnormalities and genetic predispositions. (2) The prevalence of autism has increased radically over the few decades for reasons not yet known. It is seen three to four times more in boys than girls. (3)

Pathophysiology of Autism

The exact etiology and pathophysiology of autism remains poorly understood. The numerous biochemical abnormalities detected in autism are oxidative stress; endoplasmic reticulum stress; mitochondrial dysfunction; decreased methylation; underproduction of glutathione; intestinal dysbiosis and toxic metal burden. (4) Brain hypoperfusion and immune dysfunctions have been postulated as the two main underlying pathologies in autism. (5,6) Research on animal brain to study the etiology of autism has shown that a major dysfunction of the autistic brain resides in neural

mechanisms of the structures in the medial temporal lobe, and, perhaps, more specifically the amygdaloid complex. Distinct patterns of memory losses and socioemotional abnormalities emerge as a result of extent of damage to the medial temporal lobe structure.

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. This suggests that this heterogeneity can be attributed to a previously unrecognized autism feature; "idiosyncratic distortions of the functional connectivity pattern relative to the typical, canonical template".

Autism, similar to other neurodevelopmental disorders, is incurable and requires chronic management. Currently, the only treatment options available for autism are behavioral, nutritional and medical intervention. These interventions facilitate development and learning, promoting socialization, self awareness, reducing maladaptive behaviors and educating and supporting families. (6)

Imaging in Autism

A limitation of MRI imaging of the brain is that it does not give an idea about the function of the brain tissue and in most cases of autism it does not reveal any significant abnormality. PET-CT scan can be utilized as a monitoring tool for autism, as it is more sensitive in analyzing the effects of cell therapy on the function of the brain as compared to MRI. It is a relatively non-invasive imaging technique that enables detection of aberrations in the brain based on changes in the metabolic activity at the molecular level.

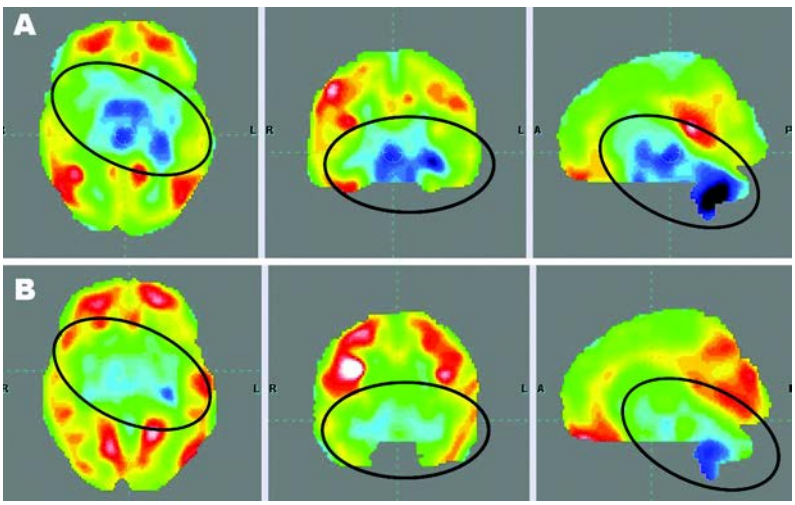


Figure 1: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image which have changed to green areas depicting normal metabolism.

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 (7) Hence, the diagnosis relies on the recognition of an array of behavioral symptoms that vary from case to case, heterogeneous and overlap with other childhood neuropsychiatric disorders. The treatment available does not address the core pathophysiology of autism but only manages the symptoms and associated medical conditions.

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. Reduced blood supply to specific areas of the brain (mesial temporal and cerebellum), could contribute to the cause of reduced functioning of that particular area. This along with overall imbalance of the activity of the brain is responsible for various symptoms of autism.

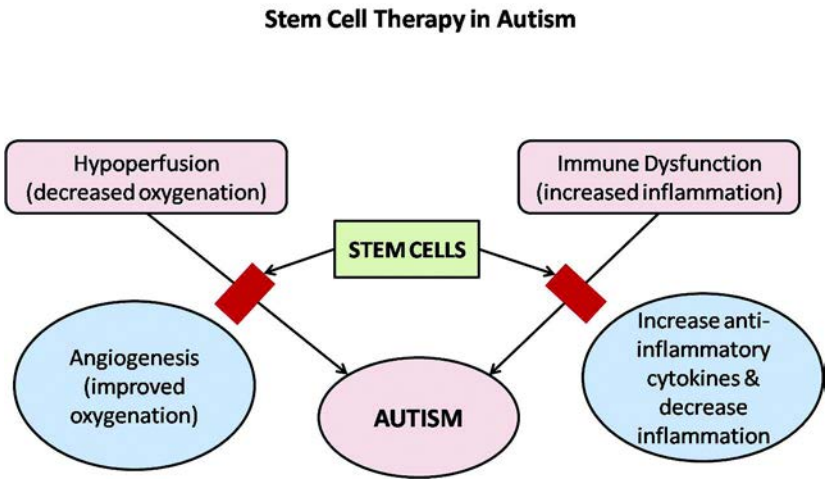


Figure 2: Stem Cell Therapy in Autism

In the past decade stem cell therapy has emerged as one of the treatment strategies for various neurological disorders. It has the therapeutic potential to repair the damaged neural tissue at molecular, structural and functional level. Recently, researchers worldwide have emphasized the potential of stem cells for the treatment of autism. (8) 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. (9) It also regulates the immune system,

balances inflammation further exhibiting beneficial clinical effects in patients with autism. 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. (10) Thus, stem cells are capable of suppressing the pathological immune responses as well as as well as stimulating neovascularisation. Cell therapy may also prove useful for the treatment of T cell defect associated with autism. (11) Overall, stem cells carry out functional restoration of specialized neural systems by neuroprotection, neural circuit reconstruction, neural plasticity, neurogenesis and immunomodulation.

Worldwide literature review

Not many preclinical studies have been conducted to study the benefits of stem cell therapy in autism as it is very challenging to study the effect of any intervention on animal models of autism due to lack of characteristic social interaction and language deficits found in autism. However, there are few clinical case reports (12,13) and studies (14,15) which are recently published and have shown beneficial effects of cellular therapy.

Sharma et al 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. (14). The next clinical study was published by Yong-Tao Lv et al where they studied use of human cord blood MNCs and MSCs. This study also showed a positive outcome. (15) In 2014, Bradstreet et al published their study using fetal stem cells in autism. The study was carried out on 45 children with autism. On follow up after 6 months and 12 months, there was a significant change in Autism Treatment Evaluation Checklist (ATEC) test and Aberrant Behavior Checklist (ABC) scores. Improvement was also seen in behavior, eye contact, appetite, etc.

Our Results

Published data

An open label proof of concept study of autologous bone marrow mononuclear cells (BMMNCs) intrathecal transplantation in 32 patients with autism followed by multidisciplinary therapies 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

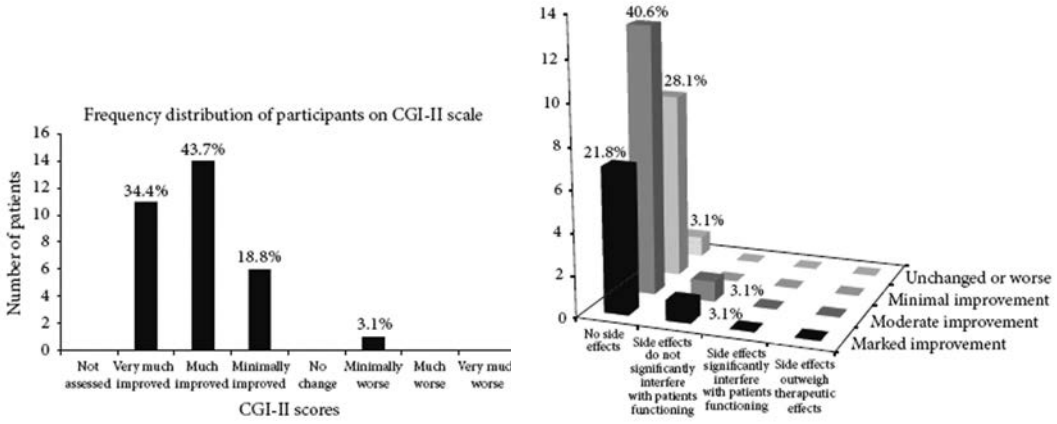


Figure 3

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.

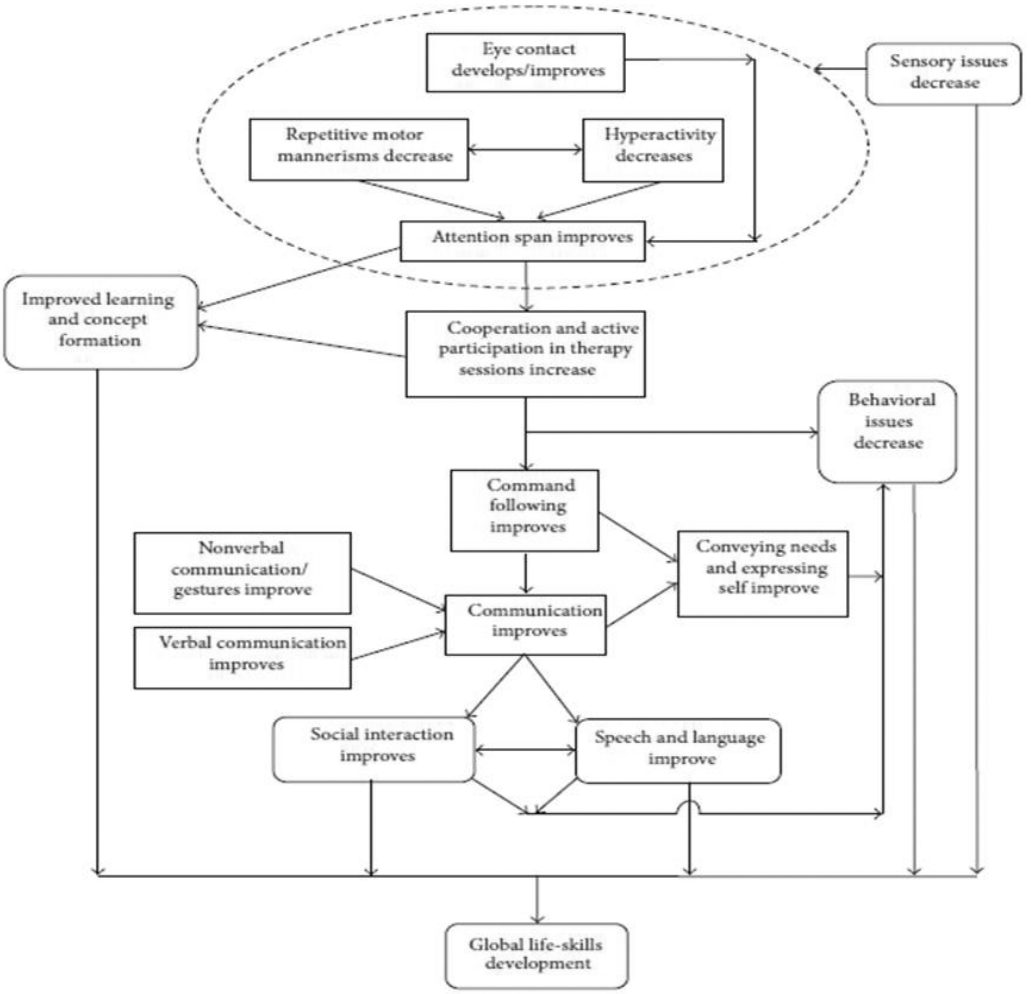


Figure 4: Schematic representation of clinical improvements after cellular therapy. This figure shows proposed theoretical outline of observed changes after cellular therapy.

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.

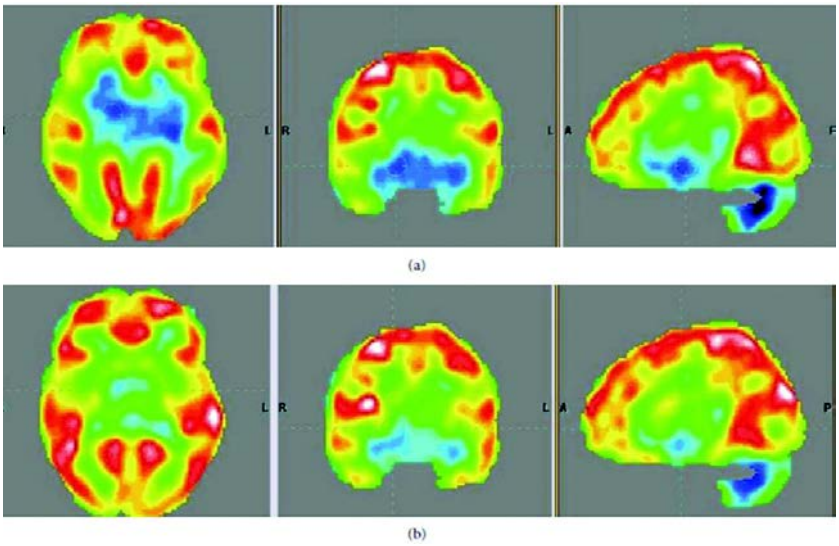


Figure 5: Findings in PET-CT scan before and after cellular therapy. (a) PET-CT scan before intervention showing reduced FDG uptake in the areas of frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus, andmesial temporal lobe. (b) PET-CT scan sixmonths after intervention comparison shows increased FDG uptake in the areas of frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus & mesial temporal lobe.

Case Reports

Neuropsychiatric Disorder Tackled by Innovative Cell Therapy-A Case Report in Autism.

An 11 year old boy diagnosed with autism was administered autologous bone marrow derived mononuclear cells intrathecally by Dr. Alok Sharma et al in 2014. A patient was assessed for a follow up period of eight months wherein his autistic features had reduced. This was supported by improvement in scores of CARS (31 to 25), ISAA (130 to 98), CGI-I (6 to 5) and FIM (104 to 110).

Autologous Bone Marrow Mononuclear Cells may be Explored as a Novel Potential Therapeutic Option for Autism.

A 14 year old male with autism with comorbid mental retardation, received intrathecal administration of autologous bone marrow mononuclear cells by Dr.Alok Sharma et al in 2013 . On regular follow up assessment of the patient over 18 months, there was significant clinical improvement in social relationship, communication and behavior. On outcome measure, ISAA score improved from 111 (Moderate autism) to 73 (Mild Autism). PET CT scans comparison of pre and post therapy showed balancing effect on brain metabolism.

Autlogous bone marrow - derived mononuclear transplantation in Rett syndrome

11 year old girl child with Rett syndrome underwent autologous bone marrow mononuclear cell transplantation. Cellular therapy was performed by Sharma et al . Post -transplantation , the patient had no side effects . In a period of one month, her

spasticity and rigidity had reduced. The results show that the treatment was safe, effective and resulted in significant improvements.

An Improved Case of Autism as Revealed by PET CT Scan in Patient Transplanted with Autologous Bone Marrow Derived Mononuclear Cells

In 2013, Sharma et al administered autologous bone marrow derived mononuclear cells intrathecally in a 14 yr old boy with severe autism to improve the quality of his life. At six months, follow up after therapy, his symptomatic improvement with shift on Childhood Autism Rating Scale (CARS) improved from 42.5 (Severely Autistic) to 23.5 (Non Autistic), which was also visualized as enhanced PET scan brain function.

Intrathecal Autologous Bone Marrow Mononuclear Cell Transplantation in a Case of Adult Autism

A 33 year old adult patient of autism was administered autologous bone marrow mononuclear cells (BMMNCs) intrathecally, twice with an interval of six months. On follow up at 3, 6 and 9 months post first intervention, no major or minor side effects were observed. At 6 and 9 months objective outcome measures of Indian Scale for Assessment of Autism (ISAA) and Clinical Global Impression (CGI) showed significant improvement. At the end of 9 months, on ISAA, the score improved from 94 to 64. The CGI showed improvement by change in severity of illness from 3 (Mildly ill) to 1 (Borderline mentally ill). Global improvement on CGI was scored 2 (much improved) with an efficacy index of 5 (moderate therapeutic effect). PET CT scan was repeated at 6 months which showed a balancing effect in the metabolism of affected areas. The changes observed on the PET CT scan correlated with clinical improvements

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 2 SDs then it is considered as abnormal brain metabolism. [15, 19]. 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.

Unpublished

150 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, 90% of patients with autism showed improvements while 10% did not show any change after intervention. 23.33% showed mild improvements, 30.66% showed moderate improvements and 36% 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.

Areas	Mean SUV (PRE)	Mean SUV (POST)	Correlating clinical functional improvements observed in the case
Frontal Lobe Left	6.86	5.85	initiation, planning, anticipation, organization, problem solving, emotions, attention
Frontal Lobe Right	6.92	5.67	
Cingulate and Paracingulate gyri Left	6.72	5.57	Social brain processing
Cingulate and Paracingulate gyri Right	6.62	5.39	
Temporal Left	6.84	5.44	Social Interaction, memory and categorizing objects
Temporal Right	6.95	5.85	
Mesial Temporal Left	4.96	4.01	Social brain processing
Mesial Temporal Right	5.34	4.48	
Middle Cingulate Left	7.23	5.88	emotion processing, learning and memory
Middle Cingulate Right	7.31	5.95	
Amygdala Left	4.7	3.97	emotions, memory, social interaction, behavior
Amygdala Right	4.85	4.18	
Hippocampus Left	4.49	3.77	social interaction
Hippocampus Right	4.58	3.97	
Para hippocampus Left	5.47	4.25	Scene recognition and social context
Para hippocampus Right	6.08	4.97	
Parietal lobe Left	6.69	5.7	integration of sensory information and language
Parietal lobe Right	6.49	5.3	
Posterior cingulate Left	6.71	5.76	emotional and motor tasks
Posterior cingulate Right	5.78	4.24	
Anterior cingulate Left	6.03	5.07	attention, motivation, anticipation of tasks, emotional responses
Anterior cingulate Right	5.69	4.76	
Basal Ganglia Left	7.63	5.71	voluntary movement, coordination
Basal Ganglia Right	7.73	6.7	
Cerebellum Left	6.08	5.4	Coordination, memory, emotions
Cerebellum Right	5.6	4.56	

Table 3: Comparison of pre and post intervention SUV values in 18 FDG PET CT scan and correlation with clinical symptom improvement in the case study.

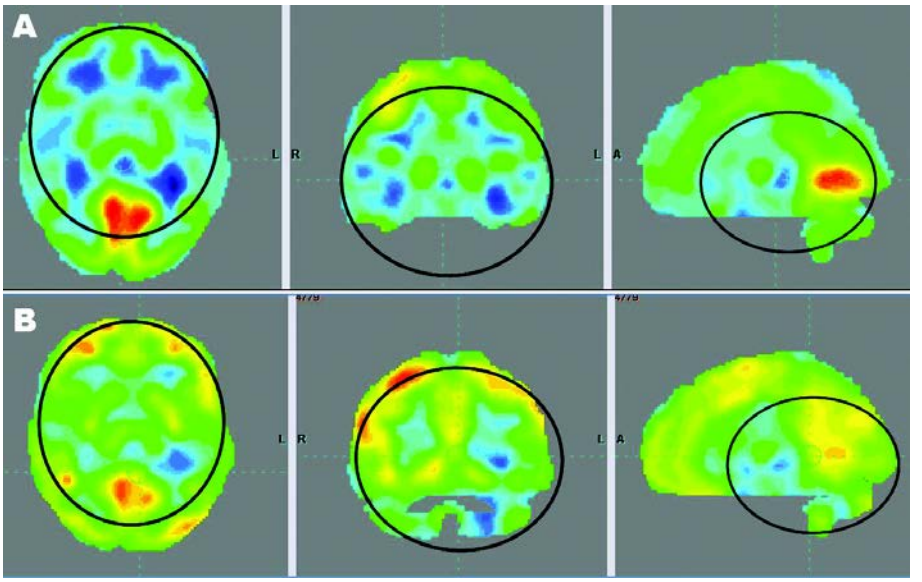


Figure 6: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image which have changed to green areas depicting normal metabolism.

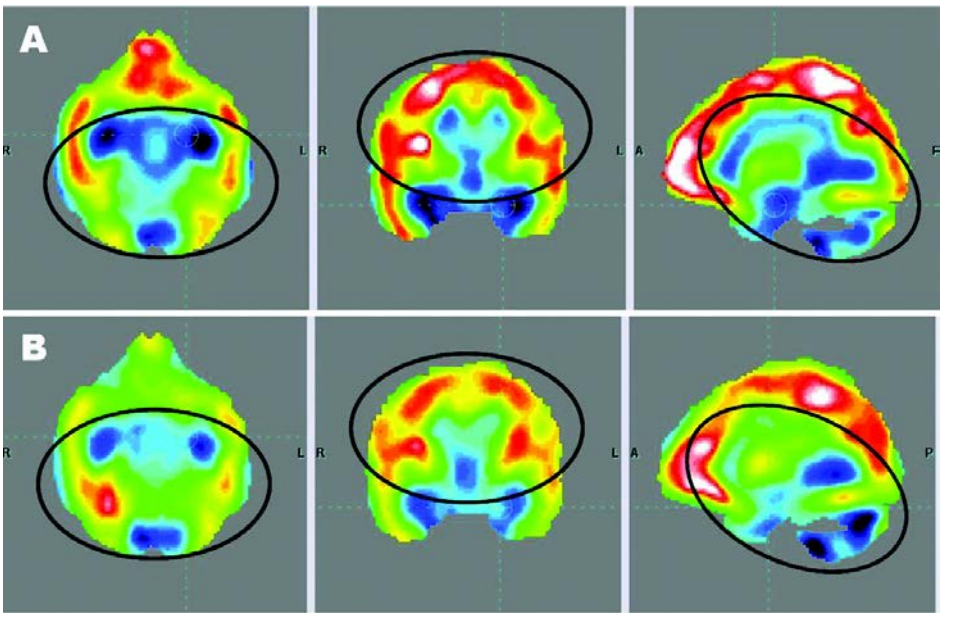


Figure 7: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows improvement in metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image have changed to green areas depicting normal metabolism. While red areas depicting hypermetabolism in the pre SCT image have become green areas which depict normal metabolism in the post SCT image. In this figure, the imbalance in brain metabolism before SCT has corrected towards balanced normal metabolism.

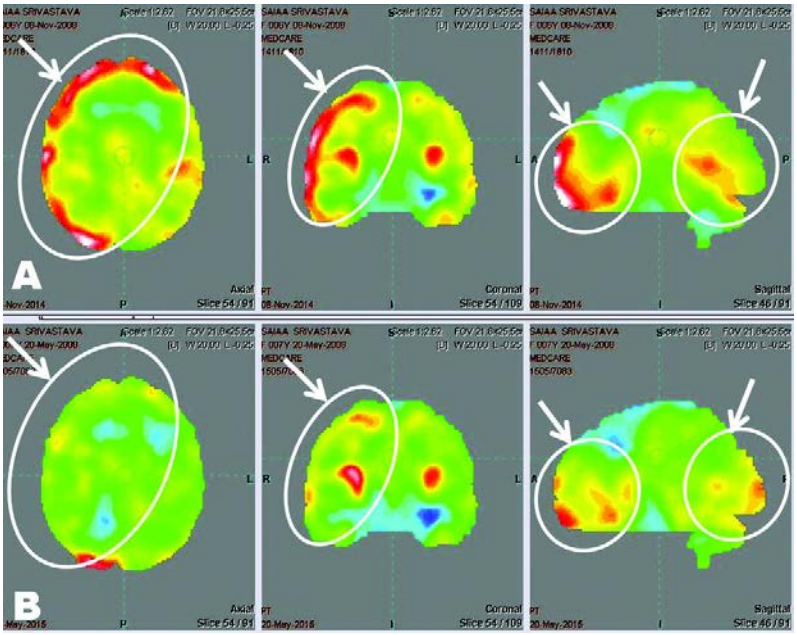


Figure 8: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows improvement in metabolism as outlined by the circles. Red areas which depict hypermetabolism in the pre SCT image have become green areas which depict normal metabolism in the post SCT image.

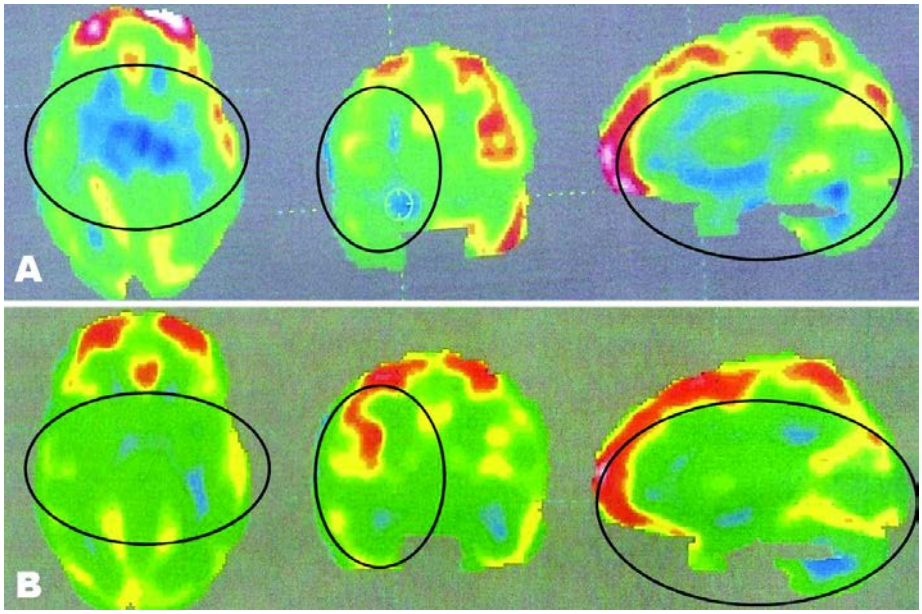


Figure 9: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image which have changed to green areas depicting normal metabolism.

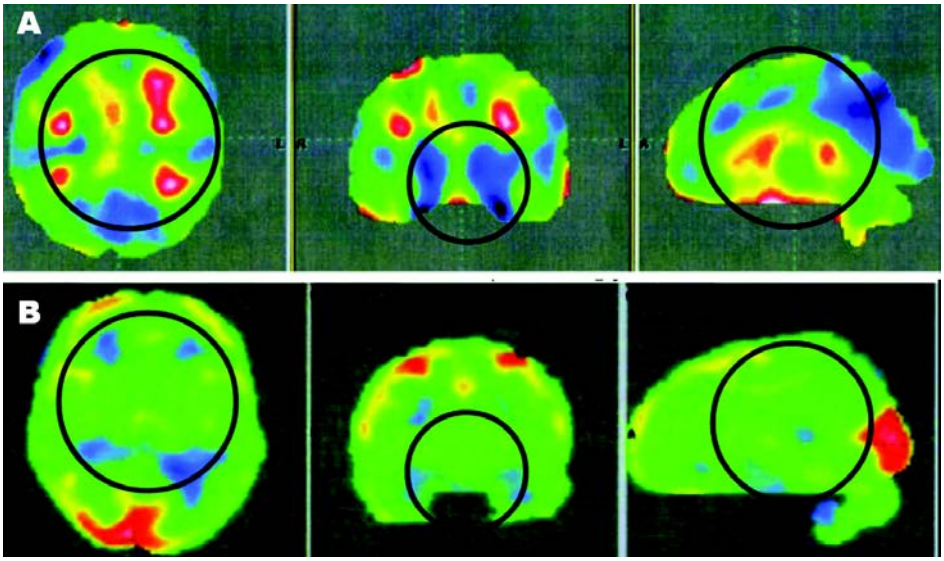


Figure 10: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows improvement in metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image have changed to green areas depicting normal metabolism. While red areas depicting hypermetabolism in the pre SCT image have become green areas which depict normal metabolism in the post SCT image. In this figure, the imbalance in brain metabolism before SCT has corrected towards balanced normal metabolism.

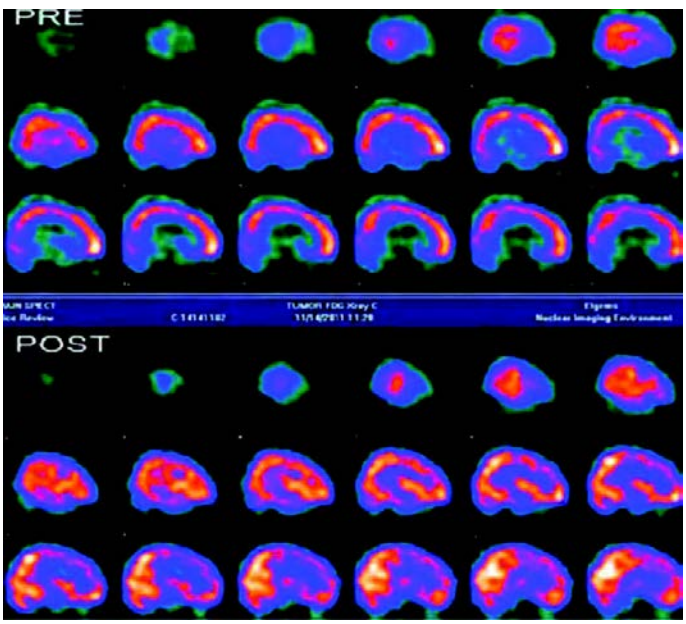
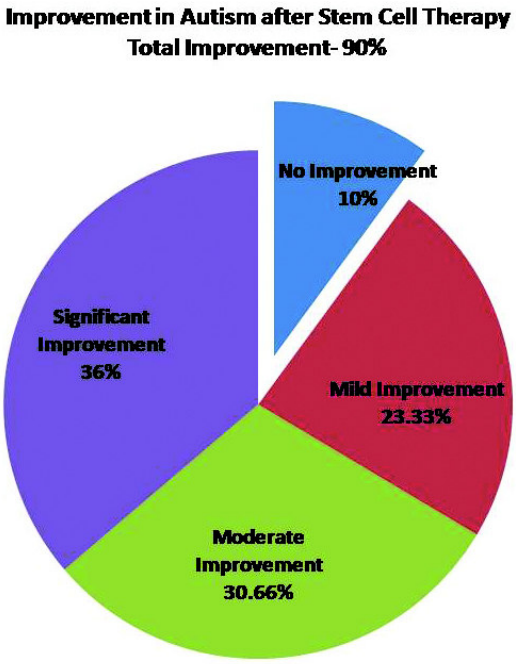


Figure 11: Comparative study of pre and post cell therapy PET CT scan shows increased FDG uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex as visualized below in the post scans. (increased orange areas in the post SCT image depicting improved brain metabolism)

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, to establish it as a standard treatment for autism, extensive trials are required. Types of cells, route of administration, quantity of cells to be injected, frequency of injections, etc need to be optimized. 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.(16)



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Security in 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

Cerebral palsy (CP) is defined as "chronic disability of central nervous system origin characterized by aberrant movement and posture, appearing early in life and associated with 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 prevalence of CP is increasing due to decrease in mortality of low birth weight infants and increase in the rate of CP in these children. Hence, establishing a standard therapeutic approach is the focus of researchers and clinicians all over the world. Although the available treatment options are helpful in managing the symptoms to

some extent, none of them repair the 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

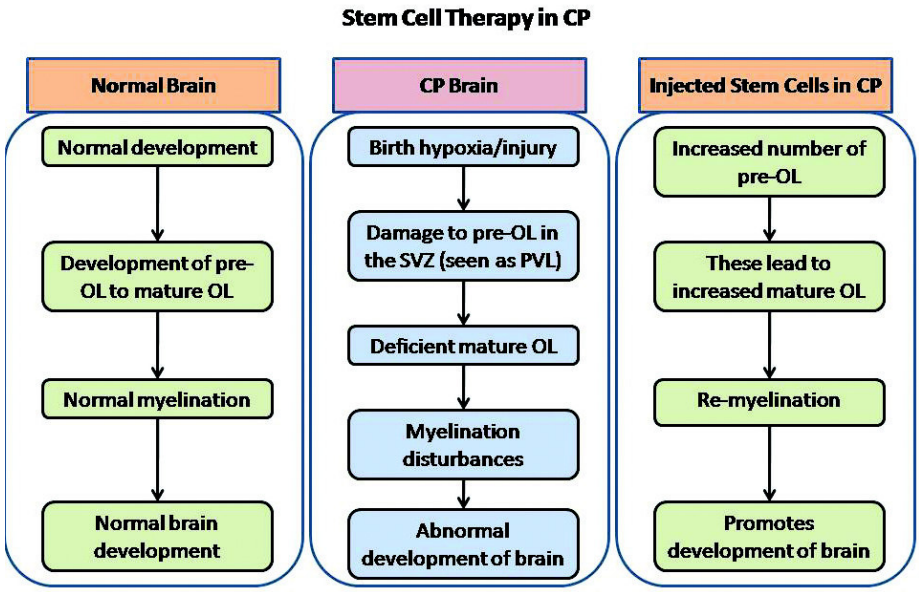

Figure 1: Stem cell therapy in cerebral palsy

Hypoxic ischemia is the most common risk factor of CP prenatally and during delivery. Periventricular leukomalcia (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-a), interferon gamma (INF-?), Interleukin -1 beta (IL-1ß), superoxide radicals, nitrogen species, glutamates, adenosine which 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- a,IL-1 ß, 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.

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,

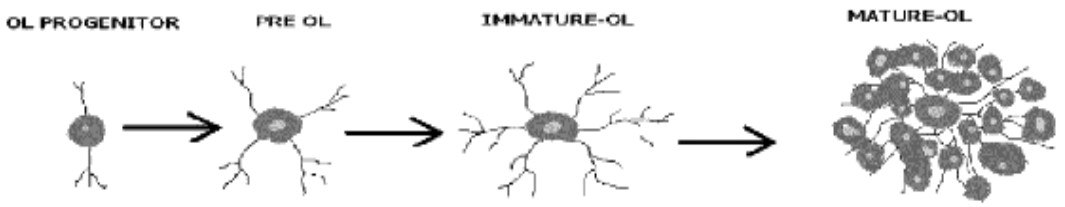

Figure 2: Phases of Oligodendrocyte development

homing and differentiation into neurons, oligodendrocytes, astrocytes, etc. (13,14) The homing property of these cells was confirmed by Chen et al, who transplanted magnetically labeled mesenchymal stem cells in a model of perinatal brain injury and found that these cells migrate to lesion sites and proliferate.(15)

Woodbury et al. have 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. (17) Park et al reported differentiation of clonal neural stem cells (NSCs) into neurons and oligodendrocytes. (18) Titomanlio et al implanted neurosphere-derived precursors in neonatal mouse models which migrated to the lesion site and differentiated into oligodendrocyte and neurons and triggered reduction in lesion size alongwith improvement in memory performance. (19) Transplantation of umbilical cord blood cells in rat models have shown to improve sensorimotor deficits along with other neurological functions. (20-25) Other cells such as multipotent progenitor cells (MPCs) and oligodendrocyte precursor cells were also found to be efficacious in rat models. (26, 27)

Human studies

Not many human clinical studies have been performed till date in CP. Few researchers have reported a positive outcome of intravenous and intrathecal administration of cord blood (CB) cells. Similarly, other studies involving intrathecal autologous BMSCs have also demonstrated motor and functional improvements. (28-47)

In 2005, a controlled study was carried out which used 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. (28)

In 2006, a study was carried out transplants with 1.5 million stem/progenitor cells (CD34+ and CD133+) in eight children (3-12 years of age) diagnosed with cerebral palsy. All the eight children showed some improvement in mobility and/or cognitive function and no graft versus host reactions. Six children (75%) showed improvement in muscle tone, hip movement, leg movement, rolling to the side, sitting and standing balance by the end of six months. (29)

In 2011, two toddlers diagnosed with CP were treated with autologous umbilical cord blood (UCB) transfusion. Improvements were seen on Gross Motor Function Classification System (GMFCS) in both with no side effects. (30)

In 2012, a pilot study was carried out conducted a pilot study, wherein autologous cord blood (CB) cells were infused intravenously in 20 children with cerebral palsy. On follow up after 6 months, 5 patients (25%) showed improvements on developmental evaluation tools as well as by fractional anisotropy values in brain MRI-DTI. (31)

In 2012, a case report of an 11 year old boy with CP and posterior visual pathway injury was published who was treated with intravenous autologous BMSCs transplantation. Six months after the treatment he could walk better and his vision had significantly improved. These findings were further supported by electrophysiological examinations. (32)

In 2012, a study was carried out on fifty two Egyptian patients with cerebral palsy who were divided into: group I (26 patients who underwent stem cell transplantation) and group II (26 patients who did not undergo stem cell transplantation). These patients underwent intrathecal autologous bone marrow derived stem cell transplantation. On follow up of 1 year, statistically significant improvements were noticed in motor, independence and communication skills using Boyd's developmental progress scale and 100 points scale. (33)

In 2012, a study was carried out in twenty-five patients with CP treated with UC-MSC intravenous and intrathecal transplantations. Six months after the transplantation all patients objectively had improvement in scores of GMFM and BSS post-treatment. (34)

In 2012, a study was carried out in 45 CP children treated with neural progenitor cells (NPCs) transplantation derived from fetal tissue into the lateral ventricle. After 1 year, the developmental level in gross motor, fine motor, and cognition of the treatment group was significantly higher compared to the control group. No delayed complications of this therapy were noted.(35)

In 2012, a study evaluated the safety and efficacy of autologous bone-marrow-derived mononuclear cell (BMMNCs) transplantation in a 6 year old CP patient. Significant motor, sensory, cognitive, and speech improvements were observed along with achieving of bowel and bladder control. On the GMFCS-E&R level, the patient was promoted from grade III to I.(36)

In 2013, a study used neural stem cell-like (NSC-like) cells derived from autologous BMSCs as a treatment for 60 patients with moderate-to-severe cerebral palsy (30 control group, 30 treatment group). On a 6 month follow up, the GMFM scores in the transplantation group were significantly higher. All the 60 patients survived, and none of the patients experienced serious adverse events or complications. They concluded that NSC-like cells are safe and effective for the treatment of motor deficits related to cerebral palsy.(37)

In 2013, allogeneic umbilical cord blood (UCB) cells potentiated with recombinant human erythropoietin (rhEPO) were administered in 96 children with CP. Compared with the EPO (n =33) and Control (n=32) groups, the pUCB (n = 31) group had significantly higher scores on the GMPM and BSID-II Mental and Motor scales at 6 months. DTI revealed significant correlations between the GMPM increment and changes in fractional anisotropy in the pUCB group. 18F-FDG-PET/CT showed differential activation and deactivation patterns between the three groups. UCB cell treatment ameliorated motor and cognitive dysfunction in children with CP accompanied by structural and metabolic changes in the brain.(38)

In 2013, a 2.5 years old normally developed boy suffering from global ischemic brain damage following a cardiac arrest in a persistent vegetative state was treated with autologous cord blood (91.7umL, cryopreserved, mononuclear cells) intravenous transplantation followed by active rehabilitation. At 40 months, he could independently eat, walk in gait trainer, crawl, and move from prone position to free sitting with improvement in receptive and expressive speech competence. (39)

In 2013, a 5-year-old girl with cerebral palsy was treated with intravenous and intrathecal administration of umbilical cord mesenchymal stem cells (MSCs) derived from her young sister. At 28 months follow up, gross motor dysfunction had improved along with enhanced immunity, increased physical strength, and adjusted speech and comprehension. Temporary low-grade fever was the only side effect during the treatment.(40)

In 2013, a study was carried out in 52 patients with CP who received BM-MSC transplantation. The total GMFM-88 and GMFM-66 scores increased at 1 month, 6 months and 18 months post-transplantation indicating that the treatment is feasible, safe and effective for improving motor function in CP.(41)

In 2014, a study was carried out in 12 children with CP to evaluate the safety of autologous bone marrow-derived CD133(+) cell intrathecal injection. No adverse events were detected by except for a seizure in 1 patient. Significant improvements were observed in GMFM, GMFCS, FIM+FAM, Ashworth Scale, and BBS outcome measures at 6 months after cell transplantation.(42)

In 2014, a phase I open-label clinical trial was carried out in 18 patients with CP to assess the safety of autologous bone marrow-derived total nucleated cell (TNC) intrathecal and intravenous injection. Headache, vomiting, fever and stiff neck were some of the early adverse effects noted in three patients. No serious complications were documented. According to the Battelle Developmental Inventory an overall 4.7-month increase was observed in developmental age including all areas of evaluation. (43)

In 2015, 80 patients with CP received up to 6 intravenous infusions of AB0/Rh-identical, red blood cell-depleted UCB cells. Patients were followed for 3-36 months, and significant improvements in neurological status and/or cognitive functions were observed with no complications or adverse events. (44)

In 2015, a retrospective study assessed the safety of allogeneic umbilical cord blood stem cells (UCBSCs) treatment in 47 patients with severe cerebral palsy (CP) at Guangdong Provincial Hospital of Chinese Medicine. Some adverse events during treatments were found in 26 (55.3%) patients, including fever (42.6%) and vomiting (21.2%) which were managed by symptomatic treatment, whereas no treatment related adverse events were found at follow-up within 6 months. Intrathecal infusion and the ages at the initiation of treatment (=10 years old) were termed risk factors for the occurrence of adverse events. (45)

In 2015, a report presented a case of a 6 year old girl with CP treated with 4 transplantations of hUCB-MSCs combined with rehabilitation beginning from 6 months with follow-up till 5 years. Her motor function had recovered to normal level according to the objective scales with significant improvement in language function. No adverse events were noted till 6 years of age. (46)

In 2015, a study was carried out in eight pairs (16 individuals) of identical twins with CP treated with allogenic UCMSC subarachnoid injections. Significant improvements were observed in the GMFM at the end of the 1st and 6th months. Motor function improvements in the GMFM between two individuals of an identical twin were closely correlated, but improvements among twin pairs were not correlated. Therefore, it was hypothesized that hereditary factors contribute to the mechanisms of UCMSC transplantation in motor function improvement in children with CP. (47)

Our results

Published data

A study on 40 cases was carried out to evaluate the benefits of stem cell therapy in cerebral palsy. (48) 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. Amongst 11 diplegic CP patients, 100%showed improvement in sitting balance, 90.91% in standing and walking balance, 90% in distal hand movements, 83.33% in oromotor skills, 80% in cognition, 70% in leg movements, 66.67% in speech, 50% in ambulation, 45.45% in muscle tone of the lower limb, 44.44% in overhead activities, 40% in the muscle tone of the trunk, and 36.36% in muscle tone of the upper limb. Amongst 23 quadriplegic CP, 83.33% showed improvement in neck holding, 78.95% in sitting balance, 63.16% in cognition, 60% in oromotor skills, 54.55% in ambulation, 52.38% in muscle tone of the lower limbs, 50% in muscle tone of upper limbs, 45.45% in speech, 45% in muscle tone of the trunk, 36.36% in standing balance, and 31.58% in walking balance. Amongst 6 cases in the miscellaneous group of CP, 3 were dystonic CP, 2 were athetoid CP, and 1 was ataxic CP. Overall, 83.33% showed improvement in speech and standing balance, 66.67% in walking balance, 60% in oromotor skills, 50% in sitting balance and muscle tone of lower limb and trunk, and 33.33% in muscle tone of upper limb. The patient with dystonic CP also showed an improvement in the dystonia.

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.

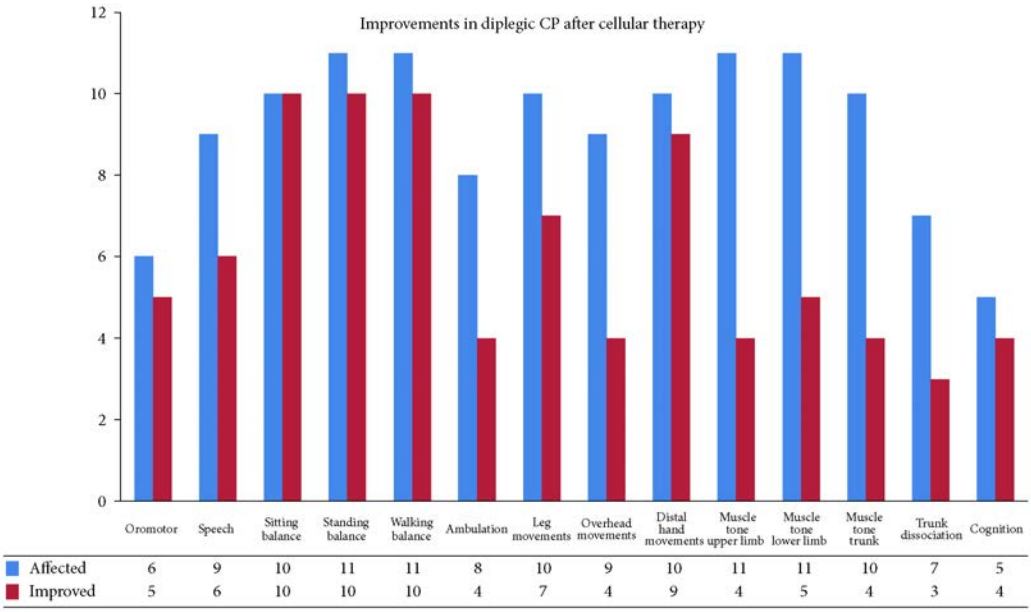

Figure 3

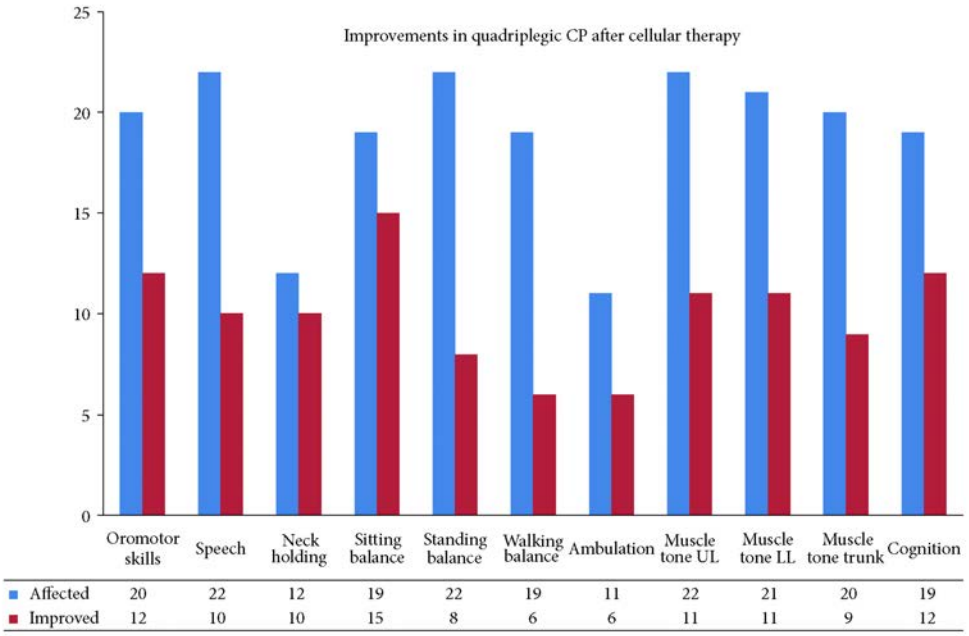

Figure 4

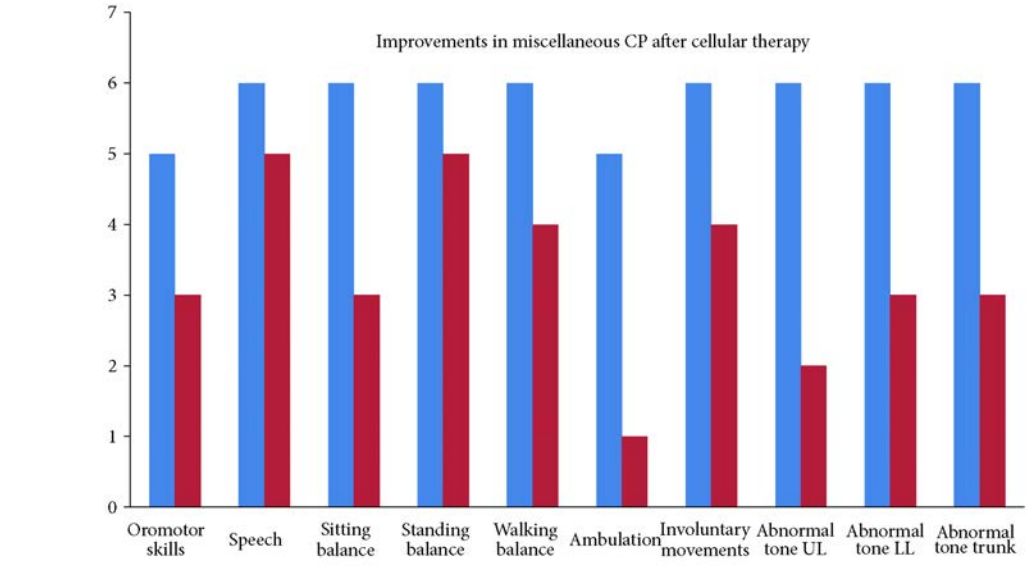

Figure 5

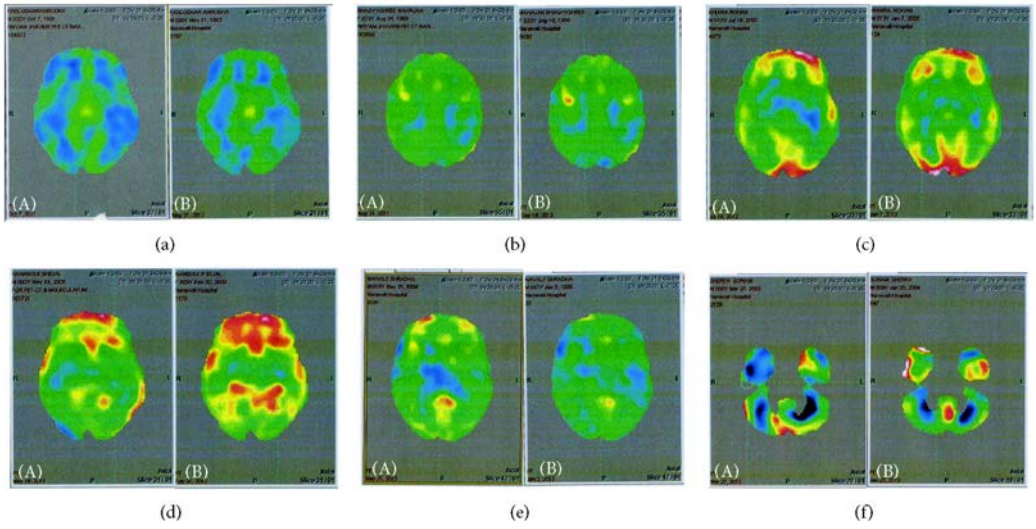

Figure 6: Improvement in PET CT sscan

Case reports

A 20 year old male suffering from CP and Mental Retardation (MR) was treated with autologous BMMNCs transplantation followed by multidisciplinary rehabilitation. He had diplegic gait and with affected fine motor activities, balance, and speech and an IQ score of 44. At six months follow up, PET-CT scan showed significant improvement in metabolic activity in all four lobes, mesial temporal structures and

left cerebellar hemisphere, which correlated with improvement in IQ, social behavior, speech, balance and daily functioning. (51)

A two year old girl with spastic cerebral palsy was administered autologous BMMNCs intrathecally. Significant functional improvements were seen six months after the therapy which correlated with significant changes seen on PET-CT scan, providing objective evidence of functional restoration of affected brain areas by cellular transplantation.(52)

A 12-year-old boy with CP was treated with autologous BMMNCs intrathecal transplantation. At six months post therapy, his trunk strength, upper limb control, hand functions, walking stability, balance, posture and coordination had improved which remained till one year follow up. His FIM score had increased from 90 to 113. A repeat PET-CT scan of the brain six months after intervention showed progression of the mean standard deviation values towards normalization which correlated with the functional changes. Thus, this report suggested that cellular therapy may improve quality of life of children with CP and emerge as a potential for option.(53)

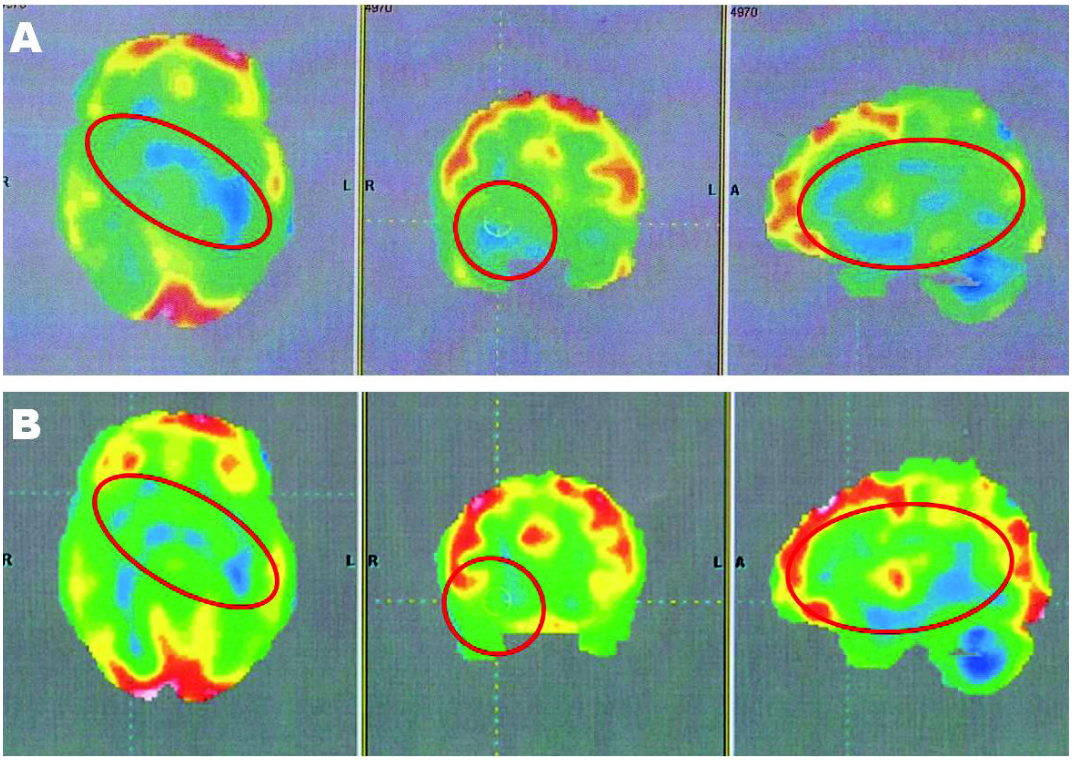

Figure 7: PET CT scan showing improved metabolic activity 6 months after stem cell therapy which is indicated by decrease in blue areas.

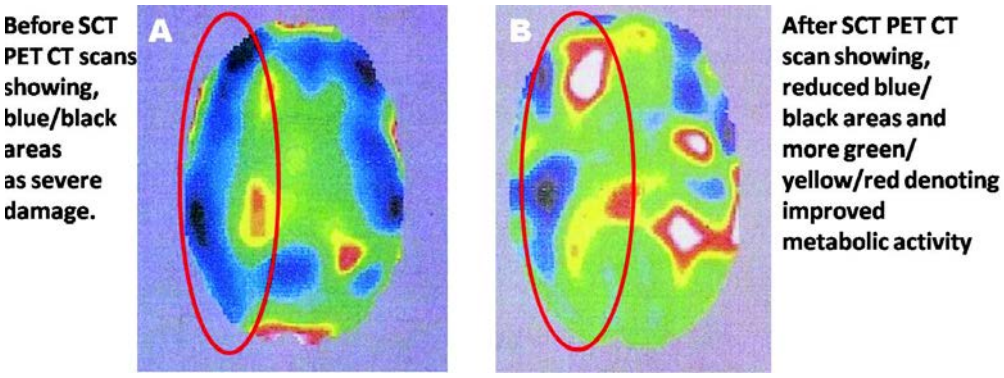

Figure 8: Blue areas representing hypometabolism have reduced suggesting a positive response to the treatment

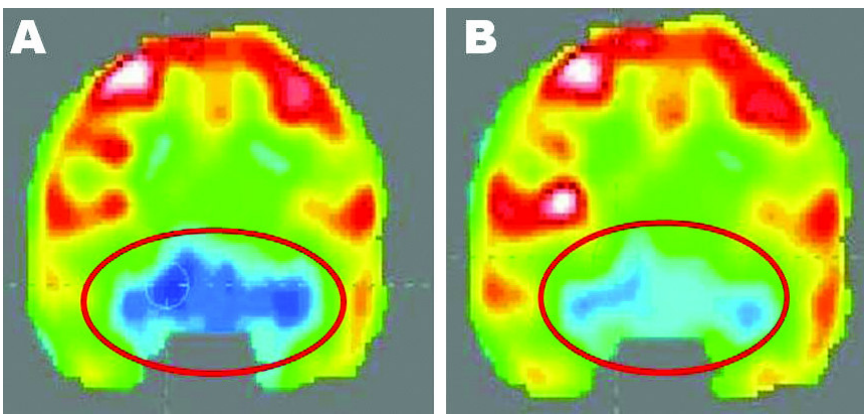

Figure 9: Blue areas representing hypometabolism have reduced suggesting a positive response to the treatment

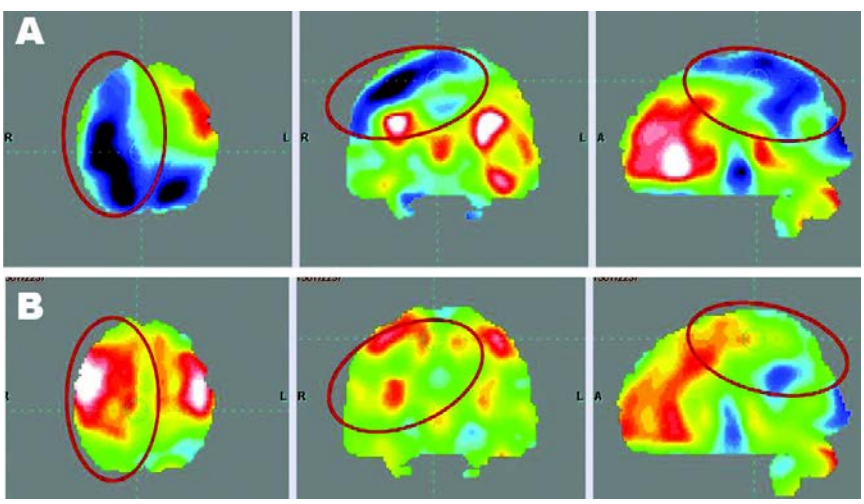

Figure 10: The blue damaged areas seen in the pre SCT image have almost disappeared after SCT. This shows improvement in the metabolism/funcitoning of the damaged areas after SCT.

Unpublished data

We analysed the effect of stem cell therapy in 193 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. Mild improvement was defined as improvements till 3 of the symptoms mentioned. Moderate was considered when 4 to 6 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 7 to 8 symptoms. Analysis revealed that out of 193 patients, Overall 91.71% patients showed symptomatic improvements. 8.29% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 31.08% of patients, moderate in 47.15% of patients, whereas, 13.47% of patients showed significant improvements.

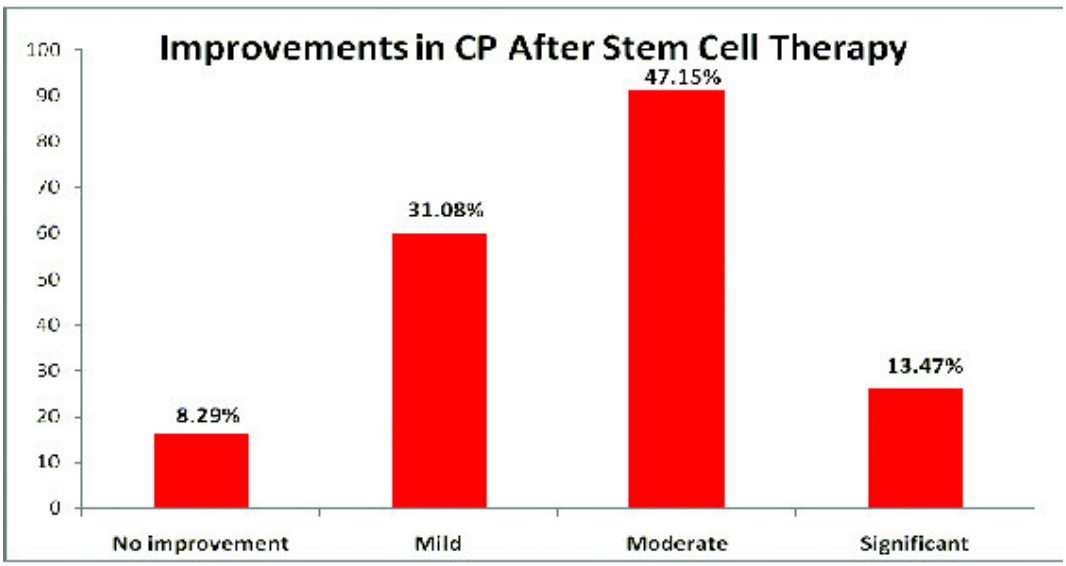

Figure 11:Improvements noted in 193 patients of CP treated with intrathecal autologous BMMNCs.

Future directions

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. Research should also focus on standardizing the outcome measures and monitoring tools need to be standardized to study the effect of intervention. Cell therapy may be used as an adjunctive treatment modality to 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

Muscular dystrophy (MD) is a heterogeneous group of genetic disorders that weaken the the striated 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 which results 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.

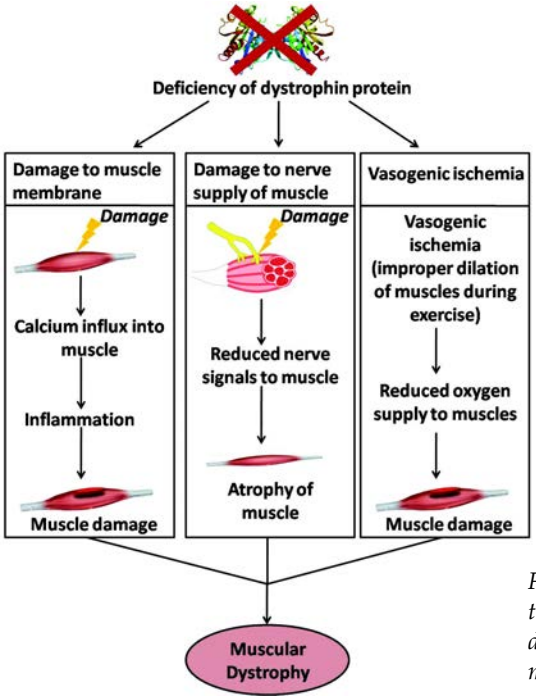


Figure 1: Pathophysiology of the most common muscular dystrophy type Duchenne muscular dystrophy (DMD).

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 (4) 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.(5)

Inspite of extensive studies being carried out in this field, there is currently no effective treatment for the same. (6) The conventional treatments include medical intervention such as corticosteroids, physical and occupational therapy, assistive devices, etc.

Unmet Medical Needs

The treatments available currently for muscular dystrophy alleviate few symptoms of the disorder but do not act at 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 but has not yet been established as a clinical application. No standard therapeutic modality has been successful to halt the progression of the disease or increase the survival.

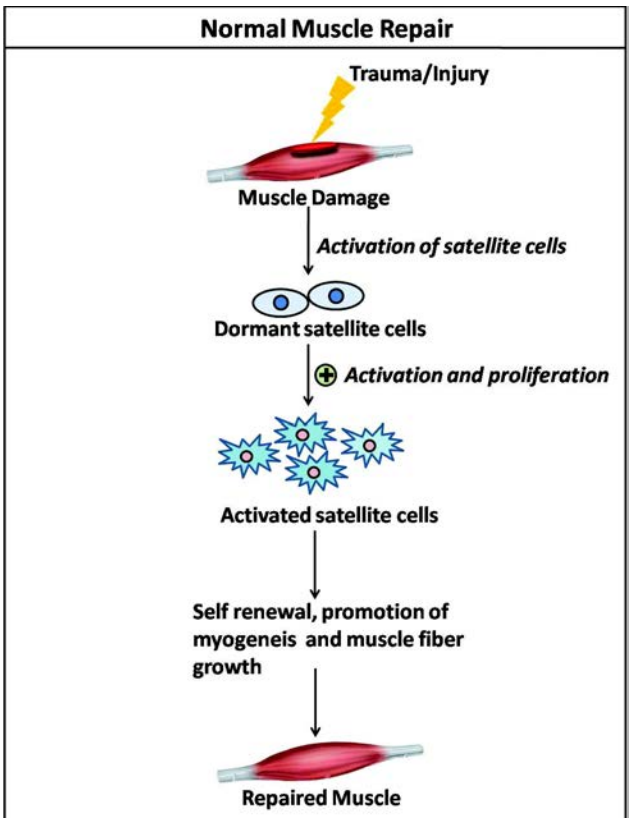


Figure 2: Normal process of muscle repair.

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. (7) Continuous damage to the cytoskeleton of muscle fibres leads to premature exhaustion of the muscle stem cell pool that maintains muscle integrity during normal use and exercise. 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 (8)

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. (9-11) 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. (16) 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.

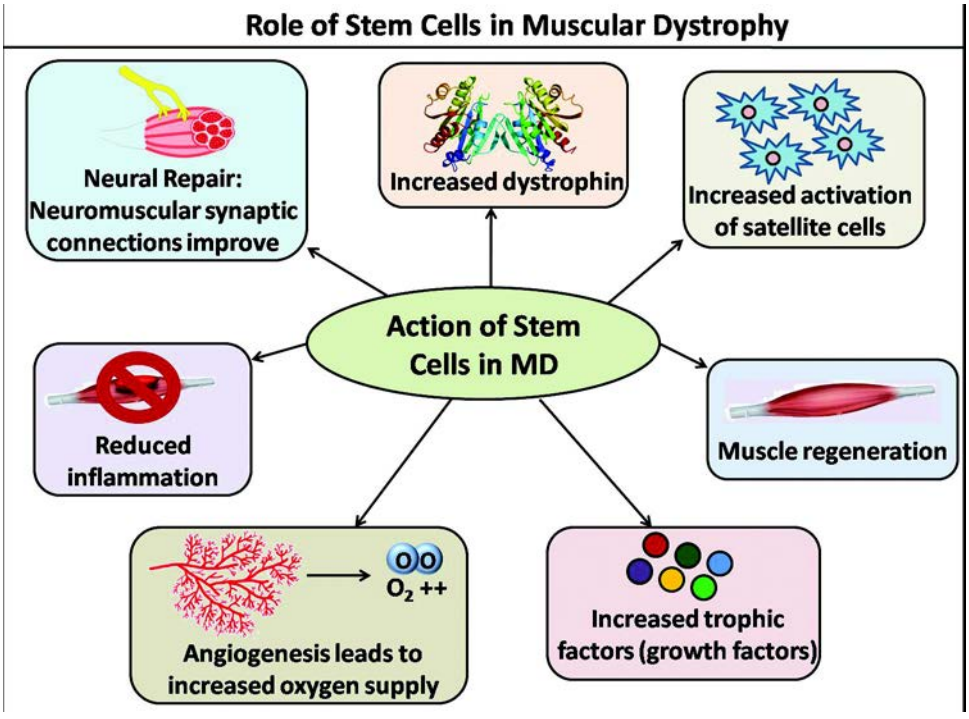


Figure 3: Role of stem cells seen in DMD (in other forms of muscular dystrophy, there is an increase in the deficient protein based on the type).

Animal studies

Preclinical studies in mouse models of various muscular dystrophies have demonstrated that myoblasts on transplantation into dystrophic muscle; could repair damaged myofibres. Although, myoblast transplantation did not show effective results due to rapid death of most injected myoblasts and the failure of injected myoblasts to migrate more than ~0.5 mm away from the injection site (12) Hence 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 (13-20)

In 2008, Wallace et al transplanted adult muscle mononuclear cells (AMMCs) in -sarcoglycan-null dystrophic mice. They found that AMMCs were 35 times more efficient at restoring sarcoglycan compared to cultured myoblasts. (21) Similar studies were carried out using side population (SP) cells (22)

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.(23) Similar test was carried out using 3H-thymidine labeled human bone marrow derived MSCs. (24) 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. (25-27) 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. (28,29)

Human studies

Stem cell transplantation using satellite cells or myoblast progenies have been carried out extensively in MD (30) this was performed by different groups. Huard et al reported presence of dystrophin positive fibres along with improvement in muscle strength. But, these improvements faded over time. (31) Gussoni et al performed a series of studies to test the potential of myoblast transplantation. (32-34) These studies individually demonstrated that the transplanted myoblasts persisted after injection but their microenvironment influenced whether they fuse and express dystrophin. They also documented the ability of exogenous human bone marrow cells to fuse into skeletal muscle and persist up to 13 years after transplantation. Similar results were recorded for various other studies on myoblast transfer. (35-40) Although, they enable transient delivery of dystrophin and improve the muscle strength to some extent, they have various limitations, such as immune rejection, poor cellular survival rates, and limited spread of the injected cells.

Hence, other sources of stem cells such as bone marrow and umbilical cord are being explored by the researchers. Zhang et al performed umbilical cord stem cell transplantation in DMD and found it to be feasible. (41) Yang et al (2009) investigated

the feasibility of employing double transplantations of autologous bone marrow mesenchymal stem cells (BMSC) and umbilical cord mesenchymal stem cells (UMSC) in the treatment of progressive muscular dystrophy (PMD). Total effective rate was 82.9% concluding it as a safe and effective treatment. (42)

Hematopoietic stem/progenitor cell populations from adult skeletal muscle also have a therapeutic potential for muscular dystrophy. (43) Torrente et al (2007) studied the safety of autologous transplantation of muscle-derived CD133+ cells. They recorded increased ratio of capillary per muscle fibers with a switch from slow to fast myosin-positive myofibers. (44) Sharma et al published the results of autologous bone marrow derived mononuclear cells intrathecally and intramuscularly in 2 patients with DMD and 2 with BMD as individual case reports showing functional improvements along with improvement in MRI and electrophysiological tests. (45-49)

Our results

Published data

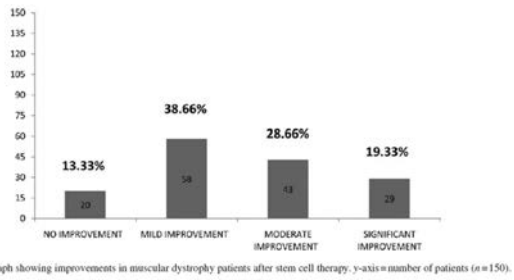


Figure 4: Graph showing improvements in muscular dystrophy patients after stem cell therapy. y-axis = number of patients (n = 150).

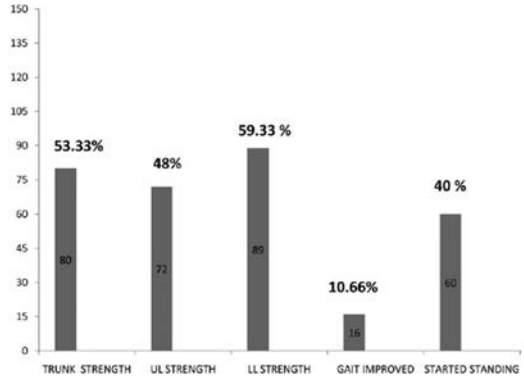


Figure 5: Graph showing symptomwise improvements in muscular dystrophy patients after stem cell therapy. Number of patients showing improvements with respect to trunk strength, upper limb (UL) strength, lower limb (LL) strength, gait, and standing are shown. y-axis = number of patients (n = 150).

1. A study was carried out on 150 patients diagnosed with Muscular Dystrophy. These included Duchenne Muscular Dystrophy, Limb Girdle Muscular Dystrophy and Becker Muscular Dystrophy variants. They were administered with autologous bone marrow derived mononuclear cells intrathecally and intramuscularly at the motor points of the antigravity weak muscles followed by vigorous rehabilitation therapy. No significant adverse events were noted. Assessment after transplantation showed neurological improvements in trunk muscle strength, limb strength on Manual Muscle Testing (MMT), with Gait improvements and a shift on assessment scales such as Functional Independence Measure (FIM) ; Brooke and Vignos scale. Further, Imaging

and Electrophysiological studies also showed significant changes in selective cases. On a mean follow up of 12 months ± 1 month, overall 86.67% cases showed symptomatic and functional improvements, with 6 patients showing changes with respect to muscle regeneration and decrease in fatty infiltration on musculoskeletal Magnetic Resonance Imaging (MRI) and 9 showing improved muscle electrical activity on Electromyography (EMG). 53% cases showed increase in trunk muscle strength, 48% showed increase upper limb strength, 59 % in lower limb strength and about 10 % showed improved gait. The results show that this treatment is safe, efficacious and also improves the quality of life of patients suffering from Muscular Dystrophy

2. An individual study comprising of LGMD patients was also published. This study was a 5 year longitudinal study including 65 diagnosed cases of LGMD who underwent autologous bone marrow mononuclear cells intrathecally and intramuscullarly out of which 59 cases completed the study and 6 were lost to follow up. These cases were divided into 3 groups depending on the number of treatments administered. Group 1 included the cases who underwent one treatment of stem cell therapy (N=31). Group 2 included cases who underwent two treatments (N=24). Group 3 included cases who underwent 3 treatments (N=4). In group 1, overall 97% of patients showed better functional status. The total percentage of improvement ranged from 84% to 100% in all the muscles. There was a statistically significant difference seen in the hip extensors, knee extensors, peronei, tibialis anterior, upper abdominals, and deltoid, indicating improved muscle strength after 6 months of stem cell therapy. In group 2, 96% of patients improved in their functional status. The total percentage of improvement ranged from 90% to 100% in all the muscles. There was a statistically significant difference seen in hip extensors, knee extensors, peronei, tibialis anterior, lower abdominals, deltoid, biceps, and triceps. In group 3, of the four patients, one patient deteriorated in his FIM score, whereas two patients improved, and one maintained functional status. With respect to muscle strength, most of the patients were stabilized. Overall, it was found that patients who underwent two treatments of cell therapy had better outcomes with respect to muscle strength and functional status. Almost all patients in this group have maintained their functional status 3 years after the second cell therapy, and have achieved a plateau in their disease process. This study showed that stem cell therapy may help control the disease progression along with providing functional improvements, thereby enhancing quality of life.

3. Autologous Bone Marrow Derived Mononuclear Cell Transplantation in Duchenne Muscular Dystrophy.

In year 2012, Sharma et al conducted autologous bone marrow derived mononuclear cell transplantation intrathecally and intramuscularly on 18 year old boy with Duchene muscular dystrophy which is the most common childhood muscular dystrophy. After six months of procedure, he showed the significant functional improvements along with improvements in his muscle strength.

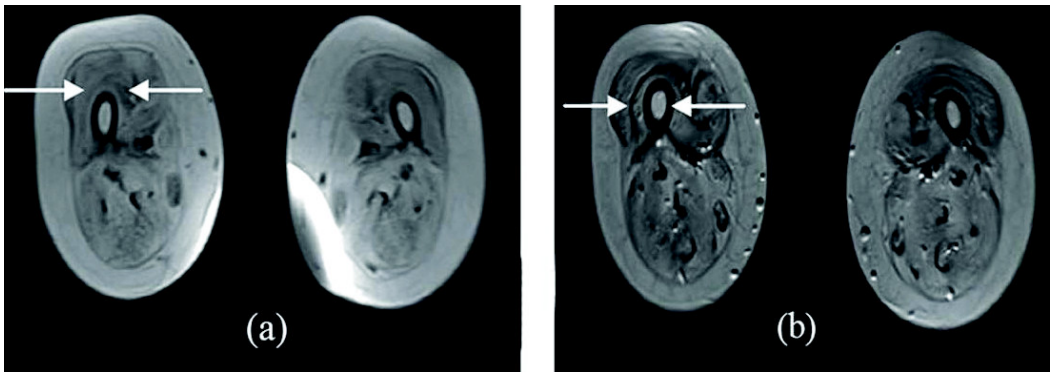
4. Autologous Bone Marrow-derived Mononuclear Cell transplantation in Duchene Muscular Dystrophy.

Sharma et al transplanted autologous bone marrow derived mononuclear cell intrathecally as well as intramuscularly in specific muscles in an 18 year old boy with Duchene Muscular Dystrophy. On follow-up at six months, he showed significant functional improvements along with improvements in his muscle strength. Clinically, his MRI showed muscle fiber regeneration with decrease in fatty infiltration.

5. Effects of cellular therapy seen on musculoskeletal magnetic resonance imaging in a case of Becker's Muscular Dystrophy.

In 2013, a 28 year old male patient of BMD treated with Autologous bone marrow nuclear cells via the intrathecal and intramuscular routes by Sharma et al .The effects were measured in the terms of clinical , functional and radiological changes . At 6 months after therapy, the comparative musculoskeletal magnetic resonance imaging (MRI-MSK) showed regeneration of muscle fibers. This observation is one of the initial evidential data for muscle regeneration, in BMD patient following cellular therapy.

Figure 6: In the figure, a & b show MRI Musculoskeletal images before and after stem cell therapy,



respectively showing regeneration of muscle in vastus medialis and vastus radialis

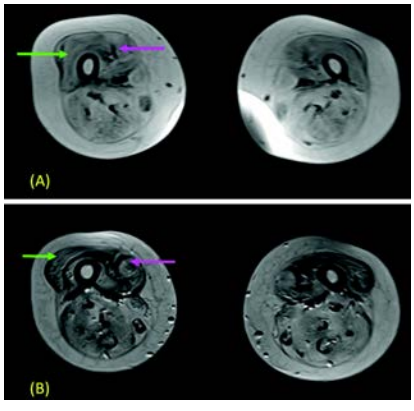


Figure 7: In the figure, A & B show MRI Musculoskeletal images before and after stem cell therapy, respectively showing regeneration of muscles

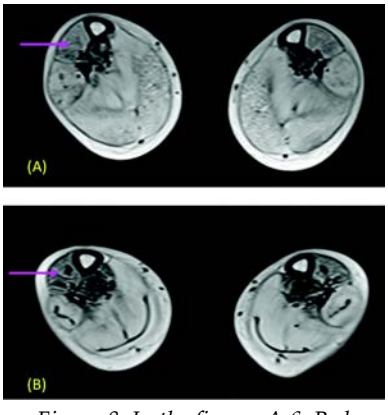


Figure 8: In the figure, A & B show MRI Musculoskeletal images before and after stem cell therapy, respectively showing regeneration of muscles

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 any 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.

Funtional 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 analysed 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 9, 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 10). 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 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

Table 1. 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

Table 2. 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

Table 3. Kaplan-Meier analysis of time till loss of ambulation for patients with and without stem cell therapy

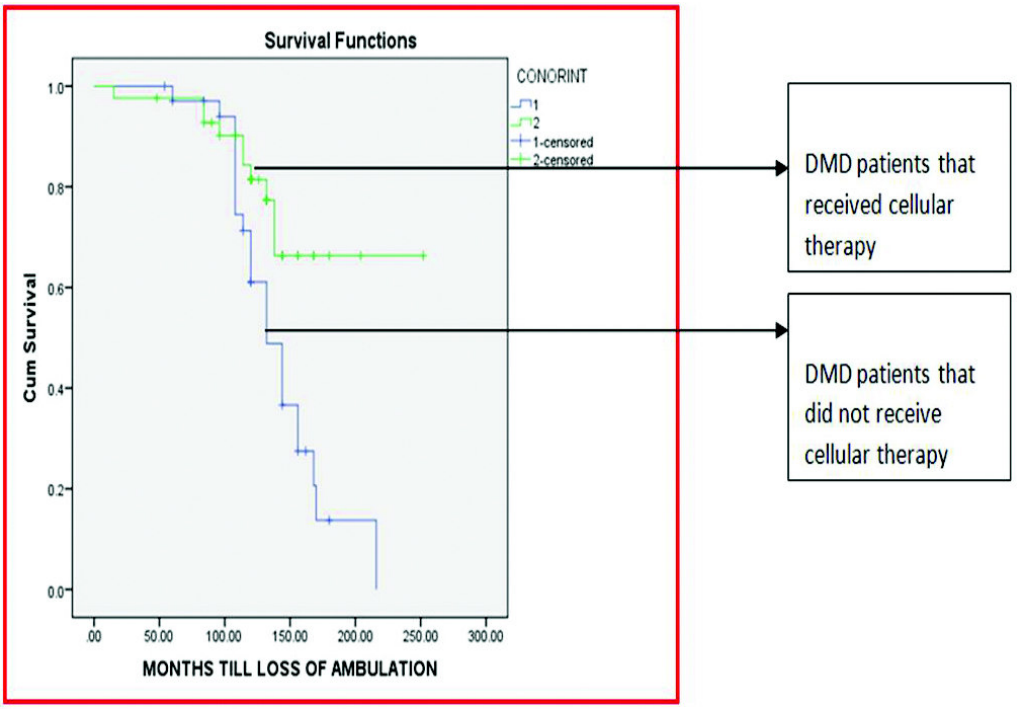


Figure 9: Kaplan-Meier curve analysis of time till loss of ambulation in patients with and without stem cell therapy

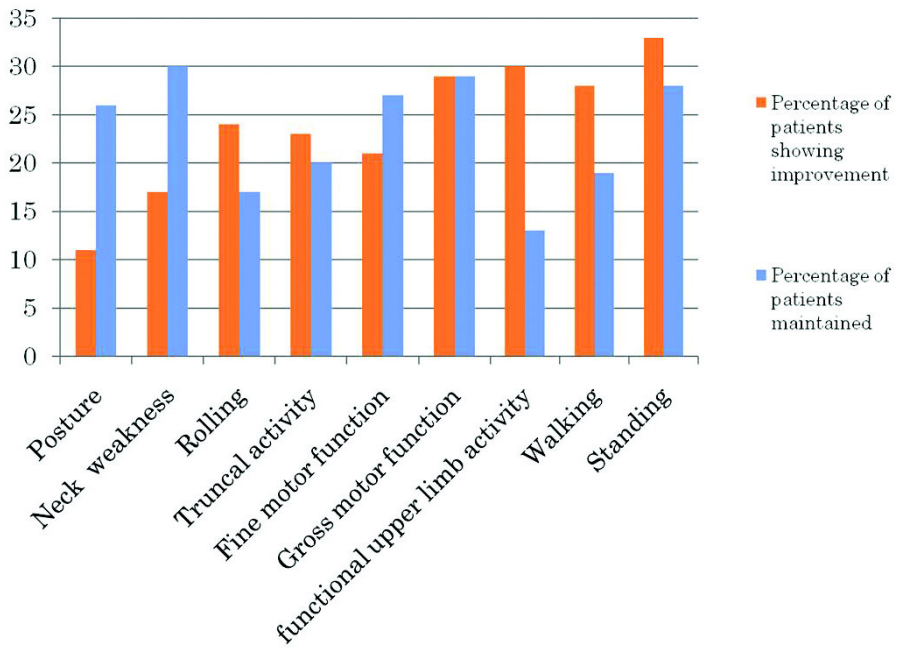


Figure 10: Percentage analysis of symptomatic improvement in the patients with stem cell therapy

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

Table 4. Percentage analysis of modified manual muscle testing scale

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. (50) 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. Recently, dental pulp stem cells have also proved their capability in regenerative research. Thus, more clinical studies for MD should be designed involving them. Studies carried out so far lack the inclusion of imaging techniques. These techniques may monitor the disease activity and assess the effectiveness of therapeutic intervention. Furthermore, facilitating the translation of stem cell therapy from bench to bedside.

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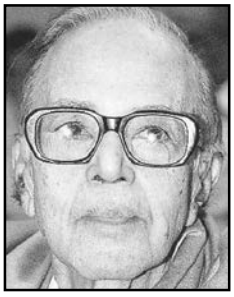
"Neurosurgeons would be happy if they could make the spinal cord regenerate thus helping thousands of paraplegics all over the world.

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

Spinal cord injury (SCI) is a devastating disease 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 its 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 neurological damage completely, leaving behind few deficits. Presently, all modalities aim at repairing the spine 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. However, the dependence level of the patients does not reduce to a great extent. Affected sensations, loss of bladder bowel control, etc are major complications observed in SCI. The currently available treatment modalities fail to improve these complications in case of severe injuries. It is also important to track the cellular changes occurring in the 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 in the past few decades for stem cell transplantation as a therapeutic intervention for SCI. in the field of regenerative medicine 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)

Animal 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.

Human Studies

One of the earliest studies used cells from the fetal nervous and haemopoietic tissues in 15 SCI patients with no side effects. (55) However, due to various ethical and medical concerns over embryonic and fetal stem cells, adult stem cells have been tried extensively. In a comparison between a) transplantation of autologous bone marrow cells directly into the SCI sites and administered granulocyte macrophage colony stimulating factor (GM-CSF) {n=2} and b) only administration of GM-CSF{n=1}, sensorymotor improvements were noticed in all three patients at varied time points (56) Safety and feasibility studies for different cells showed these cells to be safe (57-

60) Comparitive studies carried out to find the optimum route of administration. Syková et al (61,62) revealed intra-arterial transplants to show more improvements as compared to those intravenous transplants. Chernykh et al, reported neurological improvements in 66.7% of chronic SCI patients who underwent autologous BMSCs transplantation intravenously as well as at the site of injury. (63) Whereas, Saberi et al. in a similar clinical trial carried out in 4 patients, found improvement in only 1 patient. (64). Geffner et al reported administration of BMSCs via multiple routes to be safe and feasible improved the quality of life in most patients. (65) O.S Abdelaziz administered autologous adult bone marrow mesenchymal stem cell through open surgical intraparenchymal and intralesional injection into the site of cord injury followed by monthly intrathecal injection of stem cells through lumbar or cisternal punctures. Clinical improvement was observed in 30% treated patients. (66) Intramedullary direct injection of MSCs into the injured spinal cord also resulted in changes in MRI and electrophysiological tests along with other functional improvements (67) However, direct injection is an invasive procedure involving risk of secondary injury. Saito et al, Pal et al and Kumar et al reported intrathecal administration to be the optimum route of administration. (68-70) Series of studies also demonstrated the benefits of bone marrow stem cells in SCI. (71-78) In a novel method, using combination of BM mesenchymal stem cells (MSC) and patient's autoimmune T cells, Moviglia et al demonstrated the regeneration phenomenon based on the controlled inflammatory activity at the injured site. Both the patients of the study showed both motor and sensory recovery with no adverse effects. (79) Peripheral stem cells and macrophages have also been reported to show improvements of motor and sensory functions without any critical complications (80,81). Other sources such as cord blood, olfactory ensheathing cells, adipose tissue derived stem cells, etc also showed improvement in sensory-motor functional improvements (82-86) Saberi et al enrolled 33 SCI cases to study the safety of intramedullary Schwann cell transplantation. After a 2 year follow up, there were no tumor formations or other adverse events recorded. (87) Co-transplantation techniques have also been tested and found to be safe. (88) Tianshen Sun et al, in their recent study have reported The synergistic effects of the combined use of olfactory ensheathing cells and Schwann cells enhancing functional recovery in SCI. (89) Similarly, Chen et al in their study of 28 cases showed beneficial effects of OECs, SCs, or a combination of them in SCI. (90) Al-Zoubi et al, demonstrated the positive effect of purified autologous leukapheresis-derived CD34+ and CD133+ stem cells in 19 cases of chronic SCI. (91) Cheng et al, in a controlled study including 34 cases of thoracolumbar spinal cord injury, stated that umbilical cord mesenchymal stem cells effectively improve neurological functional recovery after spinal cord injury, and its efficacy is superior to that of rehabilitation therapy and self-healing. (92)

To track the behaviour and the fate of the transplanted cells, the cells are labelled with magnetic particles before administration. Callera et al administered CD34+ cells labeled with nanoparticles via lumbar puncture and 6 patients received magnetic beads without stem cells. MRI done 20 and 35 days after transplantation showed that

the magnetically labeled CD34+ cells were visible at the lesion site in 5 patients out of 10. These signals were not visible in the control group (93).

Our Results

Published data:

1. A detailed analysis of chronic thoracolumbar SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells 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.

100 out of 110 (91%) patients showed improvements. Improvement in trunk control was observed in 95.6% cases, bladder management in 33% with respect to shift from indwelling and condom catheter to self intermittent catheterization, partial sensory recovery in 27% and reduction of spasticity in 26%. All the patients showed improvement in postural hypotension. 38% wheelchair bound patients started walking with assistance. Functionally, 27% showed improved activities of daily living (ADLs) and 53.6% showed a positive change in FIM score. 10% cases showed a shift in ASIA scale. 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. (9

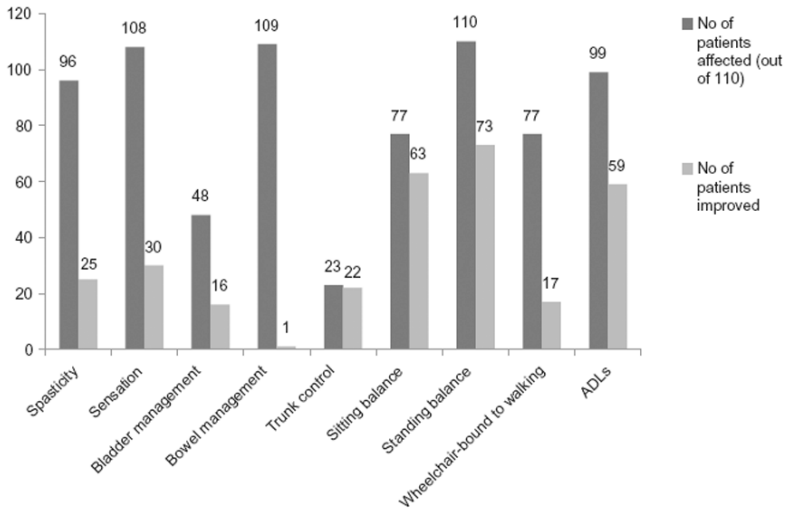


Figure 1: Symptomatic improvements in patients with spinal cord injury after stem cell therapy. The x axis denotes symptoms presented in the patient population and the y axis denotes the number of patients. (ADLs - activities of daily living).

Symptom/function	Affected patients (n of 110)	Patients improved (n)	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

Notes: #Significant at $P \leq 0.05$; †Chi-square value at one degree of freedom; *P value insignificant for improvement in bowel management.
Abbreviation: ADLs, activities of daily living.

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

(A)	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 common peroneal-EDB ankle	0.8	3.0
	R common peroneal – tibialis anterior, fibular head	1.6	3.4
	L common peroneal – tibialis anterior, fibular 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
(B)	**Functional MRI (before)**	**Functional MRI (after)**	
Patient 1	No activity in the pre and post central gyri	Activation in the right precentral gyrus	

Abbreviations: AH, abductor hallucis; EDB, extensor digitorum brevis; MRI, magnetic resonance imaging; R, right; L, left.

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. (95) This study includes 50 patients of chronic cervical SCI. 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. 37 out of 50 (74%) showed improvements. Sensation recovery was observed in 26% cases, improved trunk control in 22.4%, spasticity reduction in 20% and bladder sensation recovery in 14.2%. All the 50 cases had improvement in postural hypotension. 12.24% wheelchair bound

patients started walking with assistance. Functionally, 20.4% patients showed improved ADLs and 48% showed a positive change in FIM score. 6% cases showed a shift in ASIA scale. A statistical analysis using McNemar's test established a significant association of these symptoms with the intervention. (91) 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.

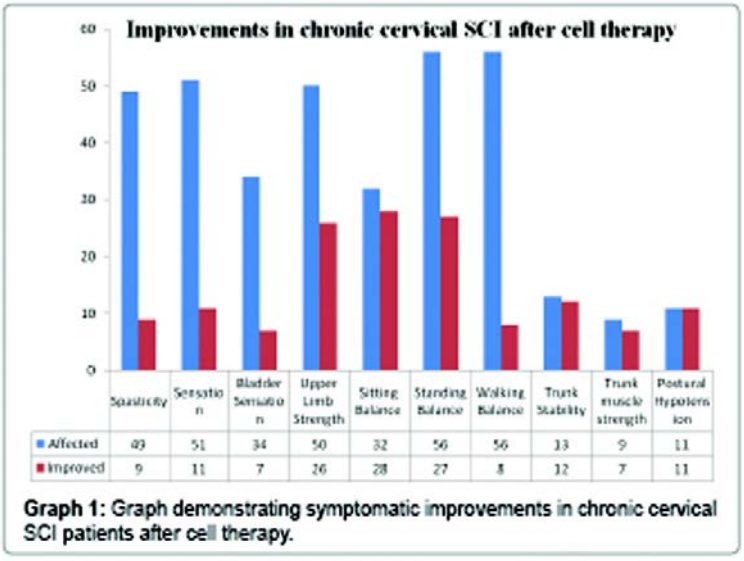


Figure 2: Graph demonstrating symptomatic improvements in chronic cervical SCI patients after cell therapy

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%

Autologous bone marrow derived mononuclear cells for the treatment of spinal cord injury.

A 35 year old female, a case of incomplete spinal cord injury with paraperesis was

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.

Functional recovery in chronic stage of spinal cord injury by Neurorestorative approach: A case report.

A 6 year old girl with traumatic SCI at the level of C7-D 14, years back underwent 2 doses of cell transplantation with Autologous bone marrow mononuclear cells with an interval of 6 months by Sharma et al in 2014.The patient did not have any major or minor side effects . The patient showed clinical improvements throughout the 6 months after transplantation, which was assessed using functional independence measure. There were patchy areas of sensory gain bilateral feet recorded, with improvements in the bladder sensation and control. Improved gait was seen as a result of better strength in abdominals and back extensors.

administered bone marrow derived mononuclear cells intrathecally by Sharma et al. After the therapy, patient had no side effects and underwent intensive rehabilitation. She showed some immediate improvement within a week and a few improvements over a period of six months which were quantified using functional independence measure and manual muscle testing.

Unpublished data

Thoracic Spinal Cord Injury:

We analyzed 165 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 165, 94.54% patients showed improvements while 5.45% of showed no improvements in any of the symptoms. Mild improvements were observed in 13.93% of patients, moderate in 55.75% of patients, whereas, 24.84% of patients showed significant improvements

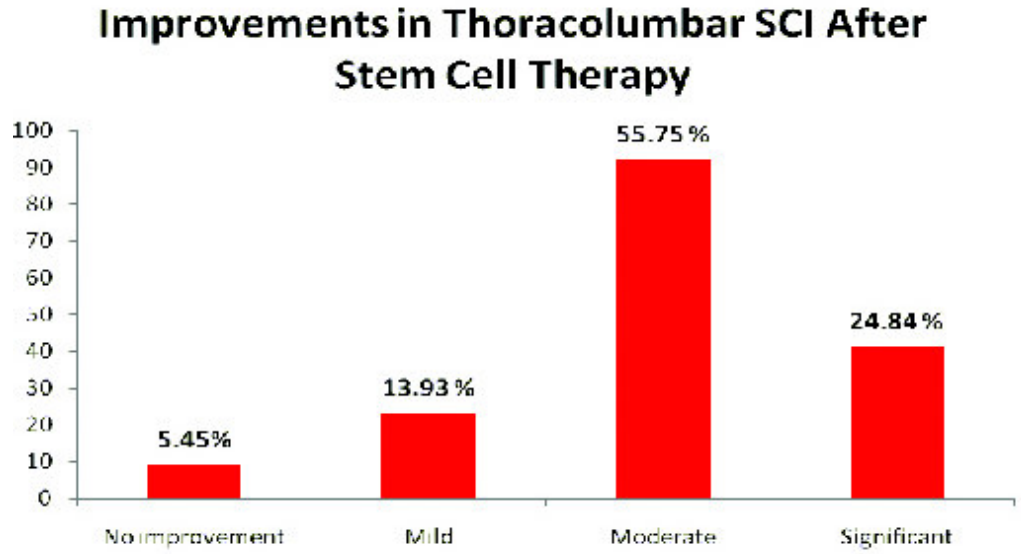


Figure 3: Improvements seen in thoracolumbar SCI after intrathecal administration of autologous BMMNCs.

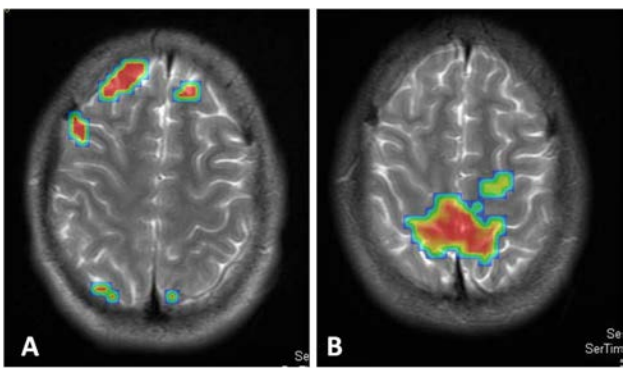


Figure 4: fMRI imaging shows new areas of activation in the brain after stem cell therapy.

Cervical Spinal Cord Injury:

70 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 70 patients, 97.14% patients showed improvements while 2.86% did not show any improvements. Mild improvements were observed in 24.28% of patients, moderate in 54.28% of patients, whereas, 18.57% of patients showed significant improvements.

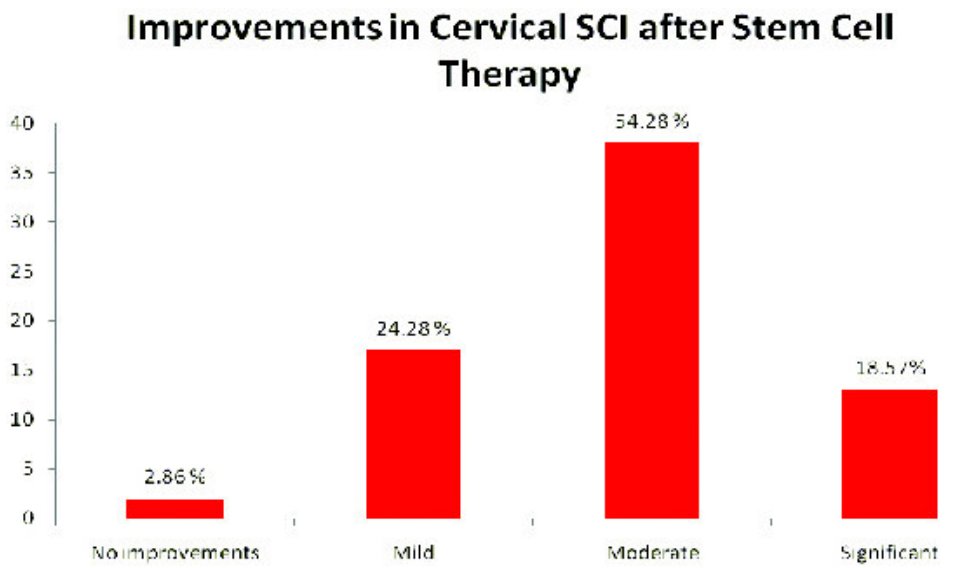


Figure 5: Improvements seen in cervical SCI after intrathecal administration of autologous BMMNCs.

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 progliogenic signals. Hence, the future research should focus on manipulating the cells before transplantation or infusing growth factors manipulating the endogenous cells and modulating them towards producing more oligodendrocytes. (96) Future of regenerative medicine is the use of stem cells along with nanodrug in SCI. (97) 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. (98-100)

Many clinical trials are being conducted in the USA, China, India, Switzerland to optimize the intervention, find the appropriate time of injection, type of cells, route of administration, etc. (101)

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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 tocreaking 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 weknow 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

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, be it 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.

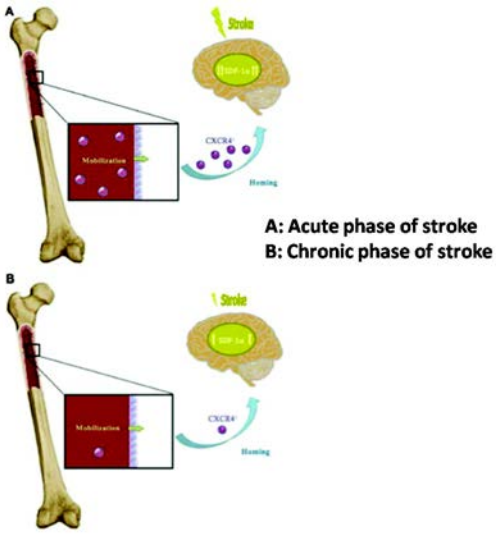

Figure 1: Natural process of mobilization of endogenous bone marrow stem cells after stroke. Courtesy: Borlongan CV, Glover LE, Tajiri N, Kaneko Y, Freeman TB. The Great Migration of Bone Marrow-Derived Stem Cells Toward the Ischemic Brain: Therapeutic Implications for Stroke and Other Neurological Disorders. Progress in neurobiology. 2011;95(2):213-228. doi:10.1016/j.pneurobio.2011.08.005.

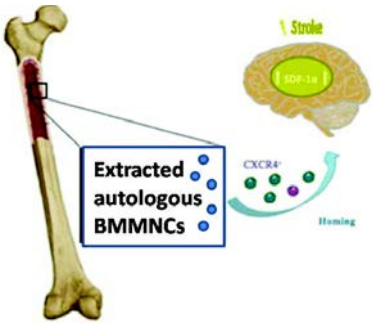

Figure 2: The transplanted autologous BMMNCs provide large number of stem cells in the chronic phase which mimic the natural pathway (depicted in figure 1) to repair the damaged brain areas.

Animal studies:

There are various clinical 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 (6-12).

Zhao et al. in 2002 conducted a trial to assess whether transplantation of human MSCs into the brain of ischemic rats demonstrated any changes functionally. Purified human MSCs were grafted into the cortex surrounding the ischemia 1 week after cortical brain ischemia in rats. Two and 6 weeks after transplantation animals were assessed for sensorimotor function. Ischemic rats that received human MSCs exhibited significantly improved functional performance in limb placement test. The authors concluded that the observed functional improvement might have been mediated by proteins secreted by transplanted human MSCs, which could have upregulated host brain plasticity in response to experimental stroke (6).

Shyu et al. in 2006 performed a clinical trial where intracerebral transplantation of peripheral blood hematopoetic stem cells was introduced in one group of rats with chronic cerebral ischemia, and compared with vehicle-treated control rats. PBSC implantation promoted the formation of new vessels, thereby increasing the local cortical blood flow in the ischemic hemisphere, enhancing the angiogenic architecture over the ischemic brain. quantitative reverse transcription-PCR analysis showed significantly increased modulation of neurotrophic factor expression in the ischemic hemisphere of the PBSC-transplanted rats compared with vehicle-treated control rats (7).

Human studies

There are a few clinical trials conducted of humans, to find the efficacy of cell transplantation after stroke. Clinical improvement was seen in the form of decreased spasticity and paresis, resulting in improved walking, improved functional recovery, and restoration of neurological deficits by the process of increased neural plasticity (13-19).

Prasad et al. in 2012 conducted a non-randomized clinical trial to evaluate the feasibility, safety and clinical outcome of administering bone marrow mononuclear cell (MNCs) intravenously to patients with sub acute ischemic stroke. 11 patients with ischemic stroke were included in this study. Intravenous administration of bone marrow MNCs was carried out. They were assessed on National Institute of Health Stroke Scale, Barthel Index, modified Rankin Scale, MRI, EEG and PET. Results demonstrated favorable clinical outcomes. The authors thus concluded that intravenous bone marrow mononuclear cell therapy appears feasible and safe in patients with sub acute ischemic stroke (17).

Friedrich eta l. in 2012 conducted a clinical trial where intra-arterial autologous BMMCs were infused in 20 patients with moderate to severe acute middle cerebral artery infarcts. Mononuclear cells were isolated from bone marrow aspirates and infused at the proximal middle cerebral artery of the affected hemisphere. National Institutes of Health Stroke Scale (NIHSS) scores, seizures, epileptogenic activity on electroencephalogram, and neuroimaging complications including new ischemic, hemorrhagic, or neoplastic lesions were the outcomes and tests on which all the patients were monitored. Satisfactory clinical improvement occurred in (30%) patients at 90 days. 40% showed a good clinical outcome. Infusion of intra-arterial autologous BMMCs appears to be safe in patients with moderate to severe acute middle cerebral artery strokes (18).

Lee et al. in 2010 undertook a study to evaluate the long-term safety and efficacy of intravenous MSCs transplantation in a larger population. an open-label, observer-blinded clinical trial of 85 patients with severe middle cerebral artery territory infarct was conducted. Patients were randomly allocated to one of two groups, those who received intravenous autologous ex vivo cultured MSCs (MSC group) or those who did not (control group), and followed for up to 5 years. 16 were included in the MSC group and 36 were in the control group. Clinical improvement was observed in the patients of MSC group on modified Rankin scale. The authors correlated this clinical improvement with serum levels of stromal cell-derived factor-1 and the degree of involvement of the subventricular region of the lateral ventricle (19).

Bang et al. in 2005 examined the feasibility, efficacy, and safety of cell therapy using culture-expanded autologous MSCs in 30 patients with ischemic stroke with middle cerebral artery infarct and severe neurological deficits. These 30 patients were divided into one of two treatment groups- the MSC group (n = 5) received intravenous infusion of autologous MSCs, whereas the control group (n = 25) did not receive MSCs. Changes in neurological deficits and improvements in function were compared between the groups for 1 year after symptom onset. Patients in the MSCs treated group showed improved outcomes in the Barthel index and modified Rankin score. Thus the authors concluded that in patients with severe cerebral infarcts, the intravenous infusion of autologous MSCs appears to be a feasible and safe therapy that may improve functional recovery (20).

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. (21) 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 thosewith 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.

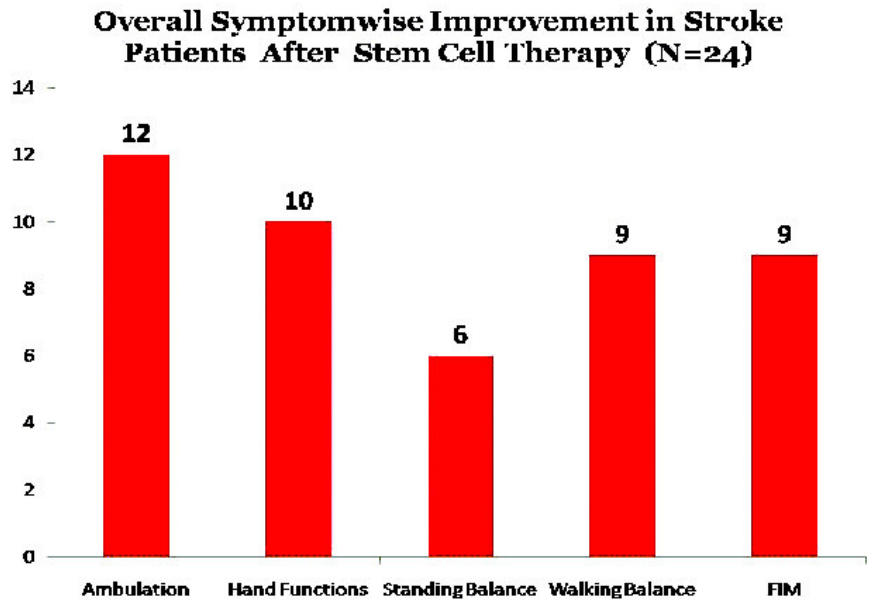

Figure 3: Symptom-wise improvements seen in patients of stroke after intrathecal administration of autologous BMMNCs.

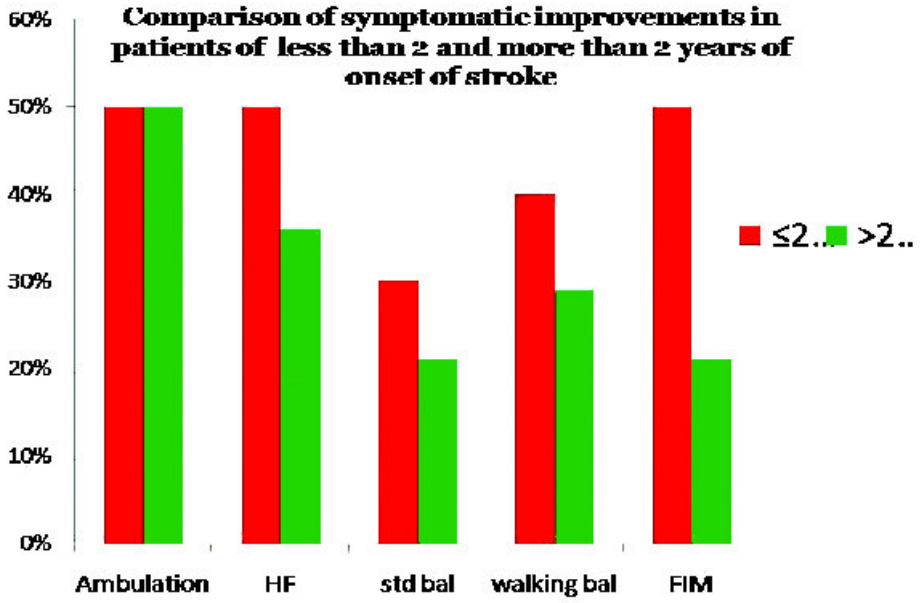

Figure 4: Comparison of symptomatic improvements in patients according to duration since onset of stroke.

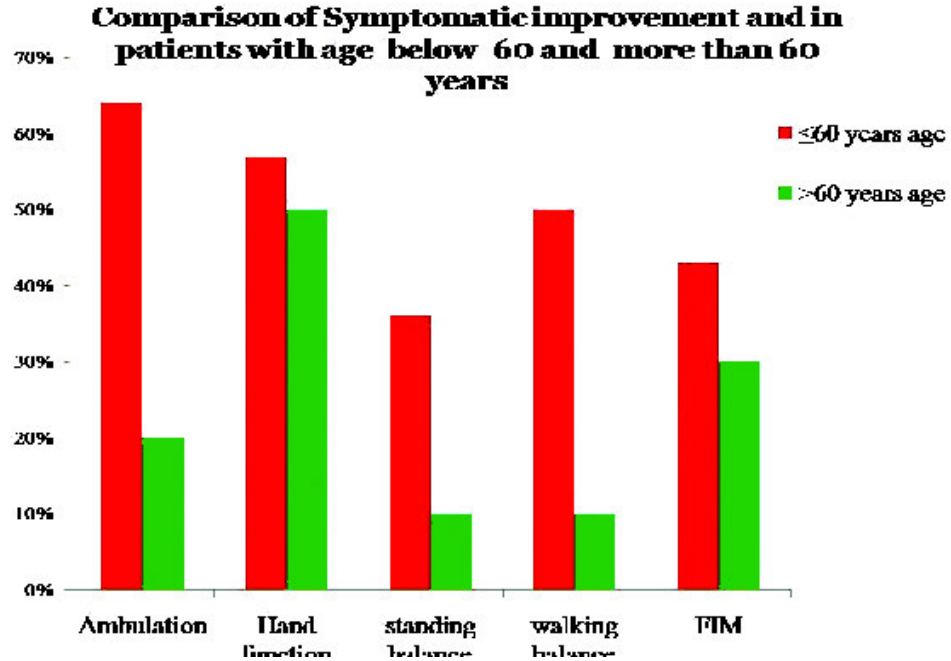

Figure 5: Age-wise comparison of symptomatic improvements in patients of stroke.

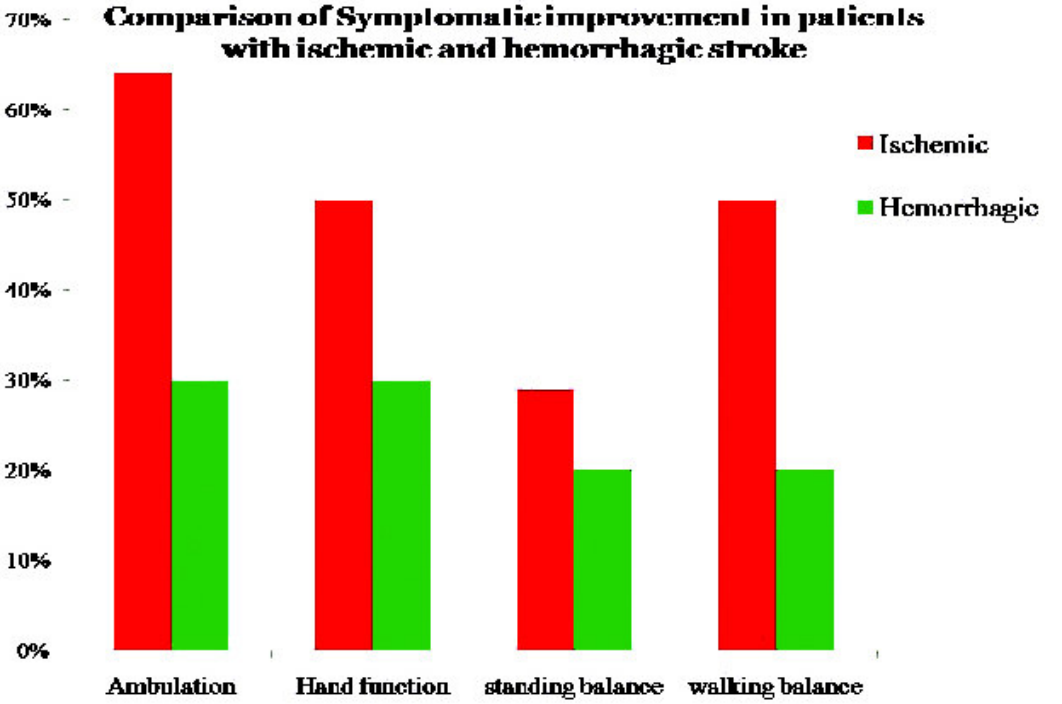

Figure 6: Comparison of symptomatic improvements according to the type of stroke.

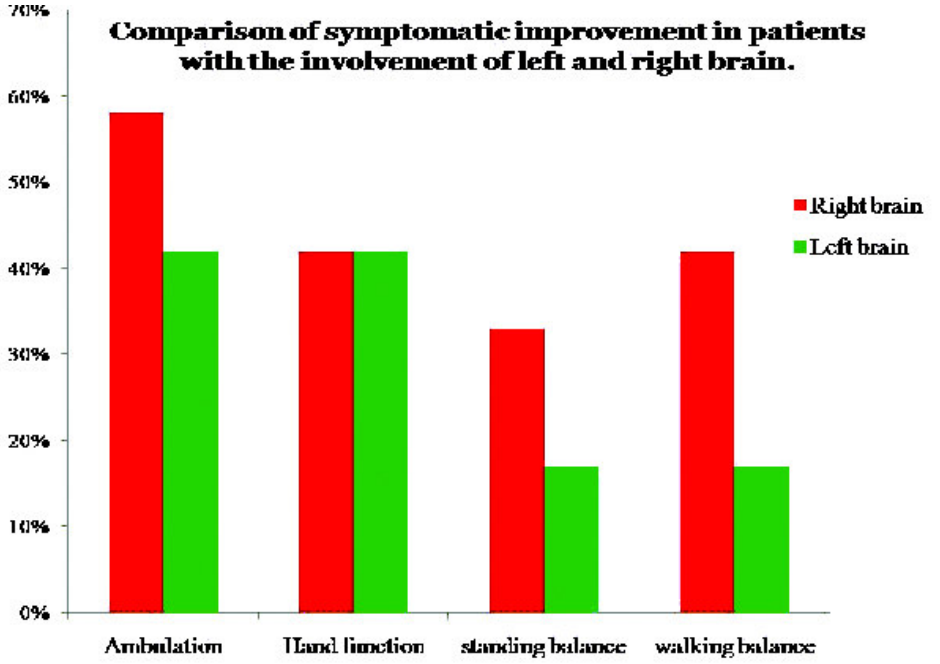

Figure 7: Comparison of symptomatic improvements according to side of the brain involved.

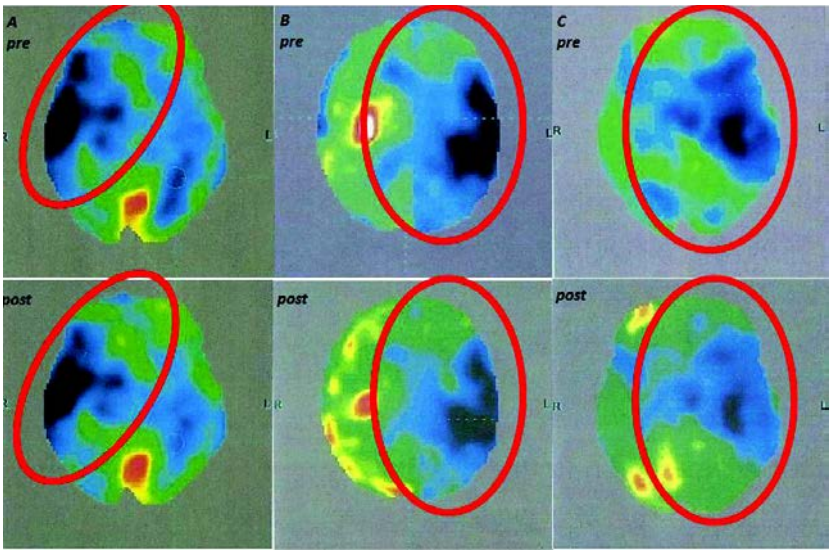

Figure 8: Increased FDG uptake seen in 3 patients. Blue areas representing hypometabolism have turned green after stem cell therapy which represents normal metabolism.

1. A case report of a male patient with a chronic right middle cerebral artery ischemic stroke was discussed in detail. (22) Inspite of rehabilitation, the patient had reached a plateau stage. He underwent autologous bone marrow mononuclear cells intrathecally alongwith rehabilitation, along with regular follow ups. Post cell therapy, at 1 month, he showed improved static and dynamic balance, decreased spasticity, improvement in his hand grip, improved ability to walk and climb stairs. Generalized fatigue reduced and improvement in speech was observed. At 3 months, the above improvements were maintained, along with better walking capacity indoors as well as outdoors. At 10 months the patient underwent second dose of cell therapy. Post second dose, he showed improvements in repertoire, control, and quality of left hand movements. Voluntary control of left hand improved. Thus, we hypothesize that cell therapy may be safe, novel and appealing treatment for chronic ischemic stroke
2. A 69 year old female patient with a history of hemorrhagic infarct was administered intrathecal autologous bone marrow derived stem cell therapy as part of the neuroregeneration and rehabilitation therapy (NRRT) along with rehabilitation. (23) She exhibited rightsided hemiplegia with impaired cognition, speech as well as bladder and bowel function. she also showed hemineglect of right side of the body and was emotionally labile. Functionally she was dependent on her caregivers for all her activities of daily living (ADLs). She was on rehabilitation previous to cell therapy, but did not show any significant changes. Post cell therapy, awareness of right side ofthe body was present and the patient tried to use it for functional activities. She showed lesser crying spells, thus less labile emotionally. Increased attention span, ability to participate

in conversations was also noted. Voluntary control of right upper extremity improved, with reduction in spasticity and better strength. All these improvements translated into her ADLs, which were quite efficient. This case report supports the concept of neuroregeneration with bone marrow stem cells as a novel strategy having great therapeutic potential.

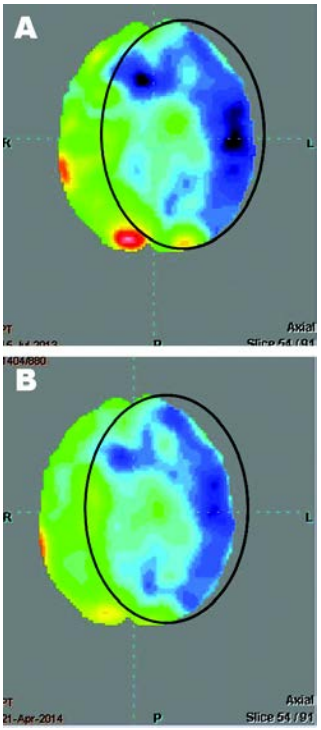

Figure 9: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image which have reduced after SCT.

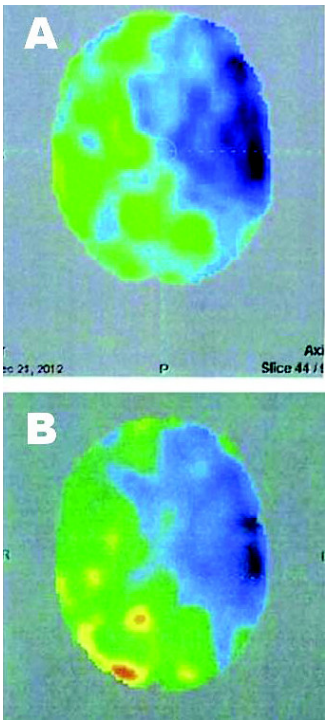

Figure 10: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism. Blue areas depicting hypometabolism in the pre SCT image which have reduced after SCT.

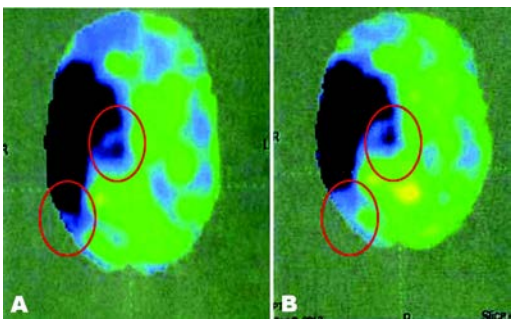

Figure 11: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue/black areas depicting hypometabolism in the pre SCT image which have reduced after SCT.

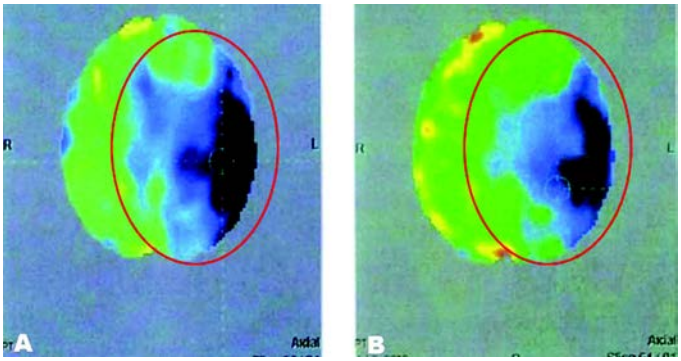

Figure 12: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue/black areas depicting hypometabolism in the pre SCT image which have reduced after SCT.

Future directions

There are many areas which need 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 (20).

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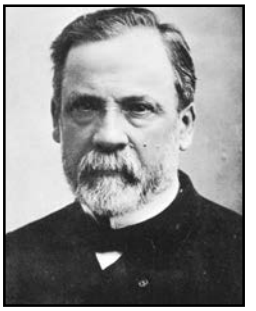

Do not fear to defend new ideas even the most revolutionary, your own faith is what counts most. But have the courage also to admit an error as soon as you have proved it to yourself, that your idea is wrong. Science is the graveyard of ideas. But some ideas that seem dead and buried away may at one 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

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 motor neurons only 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 are caused by the degeneration of motor nerves and can lead variety of upper and lower motor neuron symptoms.

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 symptoms of bulbar region. As the disease progresses the weakness spreads to all the regions. 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 varies over several permutations of patterns of weakness and rate of progression. Some factors have been identified, which may 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. Therefore for more accurate diagnosis of ALS revised El-escorial criteria are used. El-escorial system categorizes the patients into Definite, Probable and Possible ALS. For the diagnosis of 'Definite ALS' presence of both UMN and LMN symptoms in more than one body regions and/or elemyographic evidence of anterior horn involvement is required (8).

Currently ALS is incurable. 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 gastrotomy (PEG) preventing dysphagia related complications.

Current management strategies for ALS

As multiple body systems are involved, the best approach is a multidisciplinary holistic management of the disease. Currently ALS is treated with a combination of medical, surgical and rehabilitative treatments (9).

Pharmacological management

Mainstay of pharmacological management is Riluzole. Riluzole inhibits the release and modulates the post-synaptic activity of Glutamate which is found to cause excitotoxicity in ALS. It is the only medicine that has shown significant effect on the survival duration in ALS is Riluzole (10,11,12). Other medicines like Talampanel, Memantine, Cephalosporins, antioxidants, agents modulating apoptosis, anti-inflammatory medicines like Thaliomide and Celastol and Autophagy regulators like rapamycin and lithium are being tested with no conclusive results (13).

Non Pharmacological management

With no pharmacological means of altering the disease process current management of ALS is widely palliative and symptom oriented. Regular moderate intensity physical exercise (9), and in the later stages of the disease artificial ventilatory support (14) dysphagia management and percutaneous endoscopic gastrotomy (PEG) (15) are routinely used.

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.

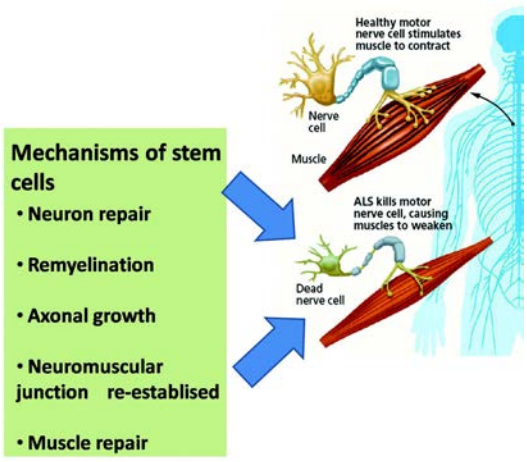

Figure 1: Mechanism of action of stem cells in MND/ALS.

Animal Studies

Zhou et al. 2013 (16) 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 (17) 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 (18) 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.

Various other pre-clinical animal studies have shown benefits in the motor function. Human clinical trials are currently being undertaken. Safety of allogenic cells is also being studied and for the use of manipulated allogenic cells strict laboratory guidelines and clinical protocols must be followed.

Human studies

Studies conducted have used various modes of administration and sources of cells. Frontal motor cortex transplantation suggested significant increase in survival. However the procedure of administration is extremely complex and can be performed only by a skilled surgeon (19).

Intra-spinal transplantation post laminectomy at the level of C1-C2 showed functional recovery of respiratory function, muscle strength and bulbar impairment. Another trial with spinal transplantation arrested the drop in the respiratory function and

improvement on neurological scales. Intra-spinal administration also showed neurotrophic effect and preservation of existing neurons (20,21,22).

Intrathecal transplantation of Autologous bone marrow mononuclear cells was found to reduce the rate of progression of the disease and drop in the ALS-FRS scores (23). A recent Phase I clinical trial, of multiple transplantations of autologous bone marrow mesenchymal stromal cells showed that there was no drop in the ALS-FRS r score. Within the 12 months of follow up patients did not develop any side effects (24).

Baek W. et al. 2012 (25) assessed the safety of delivery of stem cells through intraventricular route by insertion in reservoir of Ommaya. This delivery route was found to be safe in the said case report but larger studies for its feasibility and suggesting efficacy are lacking.

Due to lack of comparative studies between the routes of administration, the evidence for best route of administration remains scarce.

A phase I safety trial conducted by Mazzini et al. 2010 showed that autologous mesenchymal stem cells are safe to use for the treatment of ALS. The cells were injected intraspinally at the thoracic level and motor function improvement was observed (26).

Karussis et al. 2010 conducted a safety and efficacy trial with intrathecal and intravenous administration of autologous mesenchymal stem cells in 19 patients of ALS. These patients were followed up for 25 months. This trial reiterated the safety of autologous mesenchymal stem cells and also showed the immunomodulatory effects of MSCs in ALS which reflected as halting of the progression (27).

A long term safety study with a follow up of over 9 years by Mazzini et al. 2012 showed that the treatment with autologous mesenchymal stem cells was safe but clinical could not be determined (28).

All there are investigational studies for different types of cells lack of comparative studies prevents from any conclusion regarding a better cell type.

Mechanism of Action of cellular therapy in ALS

To understand how stem cell therapy benefits in ALS, it is important to understand the pathophysiology of ALS. Accelerated degeneration of axons of motor neurons is in the spinal cord as well as motor cortex is noted in ALS (29). Various mechanisms have been thought to contribute. Non-neuronal glial cell, astrocytes and T-lymphocytes involvement is also suggested in some studies (30,31). Up regulation of superoxide dismutase causes cascade of events and thereby oxidative stress. Whereas upregulation of glutamate cause excitotoxicity. Autoimmunity and widespread neuroinflammation are also stipulated contributors to the pathophysiology of ALS (32).

The maintenance of the adult tissue is achieved by large reservoirs of stem cells that have ability to regenerate over long periods of time and that can regenerate into

various types of tissues (33). Replacement of degenerated motor neurons due to these causal factors 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 (34). 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 (35). They further demonstrate immomodulatory, anti-inflammatory and cytoprotective properties (36). The factors secreted by these cells bring about neoangiogenesis (37). These paracrine effects lead to neuroprotection and subsequent alteration in the disease course and progression.

Our results

Published results

This was a retrospective controlled cohort study of 57 patients (38). 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).

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)

Table 1

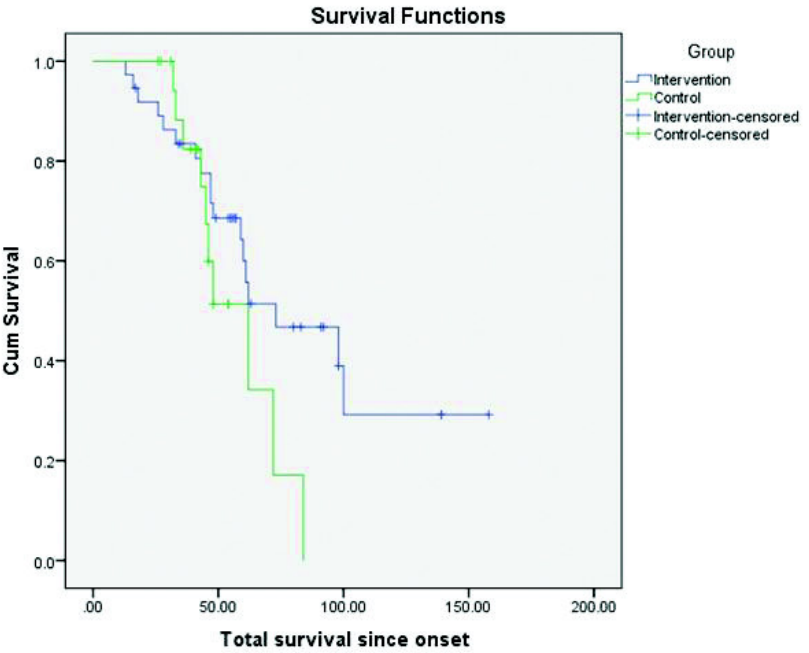

Figure 2 Kaplan Meier survival analysis comparing the mean survival duration of the intervention and control group

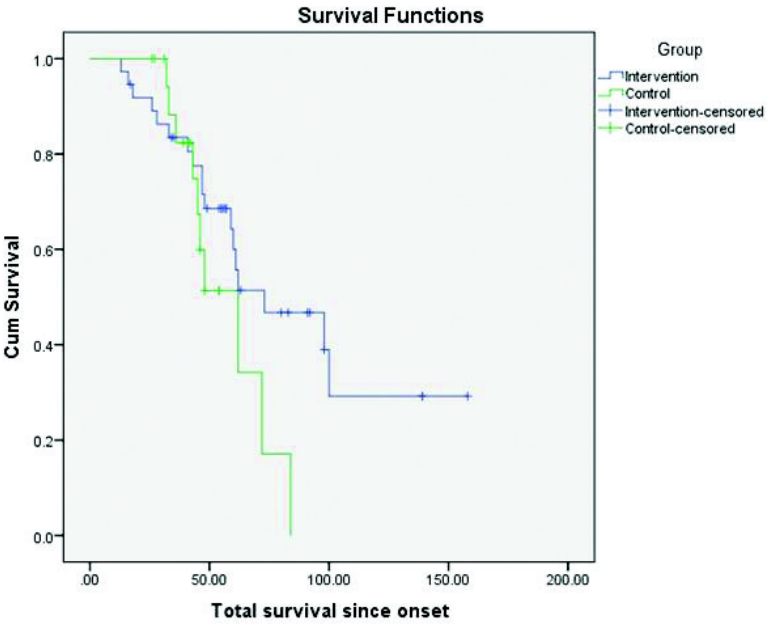

Figure 3: Kaplan-Meier Survival comparison for the patients in intervention group below and above 50 years of age at the onset

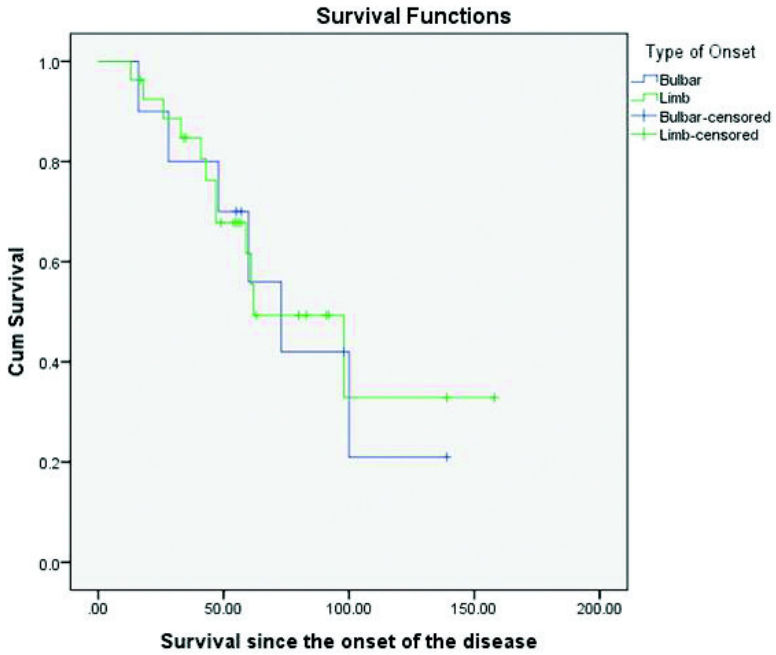

Figure 4: Kaplan-Meier Survival comparison for the patients in intervention group with bulbar onset and limb onset

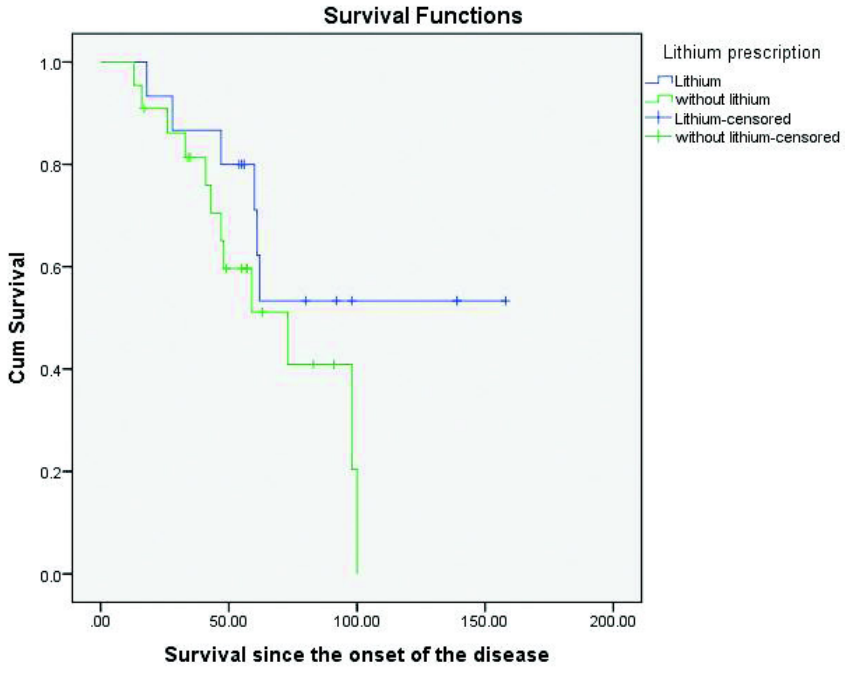

Figure 5.Kaplan-Meier Survival comparison for the patients in intervention group with or without Lithium prescription.

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. [23]	1995	USA	1200	55.7	37.5 months	88.86 months
Forbes et al. [24]	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. [25]	2005	Italy	79	64.4	39.2 months	88.86 months
Osuntukun et al. [26]	1974	Nigeria	92	39.2 (1.6)	Dead – 73 months Alive – 79 months	Dead – Alive -
Radhakrishnan K [27]	1986	Libya	23	51	42	88.86 months
Norris et al [28]	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 [29]	1997	Serbia	58	Men – 56.2 Women – 56.6	2 years survival probability – 62% 5 years survival probability – 27% 7 year survival probability –	2 years survival probability – 89% 5 years survival probability- 62.3% 7 year survival probability –

					27%	43.2%
Eisen et al. [30]	1993	USA	246	Not available	< 40 years – 98.4months 61 – 70 years – 31.2 months	< 40 years – 61 – 70 years -
Traynor et al. [31]	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. [32]	2002	USA	77	63	23 months	88.86 months
Tysnes et al. [33]	1991	Norway		60.9 Range - 34 to 82	28 months	88.86 months
Turner et al. [34]	2003	England	769	Long survivors – 43, short survivors - 57 , others - 62	43months	88.86 months

Table 2: Mean and median survival duration as observed in previous epidemiological studies and its comparison with the current findings

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. 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 3) (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 marginally 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.

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]

Table 3: Survival analysis

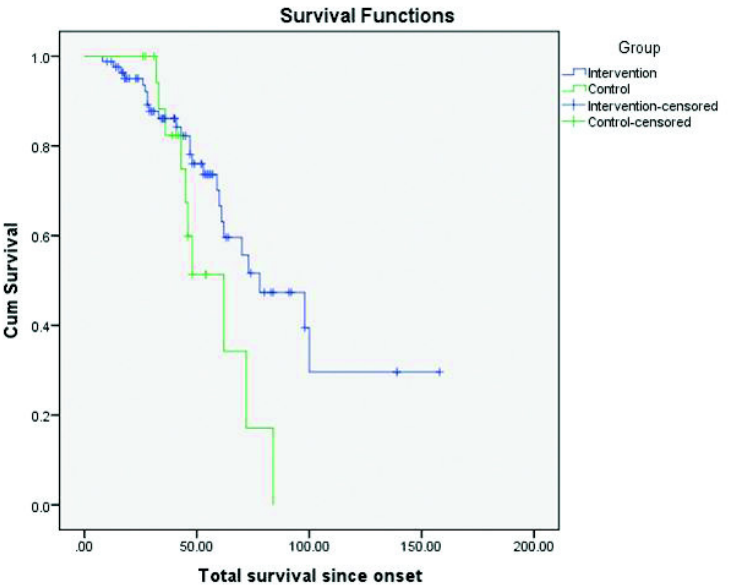

Figure 6. Comparitive Kaplan-Meier Analysis of ALS patients with and without stem cell transplantation

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 may have a positive effect on the duration of survival in ALS.

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 (39).

Nur-Own cells transplantation

Recently brain storm 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.

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

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. However, they have failed to translate into a successful treatment strategy. Among the numerous barriers to finding effective interventions to improve outcomes after TBI, the heterogeneity of the injury and identification and classification of patients most likely to benefit from the treatment are considered some of the most significant challenges. In chronic TBI, the life expectancy of the affected is normal, but there is high prevalence of the residual disability arising from the injury. These include hemiparesis, spasticity, cognitive, emotional and behavioral

issues, etc. The available pharmacological modalities manage these disabilities, but their effect wears off gradually. The rehabilitation resources are inadequate for the increasing number of survivors of TBI. There is diffuse white matter damage which cannot be addressed by current medical treatments. Also, the gliotic areas in the brain cannot be reversed.

Stem cell therapy in TBI

Due to the brain's limited capacity to regenerate the damaged neaurons, 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. (7,8)

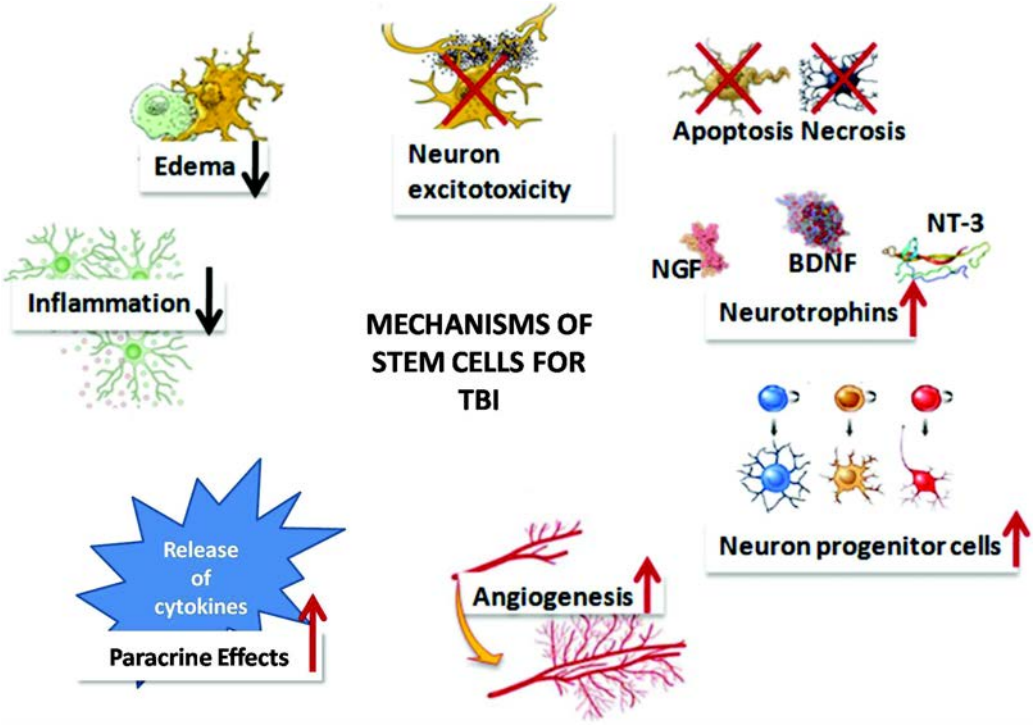

Figure 1: Mechanisms of stem cells for TBI

Animal studies

Various experiments on animal models have been carried out to test the safety and feasbility of different types of cells. Reiss et al, transplanted embryonic cells in experimental rats and recorded dramatic improvements. But, over the period of observation they also recorded tumor formation in the rats, raising serious safety concerns about the use of these cells. (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. (10-13) Bone marrow stem cells were also found to be efficacious. (14) In rat models, these cells modulated the inflammation-associated immune cells and cytokines in TBI-induced cerebral inflammatory responses. (15) Recently umbilical cord cells have also been tested in rats. In an experimental study, rats were injected with brain-derived neurotrophic factor (BDNF) gene-modified umbilical cord mesenchymal stem cell (UCMSC). These cells survived and migrated to the cerebral tissues. They led to dramatic improvements in behavior and other neurological functions. (16)

Human Studies

Stem cell therapy for TBI is still in an experimental stage. Not many clinical trials have been conducted on human subjects. In 2009, Zhang et al, conducted a study to assess the safety and feasibility of a combined procedure to deliver autologous mesenchymal stromal cells to patients with traumatic brain injury. (17) They found that neurological functions improved significantly at 6 months after cell therapy. In 2011, Cox et al, performed a study to demonstrate the effect 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. (18) Later in 2013, Wang et al published the results of the study conducted on patients with sequelae of TBI. They administered 40 patients with umbilical cord mesenchymal stem cells. They observed improved neurological functions and self care in these patients as compared to the controls. (19)

Our Published Results

We conducted a study on 14 cases who were administered with autologous bone marrow mononuclear cells, intrathecally. (20) 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.

One week after the intervention, 5 out of 13 patients improved in speech, 3 improved in trunk and upper limb activity, oromotor activities, muscle tone, voluntary control, ambulation and gait pattern and posture, 2 improved in lower limb activity, balance and psychological status, and 1 improved in cognition, memory, ADLs and communication.

Three months after the intervention, 8 showed improvements in voluntary control, 7 in trunk and upper limb activity, 6 in speech, lower limb activity and ambulation and gait patterns, 5 in posture, 4 in muscle tone, balance, ADLs, psychological status, coordination

At the end of six months, a percentage analysis was carried out for improvement in every symptom. Amongst 13 patients, 73% showed improvement in balance, 69% in voluntary control, 60% in memory, 57% in oromotor activities, 55% in lower limb activity and ambulation and gait patterns, 54% in trunk and upper limb activity, 50% in speech, posture and communication, 45% in psychological status, 38% in cognition, 36% in muscle tone and coordination and 33% in ADLs. SF8 scale was performed on 7 patients who met the eligibility criteria of the test. Six months after the intervention, the mean physical component summary (PCS8) score improved from 39.11 to 45.27 and mean mental component summary (MCS8) from 48.44 to 52.55. On the DRS, 6 out of 13 patients showed reduced scores while the scores remained the same in 7 cases. It was observed that these patients showed improved scores primarily in the cognitive component of the DRS. 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.

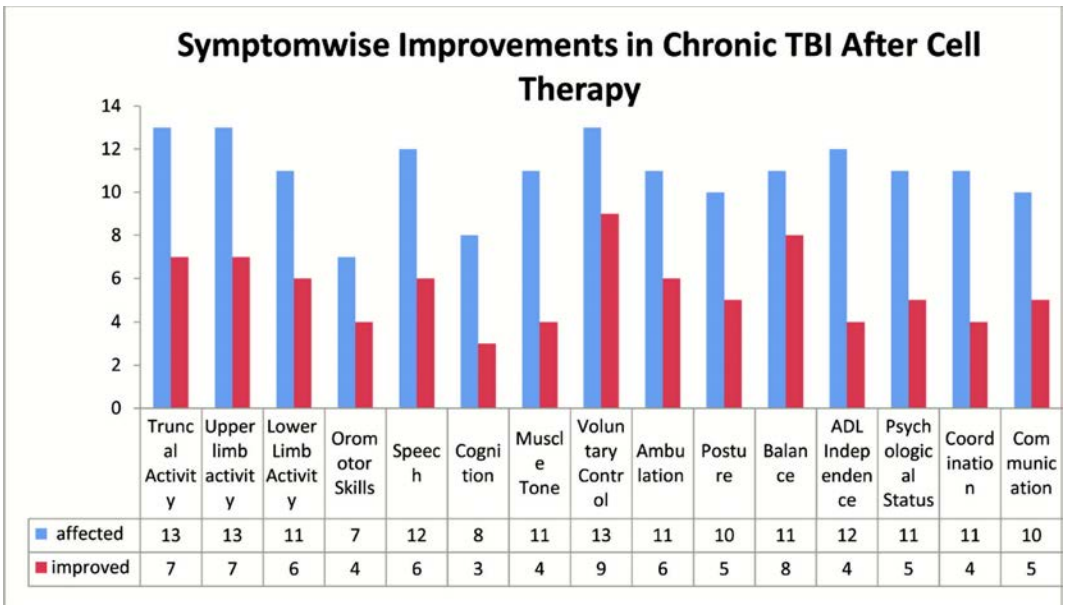

Figure 2: Symptom-wise improvements in chronic TBI patients seen after intrathecal administration of autologous BMMNCs

Patient no.	SF8 pre		SF8 post	
	PCS8	MCS8	PCS8	MCS8
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 1: SF8 scores before and after intervention suggesting improved quality of life.

Patient	Areas of the brain showing improved metabolism in PET CT scan	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

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

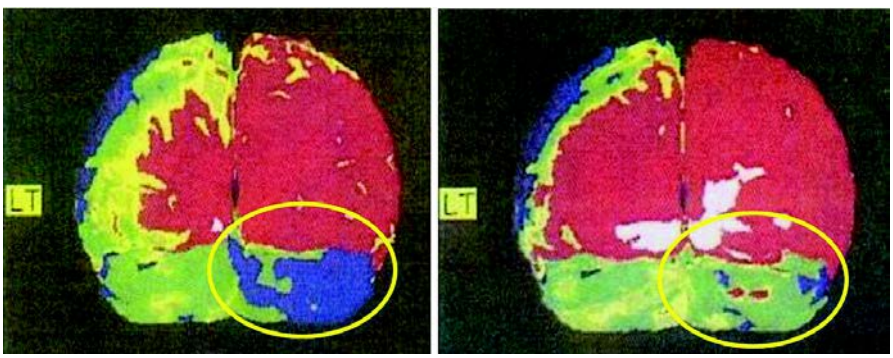

Figure 3: PET CT Scan showing improved metabolic activity which is indicated by decrease in blue areas after stem cell therapy

Future directions

Future clinical studies should be conducted to optimize this therapeutic intervention. Type of cells, route of administration, quantity of cells, frequency of doses and the time interval between consecutive doses should be established. 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 and 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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15.
Stem Cell Therapy for Intellectual Disability

Intellectual disability (ID) is a cluster of syndromes and disorders characterized by low intelligence and associated limitations in adaptive behaviour. The communication skills, home-living skills; use of community resources; health, safety, leisure, self-care, and social skills; self-direction; functional academic skills (reading, writing, and arithmetic); and job-related skills are affected in ID. The IQ score of individuals with ID is generally below 70-75. (1) It is a developmental disability which first appears in children under the age of 18. About 30% of ID cases are caused by hereditary factors. Intellectual disability may be caused by an inherited genetic abnormality, such as fragile X syndrome. Fragile X, a defect in the chromosome that determines sex, is the most common inherited cause of Intellectual disability. Single-gene defects such as phenylketonuria (PKU) and other inborn errors of metabolism may also cause Intellectual disability if they are not discovered and treated early. An accident or mutation in genetic development may also cause retardation. (2)

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

Currently, there is no treatment available for ID. But with correct support and teaching, most individuals can learn to do their daily activities. Management of the patient depends on the level of the child and the associated conditions such as epilepsy, hyperkinesis, behaviour problem and sensory handicaps. But these strategies do not answer the core neurological problems of ID.

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. Cognition appears to be particularly dependent upon both the normal synaptic connections, as well as the ability to modulate these connections in response to new stimuli, and adapt as necessary. Hence, any disruption in the normal pattern of the synaptic connectivity may result in ID. (3,4)

Unmet Medical Needs

With increasing awareness, the prevalence of intellectual disability has risen considerably. ID is an abnormality that has enormous social effects; it not only affects the people who suffer from it but also the family and society as a group. Hence, to find a cure for it is the need of the hour. None of the treatments currently available repair the damage at the molecular or cellular level.

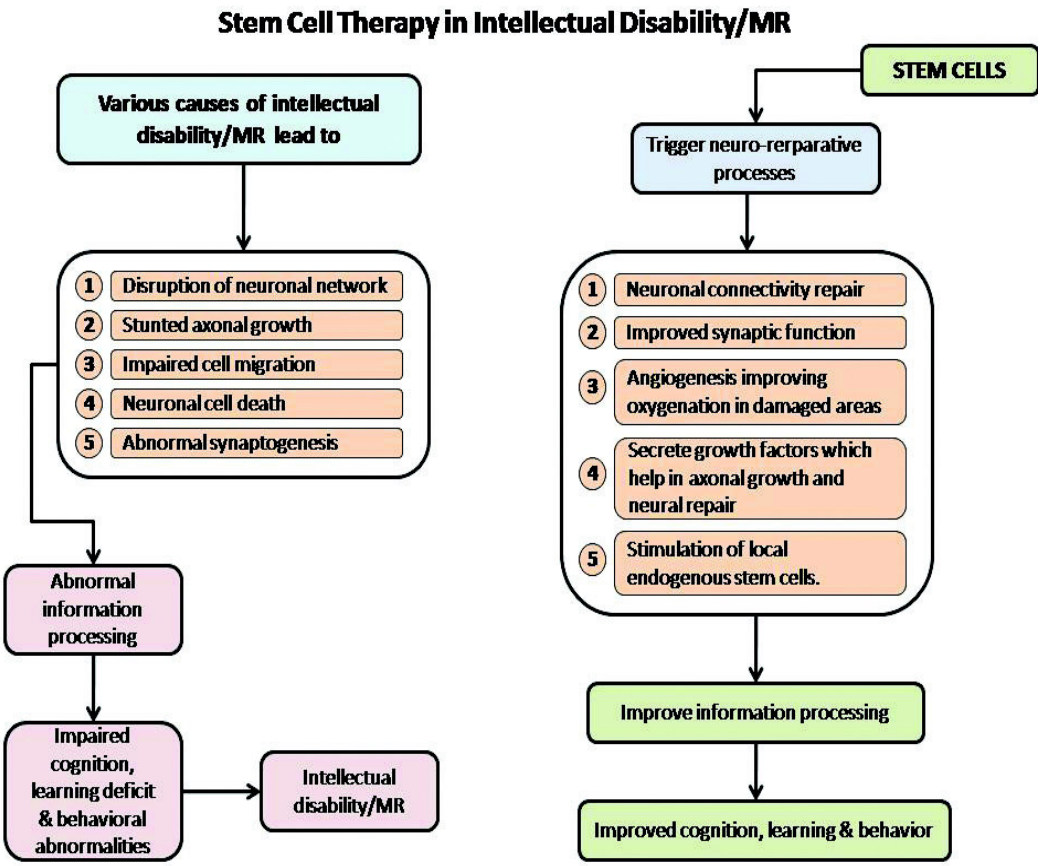

Figure 1: Stem Cell Therapy in intellectual disability

Stem Cell Therapy for Intellectual Disability

In case of intellectual disability, any damage to the brain is a permanent and irreversible damage as the neurons of the brain, once damaged, cannot repair themselves on their own. The underlying neuropathology of intellectual disability includes neuronal death along with disruption in neuronal networks, cell migration, cell multiplication, axon growth, brain plasticity, synaptogenesis, etc. Studies have shown that major defects are recorded in hippocampus and cerebral cortex areas of the brain which further lead to faulty information processing and consecutively affect the cognition and adaptive behavior.

To reverse the damage caused to the central nervous system, only a neurorestorative therapy like stem cell therapy would be beneficial. (5)

Stem cells have a unique property of migrating towards the damaged areas on administration. They survive, migrate, proliferate and differentiate into the required cell types. (6) 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. (7) This could help repair the disrupted neuronal networks in ID and hence improve the information processing.

Not many clinical studies have been carried out to study the effect of stem cell therapy in ID. But animal studies have shown that administration of stem cells may support the ability for structural brain repair as well as cognitive improvement in models with damaged brain.

Our Results

Published data

Positron Emission Tomography-Computer Tomography Scan used as a monitoring tool following cellular therapy in cerebral palsy and mental retardation.

Sharma et al treated a 20 year old male suffering from cerebral palsy and mental retardation. He had diplegic gait, an intelligence quotient score 44 with affected motor activities, balance speech and higher functions. He was given cellular therapy of autologous bone marrow derived mono nuclear cells transplantation followed by multidisciplinary rehabilitation. Six months post therapy, PET-CT scan showed significant increase in the metabolic activity in all four lobes, mesial temporal structures and left cerebellar hemisphere and it is also supported by clinical improvement in IQ , social behavior , speech , balance and daily functioning.

Unpublished data

To demonstrate the effect of autologous stem cell therapy in intellectual disability, we analysed the data of 29 patients. Symptoms such as cognition, remote memory, problem solving, understanding, social inhibition and toilette training were analysed.

Improvement in Intellectual Disability after Stem Cell Therapy (N=29, Improvement- 89.66%)

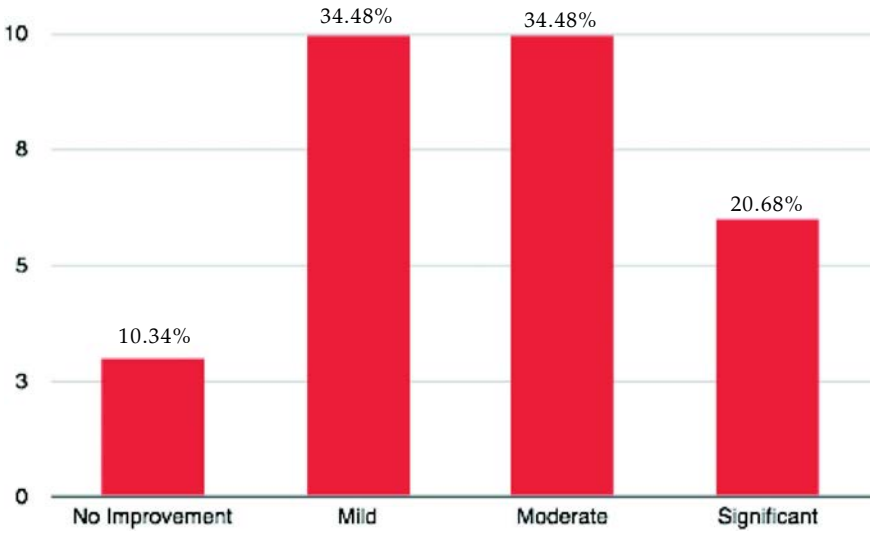

Figure 2: Improvements seen with intrathecal administration of autologous BMMNCs in patients with intellectual disability.

On follow up we found that 89.66% of patients showed improvements while 10.34% showed no change after intervention. 34.48% showed mild improvements, 34.48% moderate improvements and 20.68% showed significant improvements. No adverse events were recorded.

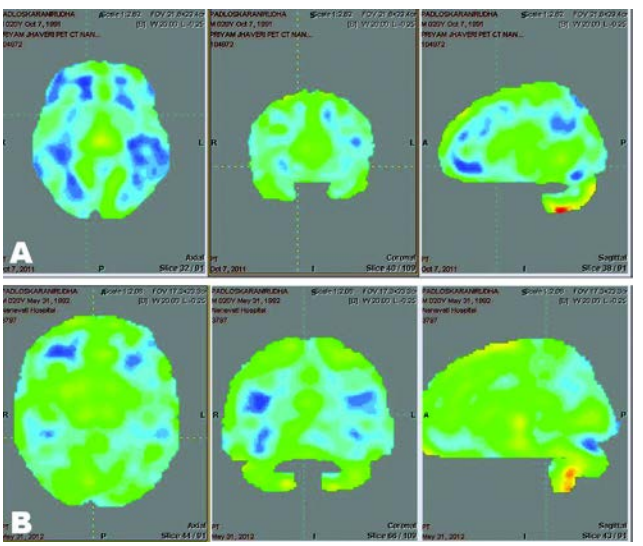

Figure 3: (A) Pre Stem cell therapy PET CT scan showing blue areas with hypometabolism (B) Post Stem cell therapy PET CT scan showing decrease in blue areas which is replaced by green areas indicating improved functioning of the brain

Future Directions

Due to the heteregenous nature of the disorder, it is a challenge to find a cure for intellectual disability. As shown by our results, stem cell therapy addresses the core damage occurring in the brain of an individual with ID. However, there are not many animal or human studies conducted until now to support the evidence. Hence, it is important to conduct large multicentric clinical trials. Use of monitoring tools like PET CT scan should be studied in depth to understand the effect of stem cells at the cellular level.

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16.
Stem Cell Therapy In Cerebellar Ataxia

Cerebellar Ataxia literally means absence of order and denotes a clinical syndrome of incoordination caused by lesions of the cerebellum and its afferent or efferent connections. (1) It is distinguished into a group of hereditary and non-hereditary disorders. Hereditary Cerebellar Ataxia can have an autosomal-dominant, autosomal-recessive, X-linked, or mitochondrial mode of inheritance. (2) Non-hereditary CA can be symptomatic or idiopathic, (3) and symptomatic Cerebellar Ataxia may be caused by multiple causes including malformations, toxic agents, endocrine disorders, and infections.

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.

Stem Cell Therapyin Cerebellar Ataxia:

Stem Cell Therapy is the recent upcoming alternative treatment option for the same. Studies have shown that stem cells migrate to the site of injury from the site of injection. It has been observed that there is improvement in the neurological function following cell therapy. Stem cells work by enhancing angiogenesis and contributing

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 may regulate the levels of VEGF. (5)

These factors have also shown to give rise to various paracrine effects resulting in neoangiogenesis which in turn enhances tissue perfusion. (6) The various mechanisms along with paracrine effects of cellular transplantation may help alleviate 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 that two months after the surgical procedure, the treated mice presented significant improvements in the motor behavior tests performed. (7) 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. (8) 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. (9)

Human Studies

Dongmei et al, studied the effect and safety of umbilical cord mesenchymal stromal cells (UC-MSC) in the treatment of 14 cases of spinocerebellar ataxia (SCA). They found improvement in symptoms like unstable walking and standing, slow movement, fine motor disorders of the upper limbs, writing difficulties and dysarthria. (10) Yang et al, treated 30 patients with umbilical cord blood-derived mononuclear cells (CBMC). They recorded improvement in functional symptoms. (11) Jin et al, in their study assessed the feasibility, efficacy, and potential toxicity of human umbilical cord mesenchymal stem cells (UCMSCs) therapy in 16 patients with spinocerebellar ataxia. On follow up they found that the symptoms were alleviated and there were no adverse events. (12)

Our Results

Published data

Cellular transplantation may modulate disease progression in spino-cerebellar ataxia - a case report

A 33 year old female with SCA was treated with autologous BMMNCs intrathecal transplantation followed by standard rehabilitation. 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 60 cases of cerebellar ataxia. Symptoms such as ambulation, hand functions, balance, stamina/fatigue/ trunk balance and standing were analysed. On follow up, 95% of patients showed improvements while 5% showed no improvement. 33% patients showed mild improvements, 20 % moderate improvements and 2% showed significant improvements.

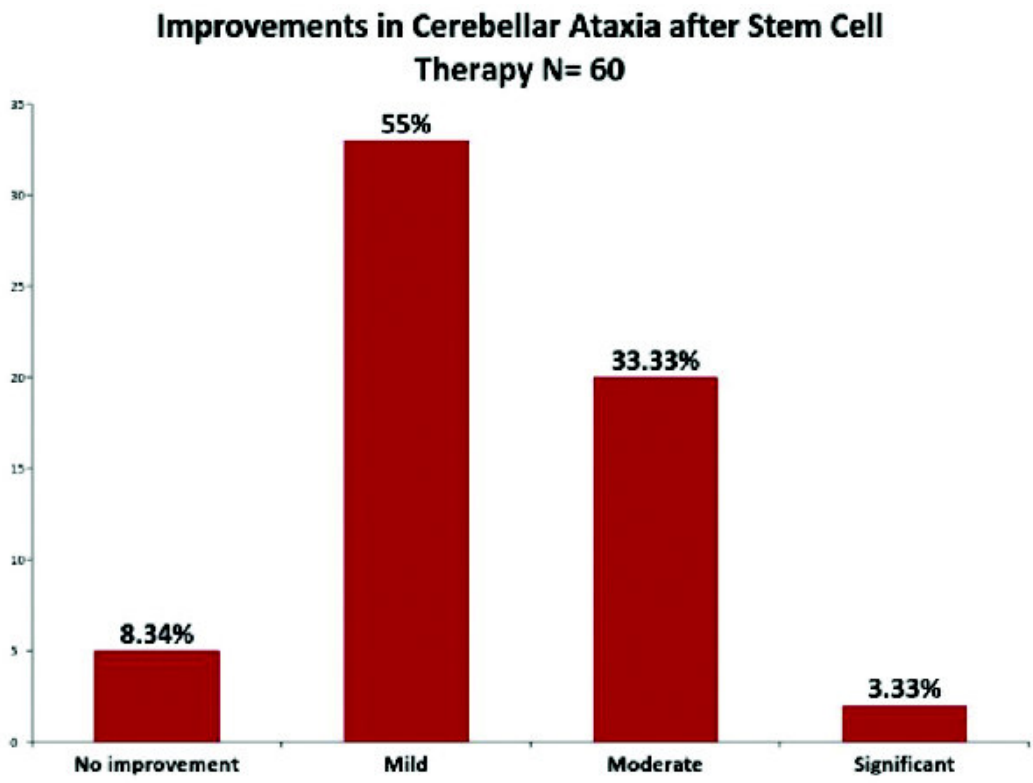

Figure 1: Improvements seen in cerebellar ataxia patients after intrathecal administration of autologous BMMNCs.

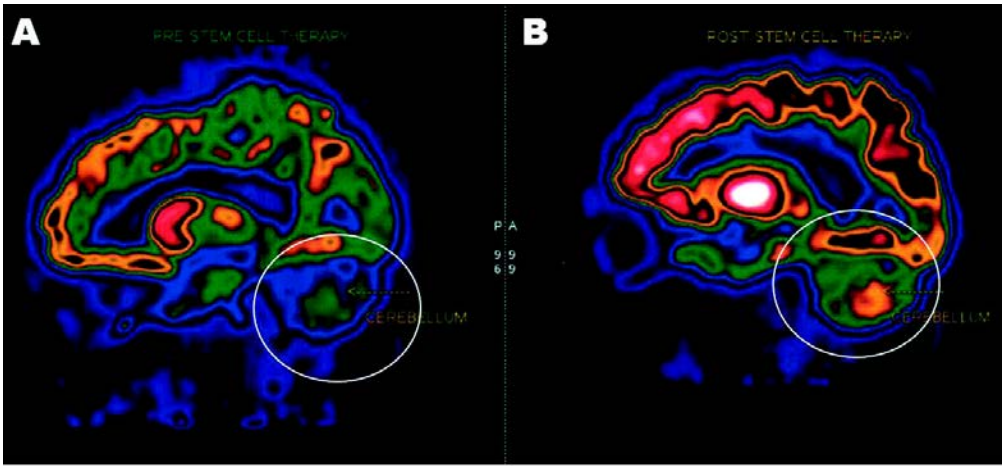

Figure 2: PET CT Scan showing improved metabolic activity which is indicated by increased orange area in the cerebellum after stem cell therapy as indicated by the circles.

Future Directions

In a progressively deteriorating condition like cerebellar ataxia, Stem Cell therapy offers a new promise as an interventional modality and as a future premise in the area of cellular research. However, many aspects of this intervention need to be analyzed in detail to optimize the outcome. Types of cells, dosage, number of interventions, timing of intervention, etc need to be studied. More multicentric randomized clinical trials need to be conducted to translate this intervention into a standardized treatment strategy for cerebellar ataxia.

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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. Musuloskeletal 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.

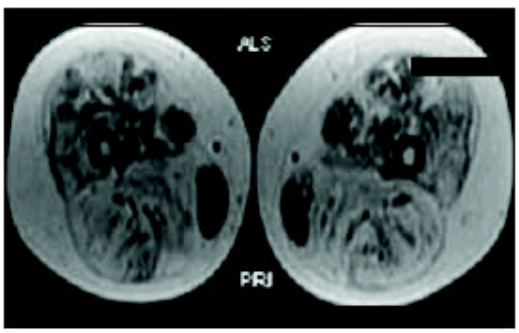

Figure 1A: T1 Weighted Axial Musculoskeletal MRI image before cellular transplantation

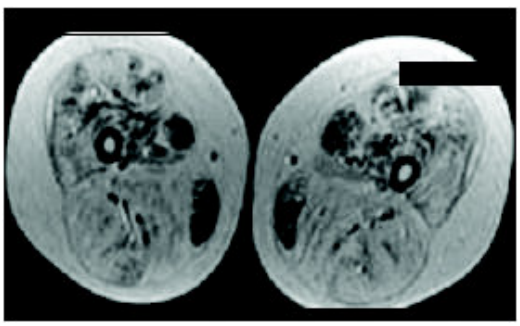

Figure 1B: T1 Weighted Axial Musculoskeletal MRI image 24 months after cellular transplantation showing no reduction in muscle mass and no increase in fatty infiltration suggestive of halting of disease progression in Duchenne Muscular Dystrophy

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 co-related with the clinical improvements.

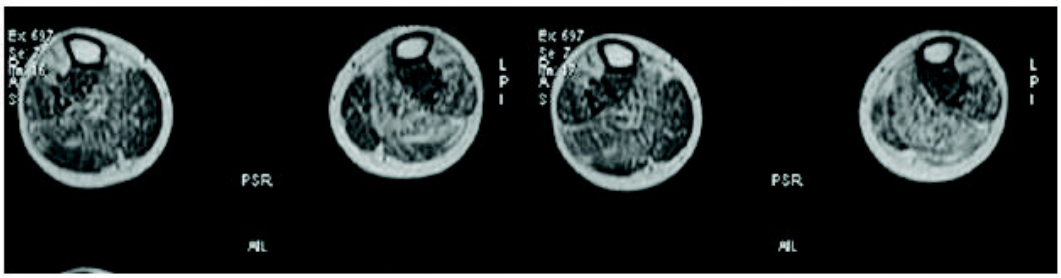

Figure 2A : T1 weighted axial musculoskeletal MRI images of Peroneous Longus and Brevis before Autologous BMMNCs transplantation

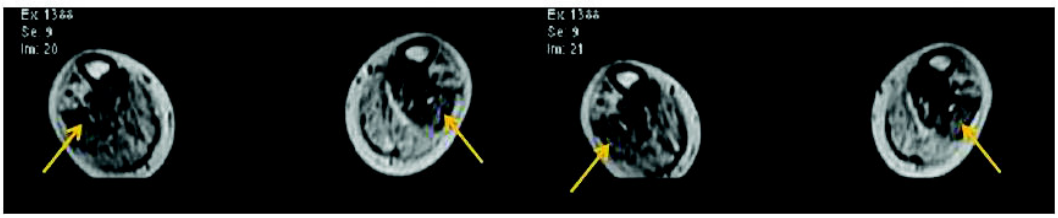

Figure 2B: T1 weighted axial musculoskeletal MRI images of Peroneous Longus and Brevis 6 months after Autologous BMMNCs transplantation, arrows showing muscle regeneration and reduced fatty infiltration

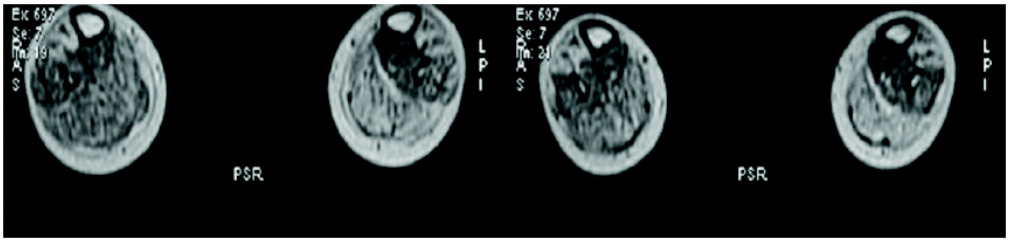

Figure 3A : T1 weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus before Autologous BMMNCs transplantation

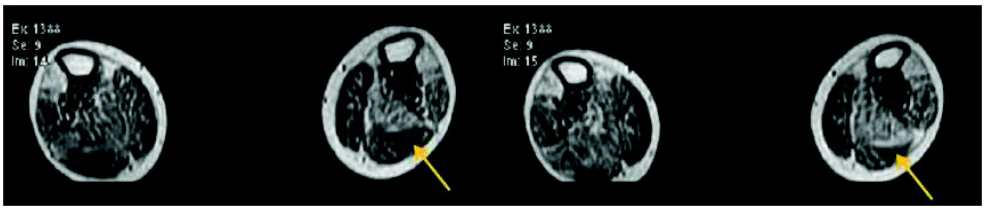

Figure 3B: T1 weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus 6 months after Autologous BMMNCs transplantation; arrows showing muscle regeneration and reduced fatty infiltration

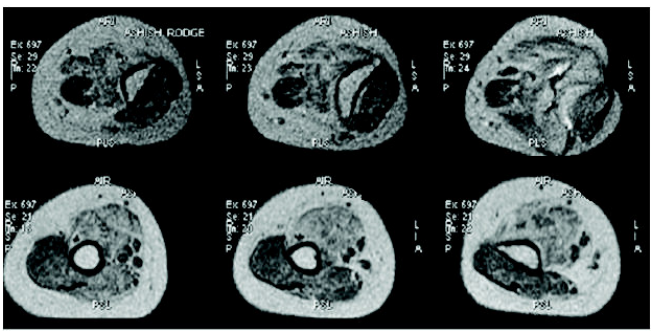

Figure 4A: T1 weighted axial musculoskeletal MRI images of Left and Right Long, Medial and Lateral head of Triceps before Autologous BMMNCs transplantation

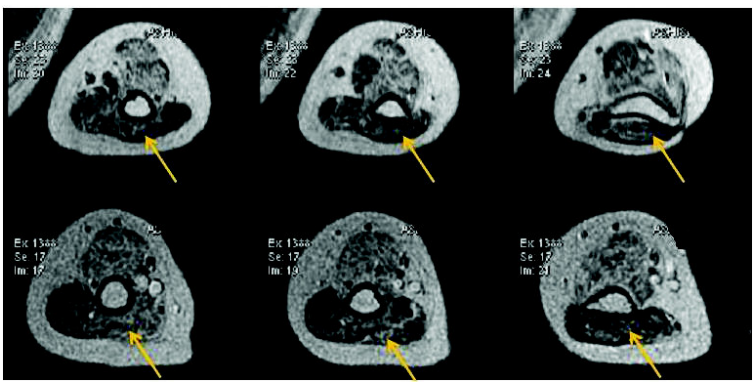

Figure 4B: T1 weighted axial musculoskeletal MRI images of Left and Right Long, Medial and Lateral head of Triceps 6 months after Autologous BMMNCs transplantation; arrows showing muscle regeneration and reduced fatty infiltration

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.

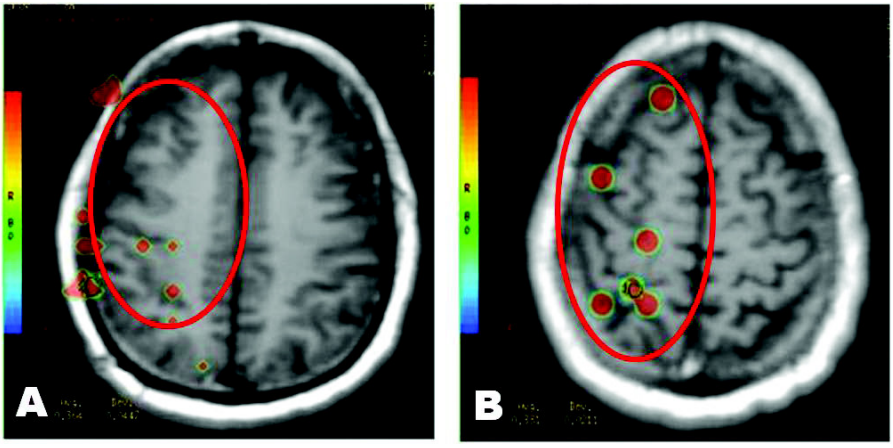

Figure 5: FMRI showing changes after stem cell therapy

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 (fluoro-deoxyglucose) 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 interation, 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.

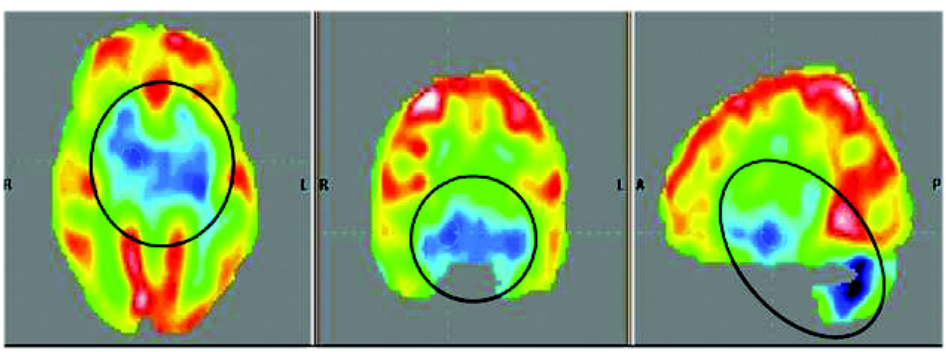

Figure 6A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hypocampus, cingulate regions and cerebellum

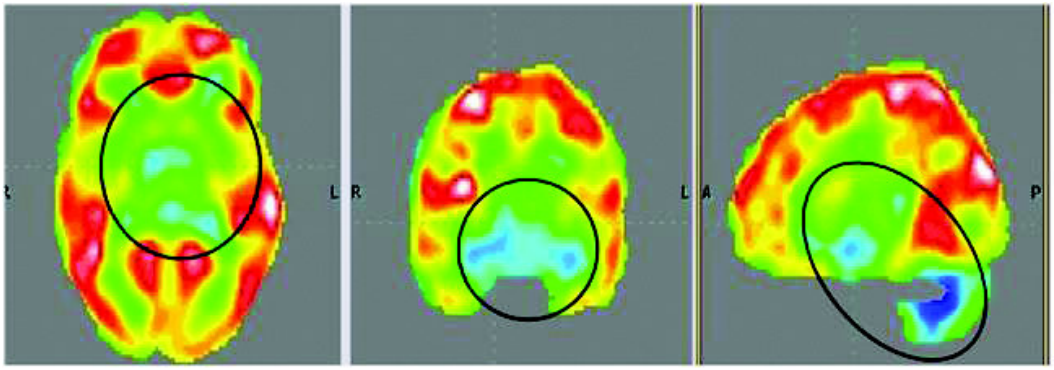

Figure 6B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of superios temporal gyrus, mesial temporal lobes, amygdala, hypocampus, cingulate regions and cerebellum; indicated by reduction in the blue and black areas

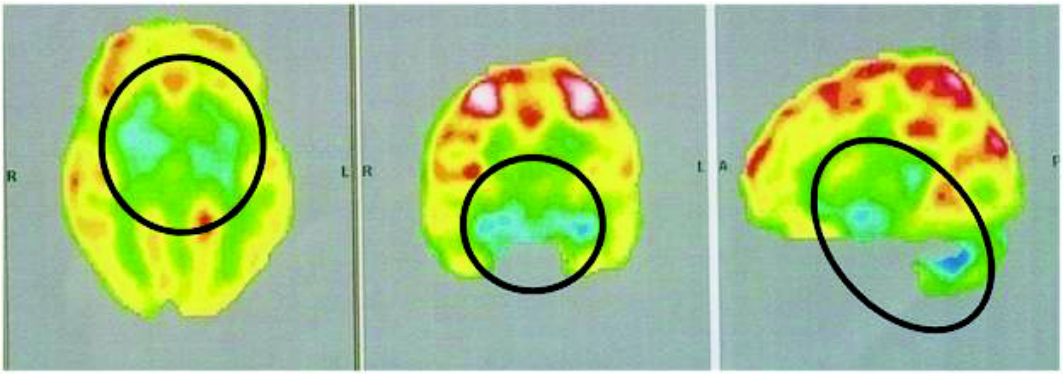

Figure 6C: PET-CT scan 8 months after second cellular transplantation showing maintained increase in the metabolism of superios temporal gyrus, mesial temporal lobes, amygdala, hypocampus, cingulate regions and cerebellum and reduction in the metabolism cortical regions showing the balancing effect

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.

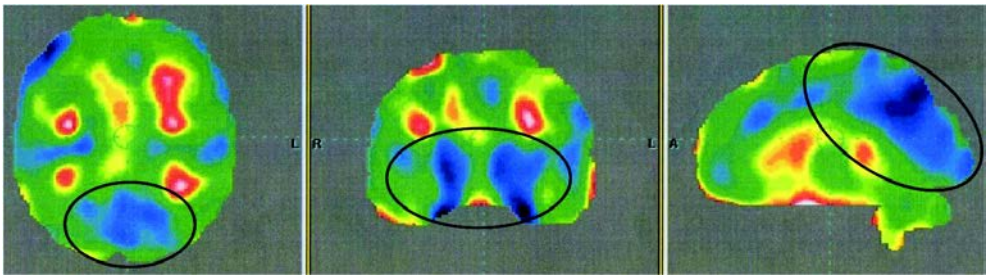

Figure 7A: PET - CT Scan before stem cell transplantation suggestive of reduction in the metabolism of occipital lobes and mesial temporal structures bilaterally

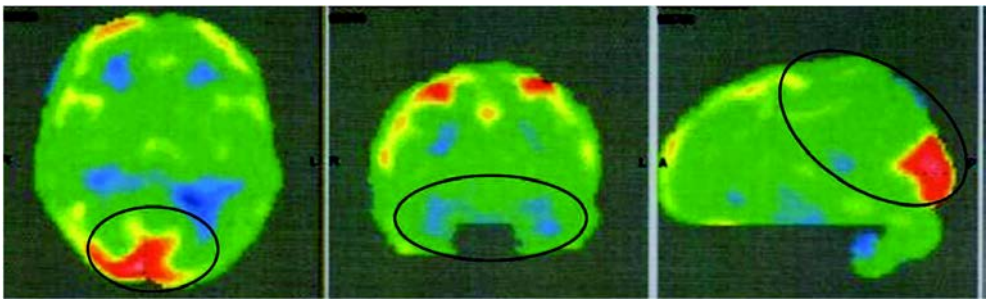

Figure 7B: PET-CT scan after stem cell transplantation showing improved metabolism in bilateral occipital lobes mesial temporal structures

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, hypocampus, and cerebellum bilaterally (Figure 8B).

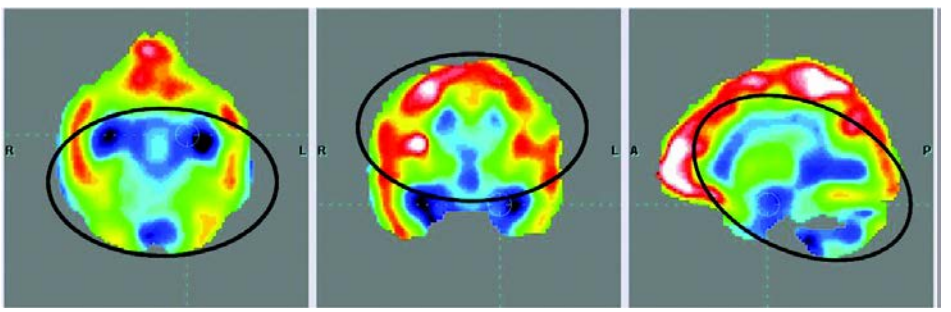

Figure 8A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hypocampus, cingulate regions and cerebellum

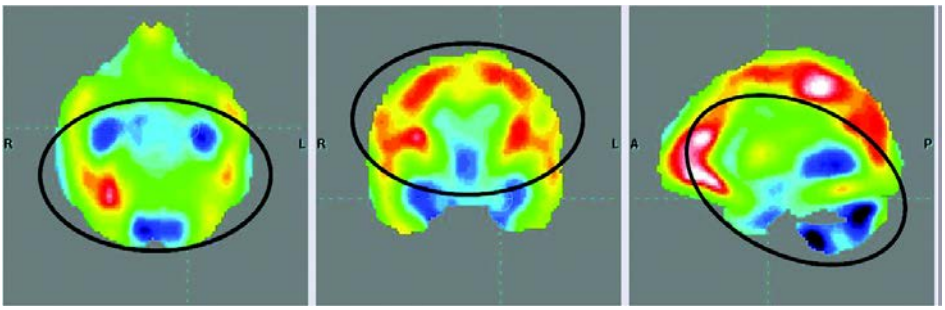

Figure 8B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of mesial temporal lobes, amygdala, hypocampus, and cerebellum bilaterally; indicated by reduction in the blue and black areas

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 PET-CT 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.

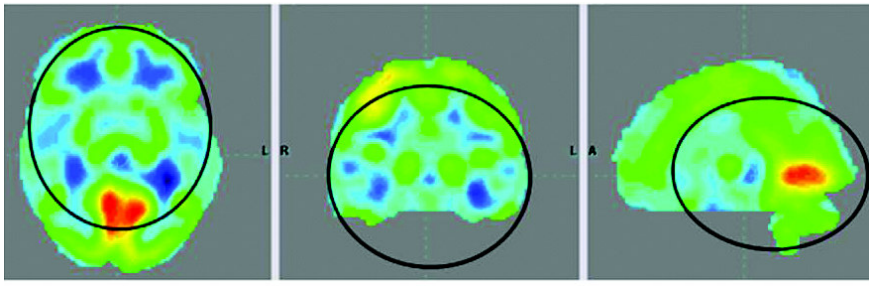

Figure 9A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hypocampus, cingulate regions and cerebellum

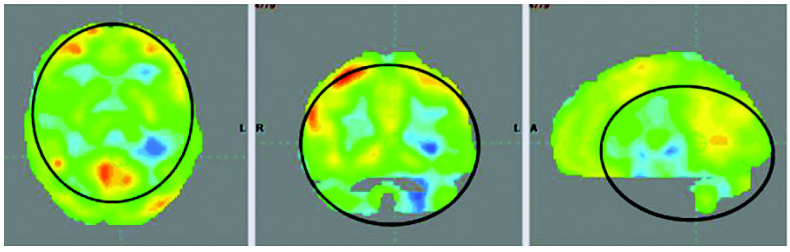

Figure 9B: PET-CT scan after cellular transplantation showing increased metabolism in the regions of mesial temporal lobes, amygdala, hypocampus & cerebellum bilaterally; indicated by reduction in the blue & black areas

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.

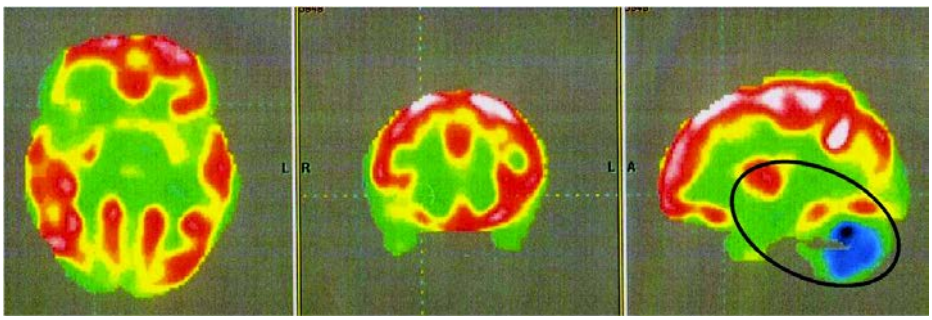

Figure 10A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of bilateral cerebellum

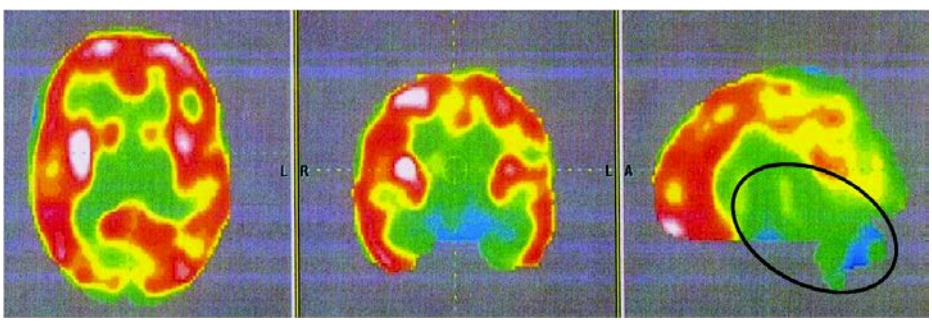

Figure 10B: PET-CT scan after cellular transplantation showing increased metabolism in the regions of cerebellum bilaterally; indicated by reduction in the blue and black areas

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 improment 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.

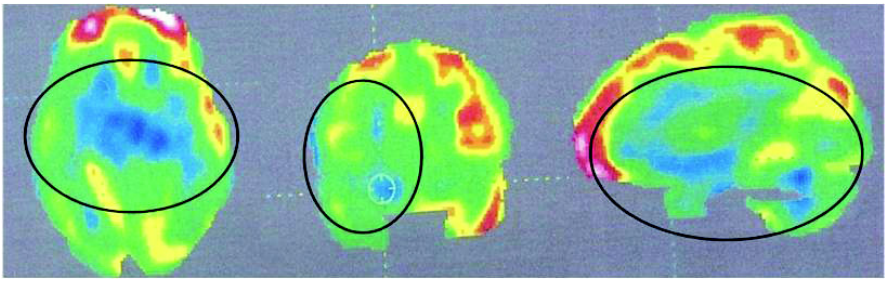

Figure 11A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour and as outlined by the circles in the regions of basal ganglia, mesial temporal structures and cerebellum

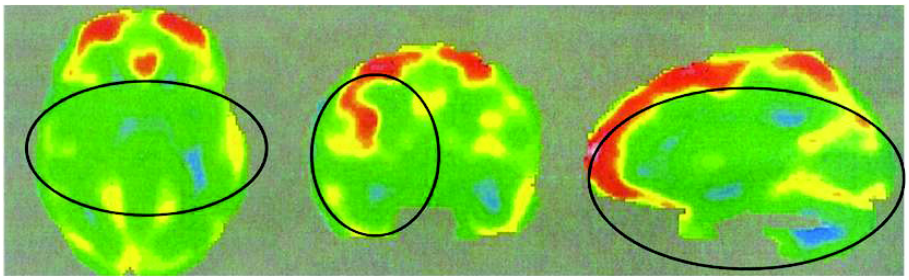

Figure 11B: PET-CT scan after cellular transplantation showing increased metabolism as outlined by the circles in the regions of basal ganglia, mesial temporal structures and cerebellum

7. A 7 year old boy diagnosed with Autism underwent autologous BMMNCs intrathecal transplantation. His main symptoms were poor social interaction, crying spella, 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 PET-CT scan chages of balancing of the brain metabolism as shown in the Figure 12B.

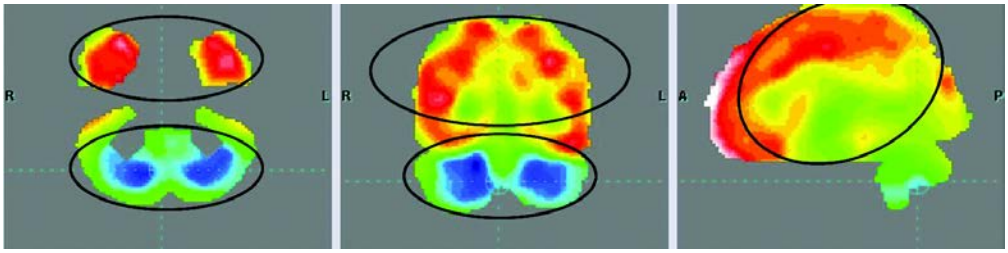

Figure 12A: PET-CT scan before stem cell therapy showing diffuse increase in the metabolic activity of the cortical lobes and reduced metabolic activity in bilateral cerebellar lobes

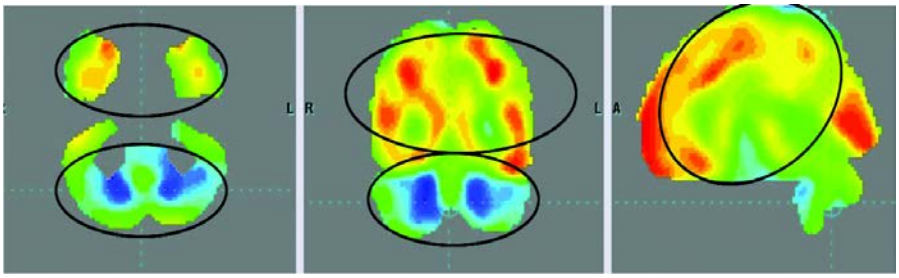

Figure 12B: PET-CT scan after stem cell therapy showing reduction in the metabolic activity of the cortical lobes and increased metabolic activity in bilateral cerebellar lobes hence highlighting the balancing effect of cellular therapy

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.

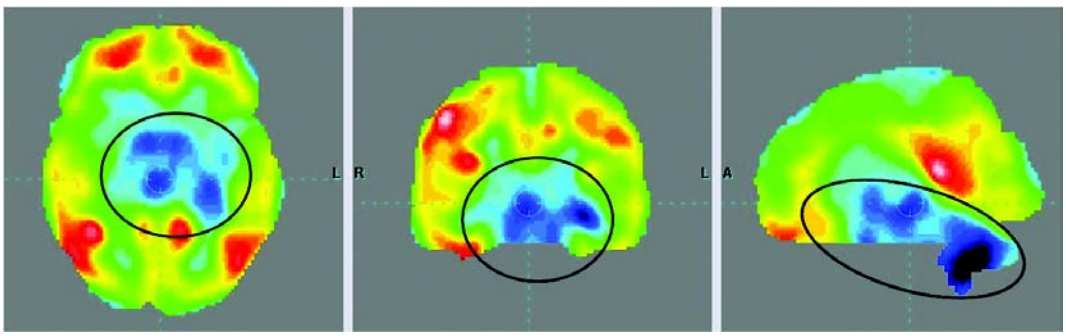

Figure 13A: PET-CT scan before stem cell therapy showing reduction in the metabolic activity of the cerebellar lobes and thalami bilaterally

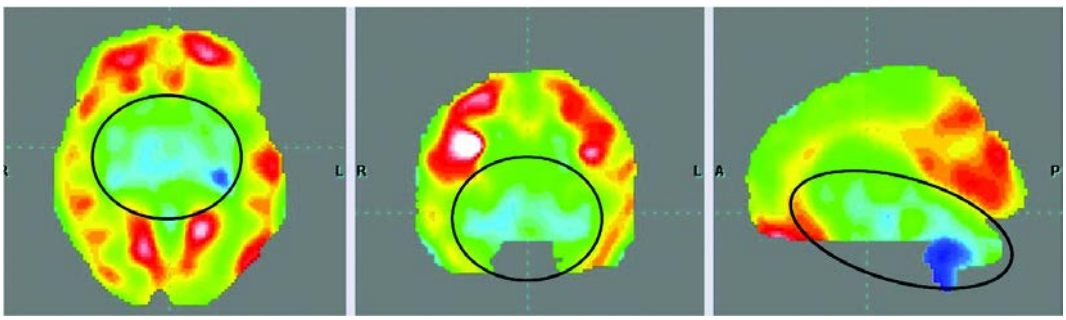

Figure 13B: PET-CT scan after stem cell therapy showing increased metabolic activity in bilateral cerebellar lobes and thalami bilaterally

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).

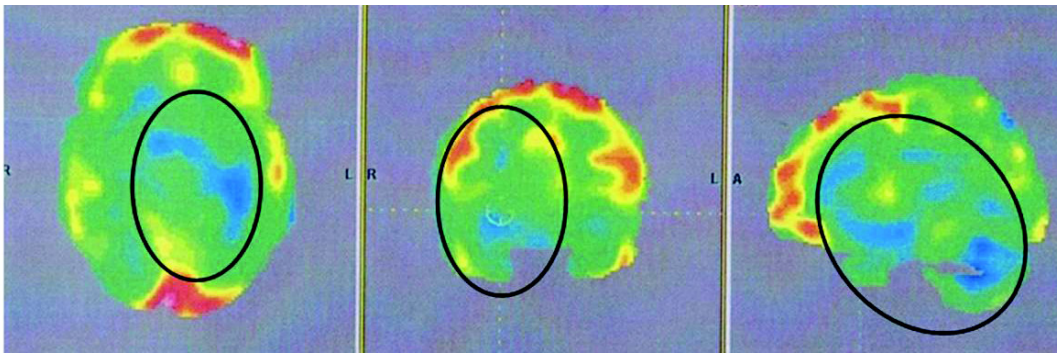

Figure 14A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of cerebellar lobes and mesial temporal structures

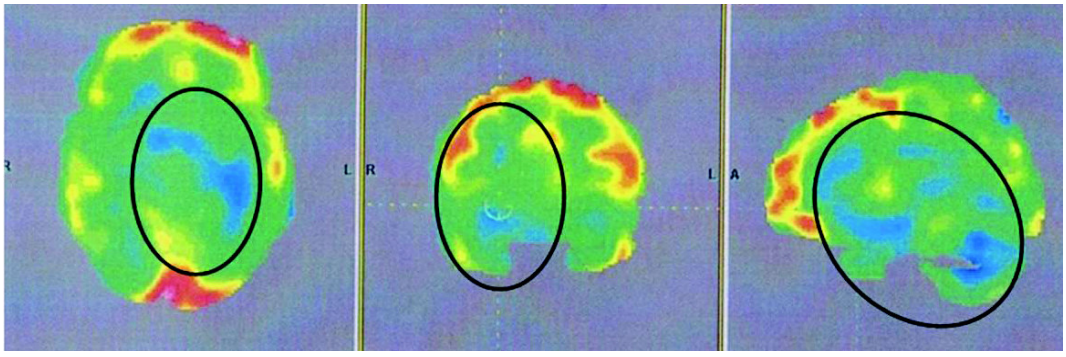

Figure 14B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of cerebellar lobes and mesial temporal structures seen as reduced blue and black areas

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).

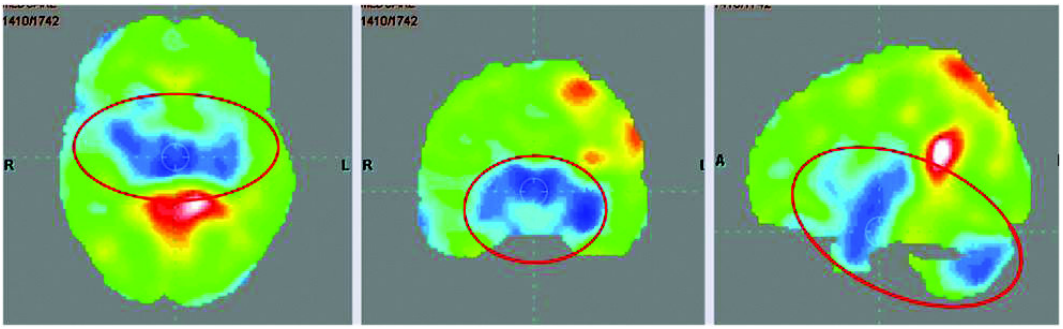

Figure 15A: PET-CT scan before cellular transplantation showing reduced metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally as indicated by the circles

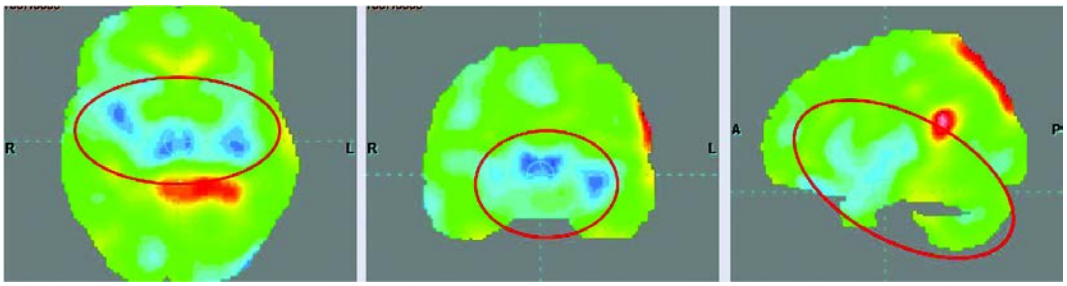

Figure 15B: PET-CT scan 9 months after cellular transplantation showing increased metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally as indicated by the circles

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).

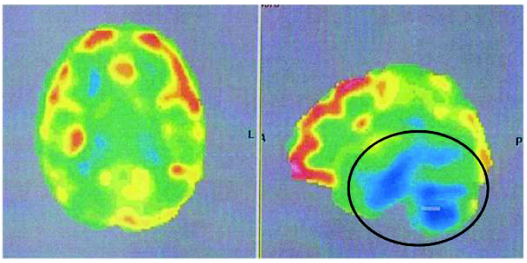

Figure 16A: PET-CT scan before cellular transplantation showing reduced metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes bilaterally as indicated by the circles

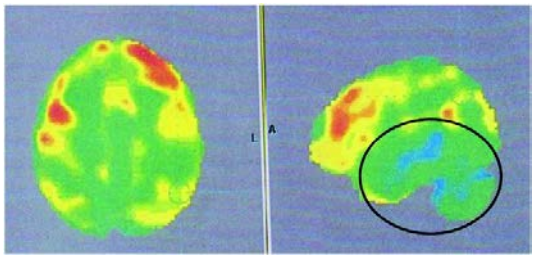

Figure 16B: PET-CT scan after cellular transplantation showing improved metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes bilaterally as indicated by the circles

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).

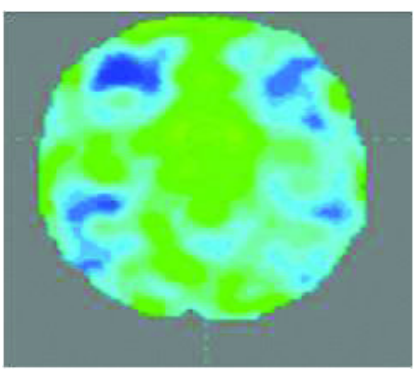

Figure 17A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus

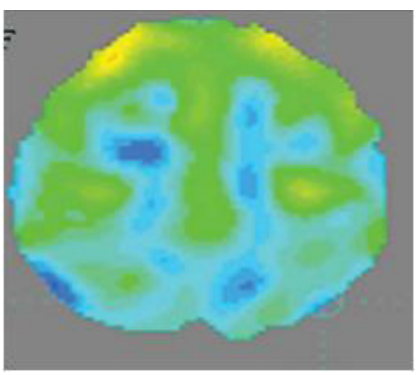

Figure 17B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus; indicated by reduction in the blue and black areas

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.

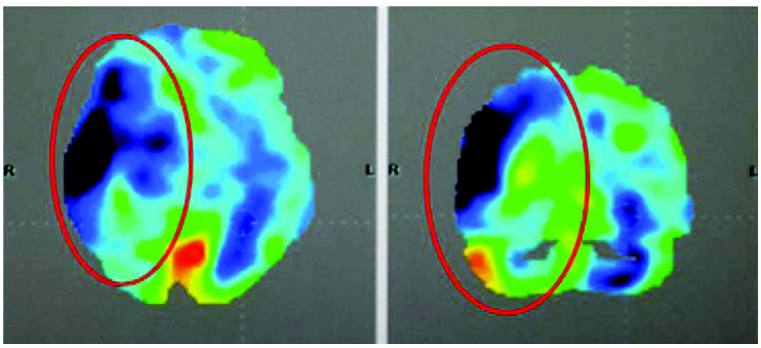

Figure 18A. PET-CT scan before Autologous BMMNCs transplantation suggestive of stroke in the region right MCA territory with black areas suggestive of gliosis where as the blue regions suggestive of penumbra

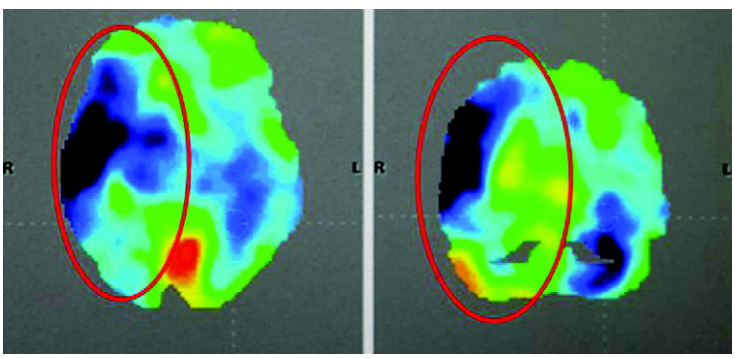

Figure 18B. PET-CT scan 1 year after Autologous BMMNCs transplantation showing improved metabolism in the parietal lobe penumbral regions seen as reduction in the blue coloured areas

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.

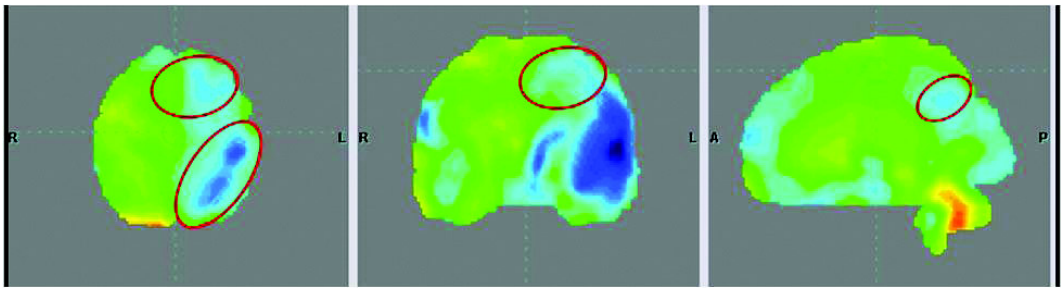

Figure 19A: PET-CT scan before Autologous BMMNCs transplantation suggestive of reduced metabolism in the regions of left frontal lobe, occipital lobe and basal ganglia

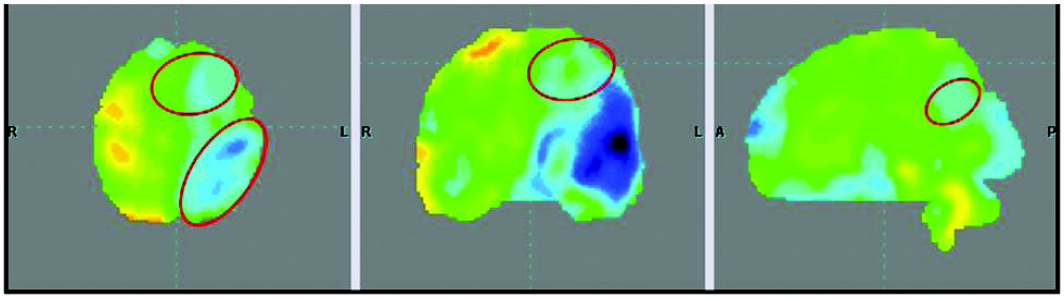

Figure 19B: PET-CT scan after Autologous BMMNCs transplantation suggestive of increased metabolism in the regions of left frontal lobe, occipital lobe and basal ganglia

Hemorrhagic Stroke

Cellular transplantation in the case of chronic hemorraghic 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.

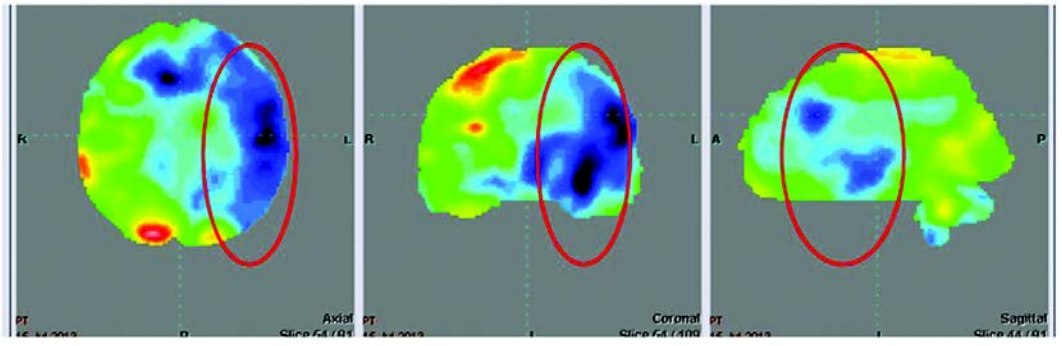

Figure 20A: PET-CT scan before Autologous BMMNCs transplantation suggestive of hemorrhagic stroke in the region left parietal lobe with black areas suggestive of gliotic areas where as the blue regions suggestive of penumbra

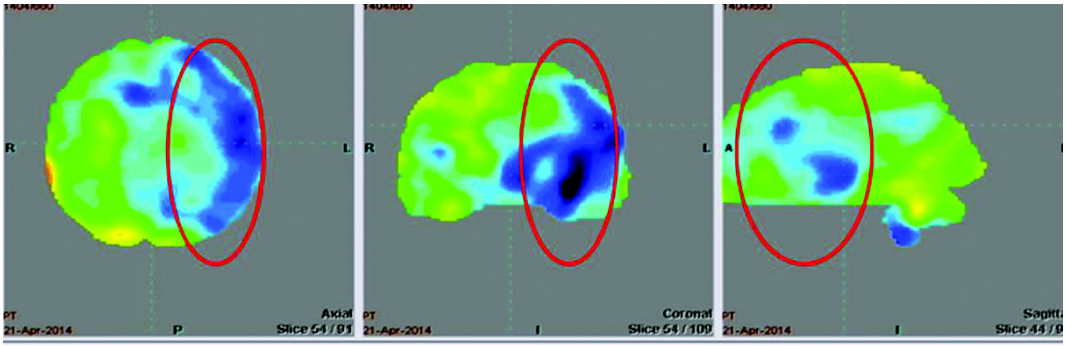

Figure 20B: PET-CT scan 6 months after Autologous BMMNCs transplantation shows reduced blue areas signifying improved metabolism of the penumbral regions

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

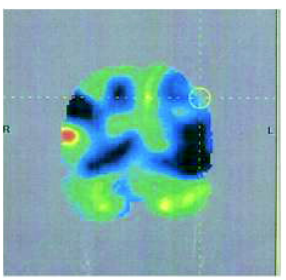

Figure 21A: PET-CT scan before Autologous BMMNCs transplantation showing gliotic areas in black with areas of reduced metabolism in blue seen in bilateral parieto-occipital&right anterior inferior temporal region

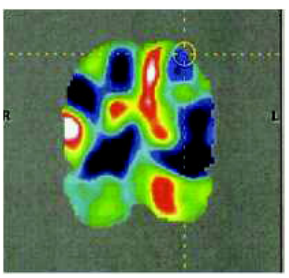

Figure 21B: PET-CT scan after Autologous BMMNCs showing improved metabolism in the regions of anterior, middle and posterior cingulate gyrus, amygdale, hypocampus and parahypocampus

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, dysrthria, poor sitting and standing blance, inability to walk without support and subnormal coginition. 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.

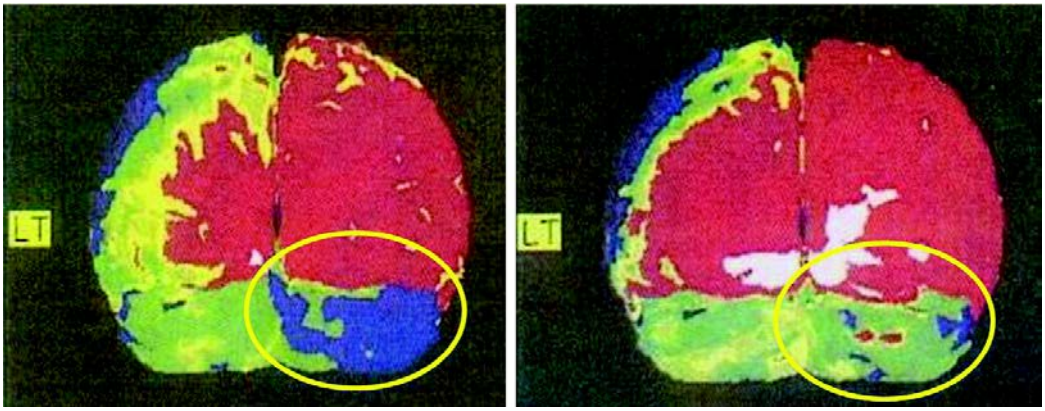

Figure 22: PET CT Scan showing improved metabolic activity which is indicated by decrease in blue areas after stem cell therapy

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).

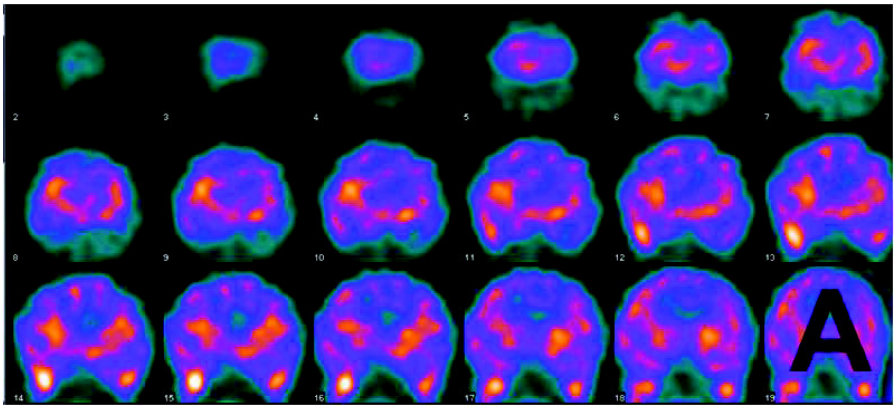

Fig 23A: Pre stem cell. There is overall hypometabolism seen in the brain, with purple areas depicting areas of hypometabolism and orange areas as areas of normal metabolism

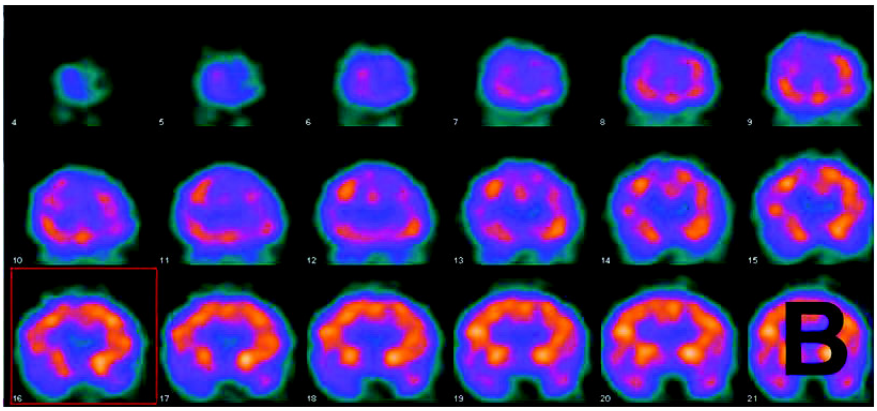

Fig 23B. Post stem cell. As compared to Fig A, there is increase in the orange area, which is the area of normal metabolism and reduction in the purple area. This suggests that after stem cell therapy, there is increased metabolic activity in the brain, thus improved neural activity.

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.

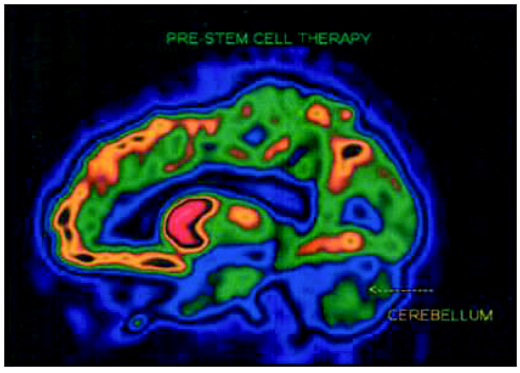

Fig 24A: Pre stem cell therapy there is hypometabolism seen in bilateral cerebellar lobes

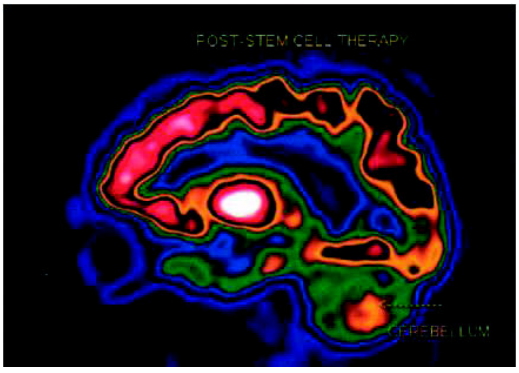

Fig 24B: Post stem cell therapy there is increased metabolism seen in bilateral cerebellar lobes

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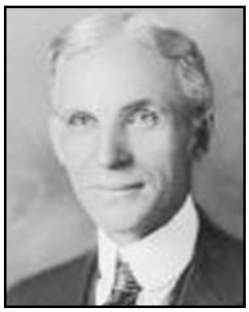


"You can do anything if you have enthusiasm. Enthusiasm is the yeast that makes your hopes rise to the stars. Enthusiasm is the sparkle in the eyes, the swing in your gait, the grip of your hand, the irresistable surge of will and energy to execute your ideas. Enthusiasts are fighters.They have fortitude. 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

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 goals of neurorehabilitation is to help patients with impairments and disabilities and to make them functionally independent, which requires team of rehabilitation specialists, such as nurses, physical therapists, occupational therapists, speech therapist, psychologist and others. (1)

Importance of Rehabilitation:

The rehabilitation team has a role to set short term goals (generally considered to be two to three weeks) and long term goals (longer than 3 weeks) which should be objective, measureable and time limited. Neurorehabilitation team has an understanding of neural regulation of movement patterns. As framework for typical motor behaviour is necessary to understand how motor behaviour is altered in persons with neurological dysfunction and how plastic properties of nervous system interact to produce change.

Motor control is the study of how an individual controls movements already acquired. Neuroplasticity is defined as brain's ability to adapt or use cellular adaptations to learn or relearn functions which are previously lost as result of cellular death by trauma or disease at any age. Neuronal sprouting is thought to be primary mechanism, allowing injured neurons, to reconnect in new ways and allowing intact undamaged neurons to form new connection and to enhance function. Motor learning will continue throughout life as long as environment asks for change and CNS has pliability and desire to learn. The rehabilitation team promotes this learning and facilitates neural plasticity (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.

The benefits of exercise and paracrine mechanisms of cellular therapy have resonating effects. 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 the repair and regeneration of the tissue [6].

Exercise stimulates the organ resident stem cells that help in repair and regeneration of the tissue locally [7]. Stem cells and progenitor cells are mobilized in the peripheral blood that bring about repair and secretion of growth factors in the distant parts of the body [7]. One of the mechanisms for exercise induced hypertrophy and neovascularization is activation of these resident stem cells. Exercise also secrets 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.

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.

The rehabilitation team sets up goals and the injected stem cells from within the body help in achieving those goals. Studies have shown that exercise induces nobility in the injected stem cells, thereby enhancing the achievable outcomes. Hence, neurorehabilitation appears to work complimentarily with stem cells therapy.

Physical therapy

As an important member of rehabilitation team a, physical therapist has a crucial role to play which includes, bed mobility, ambulation and transfer activities like, transfers from bed to chair or from chair to commode or from wheelchair to car and so on.

Their assessments emphasize measures of voluntary movement, sensory appreciation, ROM, strength, balance, fatigability, mobility, gait and functional status.

Practices in Physical Therapy includes:

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

Occupational Therapy

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

In the patient's home and workplace, the therapist provide grab bars, rails, ramps,environmental controls, computer interfaces, architectural changes such as widening a doorway to allow wheelchair access and emergency remote-control calling systems. Along with the physical and recreational therapist, occupational therapist seek out the environmental, personal, and activity-specific equipment and technologies that enhance the quality of life of patients Success in retraining during rehabilitation depends on diverse variables that include the characteristics of a task, changing contexts and environments when performing a task, psychological reinforcements including positive contextual factors like motivation, attention, memory for carryover of what is taught and negative contextual factors like environmental distractions, anxiety, sleep deprivation and family support play a significant role.

Psychology:

The word psychology is derived from the Greek words Psyche (which means soul) and logos (which means study). Hence, psychology could be defined as a "study of the soul". However, today it is defined as the scientific study of the behaviour of individuals and their mental processes (American Psychological Association).

Neuropsychological testing and evaluation is to identify the pattern of cognitive, behavioural, and emotional strengths and weaknesses and to provide specific treatment recommendations or clarify diagnostic questions.

Psychological Counseling:

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

There are several main broad systems of psychotherapy (3):

i) Psychoanalytic:
ii) Behaviour Therapy:
iii) Cognitive Behavioural Therapy:
iv) Psychodynamic
v) Existential Therapy
vi) Humanistic
vii) Brief Therapy
viii) Transpersonal Therapy
ix) Body Psychotherapy
x) Play Therapy, Gestalt Therapy, Rational Emotive Behaviour Therapy, Solution based therapies and Reality Therapy some other forms of psychotherapy. (6)

Speech therapy:

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

i) Aphasia:

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

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

ii) Dysarthria:

Dysarthria can be classified as spastic dysarthria (due to upper motor neuron lesion), flaccid dysarthria (due to lower motor neuron involvement), ataxic dysarthria (due to cerebellar involvement), hypokinetic and hyperkinetic dysarthria (due to basal ganglionic involvement) and mixed dysarthria.

Speech and language pathologist aim to improve speech intelligibility (overall clarity of speech) by:

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

iii) Apraxia:

Treatment of apraxia of speech involves phonemic drills, giving proprioceptive and kinesthetic cues to the patients. MIT (melodic intonation therapy) is another technique used (as cited in Freed, 2000).

Darley (1975) stated that the goal of treating apraxia of speech is tohelp patients relearn the motor sequences needed to produce phonemes accurately.(8)

iv) Dysphagia:

A swallowing therapist aims to work on:

a) strengthening the oral and pharyngeal structures for swallowing.
b) modify the bolus in order to facilitate adequate swallowing.
c) recommend postures and maneuvers like chin tuck/ chin down postures according to the nature of disorder.

During swallowing therapy, the therapist should ensure airway safety and rule out any silent aspiration. Children with autism, cerebral palsy, hearing impairment or mental retardation present with either absence of speech or deficient speech and language skills as compared to their age. The main aim of the speech therapist is to bridge the gap between the chronological age and the language age of the child. The speech and language pathologist tries to explore the areas which the child would respond in and facilitate communication within child's impairment. Most widely used techniques for language learning are repetitions, modeling utterances, expanding a topic and role play. However, children with higher grade of severity may have to rely on alternative and augmented communication (AAC) in order to reduce the communicative burden on the caregivers.

Various Neurological Conditions (Assessment and rehabilitation protocol)

1. Autism

Autism is one of the most common conditions which remain under diagnosed until very 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. The assessment and testing process by different professionals would comprise:

- Developmental Pediatrician would ask the parents about milestones, a complete history of the child and the problems that he faces and would advise for testing like BERA, MRI Brain, PET- CT scan, etc.
- Clinical Psychologist would take a detailed history of the child and conduct various psychological tests depending on the age and the problem areas of the child like Childhood Autism Rating Scale (CARS), Autism Diagnostic Observation Schedule (ADOS), Malin's Intelligence Scale for Indian Children (MISIC), etc.
- Occupational Therapist would collect information about the child's abilities and difficulties in performing daily occupation and activities such as play, school performance, fine motor, gross motor skills, ADL's, etc. They would assess the child on scales like Peabody Developmental Motor Scales (PDMS), Sensory Integration and Praxis Test (SIPT), etc.
- Speech and Language Pathologist would assess the child's receptive language, voice quality, vocabulary, oro-motor skills, etc. For evaluating the child they would use scales like Communication and Symbolic behaviour scales developmental profile, normative chart developed by the National Institute on deafness and other communication disorders, etc.
- Physiotherapist would closely assess the system which is responsible for all the normal physical functions of the child i.e. the neuromotor and musculoskeletal systems.

Management and treatment for a child with autism would call for a multi-disciplinary holistic approach. Each of the above mentioned professionals will target different aspects in the treatment of your child.

Interventions for Autism:

- Psychological Intervention: With behavioral issues being one of the major concerns in children with autism, a clinical psychologist/ child psychologist plays an important role as they may looks at what effect the 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 an occupational therapist with an expertise in 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 PECS.
- Physiotherapy: A Physiotherapist can help in optimal development of motor skills and develop program to address any underlying weakness in both sensory and muscular systems in children with autism.

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

Examination and Evaluation: emphasizing on following points:

Medical and social history, Aerobic Capacity and endurance, Anthropometric Characteristics, Assistive and Adaptive devices Assessment, Community and Work Integration or Reintegration, Environmental Home and Work barriers Examination, Gait, Locomotion and Balance, Integumentary Integrity, Joint Integrity and Mobility, Motor Function, Muscle performance, Orthotic, Protective and Supportive Devices, Pain, Posture, Range Of Motion, Reflex Integrity, Self-Care and Home Management, Sensory Integrity, Ventilation, Respiration and Circulation, Diagnosis of Impairment / Disabilities.

Neurological Examination:

1. American Spinal Cord Injury Association Examination: is used for specific neurological examination after spinal cord injury.
2. Assessment of muscle performance allows for specific diagnosis of level and completeness of injury. Examination includes each specific muscle and identifies subsititutions from other muscles.
3. Presence, absence and location of muscle tone should be assessed as a common tool to describe tone, using Modified Ashworth Scale.
4. Sensation is described by dermatome. The recommended tests include:
    i) Sharp-dull discrimination or temperature sensitivity to test the lateral spinothalamic tract.
    ii) Light touch to test the anterior spinothalamic tract and
    iii) Proprioception or Vibration to test posterior columns of spinal cord. Sensation is indicated as intact,impaired or absent per dermatome. A dermatomal map is helpful and recommended for ease of documentation. (10)

Functional Examination:

The Functional Independence Measure is more commonly used tools in SCI. Other tools such as Quadriplegia Index of Function(QUIF),Spinal Cord Independence Measure (SCIM), and Craig Handicap Assessment and Reporting Technique (CHART).

The goal of rehabilitation for the patients with SCI, regardless of the level of the lesion, include the following: (11)

Prevention of all secondary complications as a result of being bed ridden. Restoration of functional independency to the maximum possible limit.

Psychological counseling

Social and Vocational Rehabilitation

Family Education and Home adaptation

1. Education:
    - Patient and caregiver education plays an integral part of rehabilitation.
    - Formal education includes group and individual instruction and family/ caregiver training.
    - Preventive skin care, bowel and bladder programs, safe ways to perform all ADLS tasks, nutritional guidelines, thermoregulation precautions, pulmonary management, cardiopulmonary resuscitation, management of autonomic dysreflexia, equipment management and maintenance, transfer techniques, wheelchair chair mobility, ambulation, proper body positioning,ROM exercises,ADL basics and leisure skills should be taught.
    - Home programs to increase strength,endurance,ROM and function are taught.
    - Energy conservation techniquesand proper body mehanics should be incorporated.
2. Health Promotion and Wellness:
    - Exercise program for persons with SCI must take into consideration the musculoskeletal, respiratory, cardiovascular and autonomic nervous system changes that occur after SCI.
    - Components of an exercise program include flexibility,muscular strength and cardiovascular endurance.
    - Frequency ranges from 2-5 times per week with atleast 1 day of rest between strengthening sessions.
    - Duration of an exercise program as little as 20 minutes or as much as 90- 120 minutes.
    - Intensity ranges 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),alternate modes of exercises and get proper rest between exercises sessions.

- Equipments like weighted cuffs, elastic bands and tubing and hand cycles can be used for home exercises program.
- Health and Wellness program has potential to increase QOL, improve ADLS, decrease secondary complications, decrease depression and decrease no. of hospitalizations. (12)

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, speciality wound dressings, electromodalities and thermomodalities 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:
   - Contracture may result in postural malalignment or impede potential function.
   - 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:
   - Deformity prevention is first goal for spinting. For e.g Patients with C8 and T1 injuries or incomplete injuries may have clawing or hyperextension of metacarpophalagneal joints which is due to stronger pull of finger extensors over finger flexors. Thus splints to block metacarpophalagneal joints and promote weak instrinsic muscle function.
   - Cost, time and material should be considered when deciding between custom made and prefabricated.
   - Education regarding splint wearing schedule, skin checks and splint care should be emphasized. (13)
5. Bed mobility:
   - Rolling side to side and supine to prone, coming to sit, and scooting in all the directions while either long or short sitting.
   - Compensatory strategies and assistive devices, such as bed loops, can be used to accommodate for upper limb dysfunction.
6. Pressure Relief in the Upright Position:

- Appropriate time to maintain change in position is usually 60 seconds at intervals of 30 to 60 minutes.
- With higher tetraplegia,speciality controls like pneumatic control switch, manual recliner or tilt wheelchair are present for pressure relief.
- Mild to low tetraplegic, side or forward lean technique can be used.
- For paraplegic, push ups is performed for pressure relief.

7. Wheelchairs Transfers:
   - Type of transfer depends upon the level of injury, assistance needed, patient preference and safety transfer.
   - Appropriate body mechanisms is needed for performing transfers.
   - Dependent transfers can done by power lift, hyradulic lift, manual pivot, transfer board or manual lift.
   - Transfers are performed on different surfaces starting with easiest transfer progressing to more difficult transfer with level surfaces to uneven surfaces.
   - Transfer training should proceed with mat -bed- toilet -bath-car- floor-other surfaces (sofa,theater seat,pool). (11)
8. Wheelchair Mobility Skills:
   - Safe and appropriate use of wheelchair before getting out of bed should be taught.
   - Training such as mobility on level surfaces in open areas, setup for transfers,mobility in tight spaces,mobility in crowded places,on and off elevators, up/down ramps, in/out doors, wheelies, negotiation of rough terrain and up/down curbs and steps.
9. Ambulation:
   - Hope is important to maintain positive survival skills in SCI rehabilitation. Patients who are not candidates for ambulation should receive an explanation of why these goals are not feasible.
   - When ambulation is appropriate goal,treatment like therapeutic exercises,biofeedback, neuromuscular stimulation,balance training,standing, pregait and gait activites should be included.(14)
10. 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.(15)

Psychological Aspect in Spinal Cord Injury

Spinal Cord Injury (SCI) leaves a major impression on the person's body and mind. A new SCI patient usually has many queries regarding his future and at the same time has a sense that things are not going to be the same. 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. If not included in the team effort, a well-meaning family member could inadvertently sabotage the independence-oriented rehabilitation approach, or be too psychologically distressed to provide the emotional or physical care the patient needs.

The role of the occupational therapist is to asses' functional capabilities in all occupational performance areas and contexts. ADLs and IADLs (including self-care, home management, mobility, and work-related tasks), energy conservation, work simplification, joint protection, spiritual approaches, and appropriate humor may be used to restore to maintain function. Proper positioning, exercise programs, and pain management techniques are used as indicated to facilitate recovery and increase functional capacity.

3. Cerebral palsy:

Examination:

1) Medical history:
   a) Prenatal history:
   b) Perinatal history:
   c) Developmental history
2. Motor Performance:

a) Spasticity is examined using Modified Ashworth Scale.
b) Neck control
c) Milestones Evaluation
d) Reflexes Evaluation(Primary innate/Spinal level reflexes/cortical level reflexes/Brainstem reflexes/Mid-brain level reflexes)
e) Range of Motion of all joints (active and passive)
f) Tightness/contracture
g) Shortening/wasting
h) Gait Analysis and Posture.
i) Coordination
j) Hand functions
k) Functional Evaluation: (supine to sit;rolling,side-sitting, quadruped; crawling, kneeking, half-kneeling, standing, walking)
l) Vision: Tracking/localization
m) Oromotor Examination
n) Speech
o) Hearing

3) Specific Measures for CP

Scales and Assessment tools: Items in these are included to provide information about the disease process and outcomes and ideally document clinically meaningful change over time.

i) The Gross Motor Function Classification System (GMFCS):
ii) Functional Independence Measure (FIM)
iii) Gross Motor Function Measure (GMFM)

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

Exercises to improve grip strength

Gait Training

Over head activity while standing with walker.

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

Quadriped for trunk balance

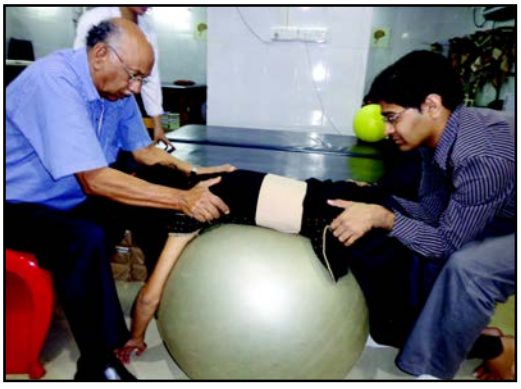
Strengthening of scapular muscles

Strenthening of back extensors

Strenthening of lower abdominals

Strenthening of neck musles

Strenthening of upper abdominals



Stretching of dorso lumbar fascia

trunk strenthning act.

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 (Performance areas, gross and fine motor skills, quality of movement), conductive education, and sensory integration.

Integrated approach for CP:

1. Developing rapport with parents and patients:
2. Normalising tone of muscles:slow passive movement, sustained stretch, cryotherapy over muscle for 15 -20 minutes, stimulation of antagonist movement and vibration are used. In cases of hypotonicity:weight bearing, joint compression, rhythmic stabilization, vibration, cryothearpyin brisk manner and tapping can be used.
3. Stretching and Mobilty
4. Developing Postural Reaction:Postural reactions consists 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. With the use of certain items with different textures, such as Styrofoam chips, water, or textured toys, 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: It has been estimated that around 65 percent of the individuals living with cerebral palsy also have some form of mental retardation. About 50% are full mentally retarded i.e. an IQ below 70. 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. About a third of individuals with cerebral palsy have mild intellectual impairments, a third have moderate-to-severe intellectual impairments, and another third have normal intellectual functioning. (17)

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.(18)

1. Frustration:
2. Communication difficulties:
3. Attention Deficit Disorder:

Treatment: Education and vocational preparation come into the foreground by school age. Concern with the physical disability should not distract attention from the emotional and social needs of childhood and adolescence.

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. Behaviour therapy might include planning activities that are rewarding which could provide a sense of accomplishment; use of reinforcements can encourage a behavior change, enhance learning and solidify gains. 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.

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. (19)

1. Assessment tools:

1. Through history and progression of disease.
2. Family History
3. Manual Muscle Testing.
4. Functional Assessment.
5. Scales : FIM and Brooke Scale

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 which are part of normal daily routine.
2. Mobility training:
   Transfers in and out of bed/ chairs/ Car transfers etc.

During therapy sessions patient is made to :

1. Perform weight bearing exercises that strengthen and tone the muscles. Stronger muscles can help to delay the impending weakness associated with muscular dystrophy.
2. Weight Bearing Activities to strengthen the trunk and in standing emphasizing on upper extremity strengthening activities.
3. Stretching Exercises to maintain flexibility, emphasing on intensity, as it has to be submaximal to avoid muscle fibre damage.
4. Engage in range of motion exercises and stretching mainly for tendo achlles, hamstrings and Iliotibila band. Flexibility can help ease the severity of joint contractures, a stiffening of the muscles around a joint. Splinting mainly advised during the night and is advisable for foot and knees to prevent contractures.
5. Emphasis is placed on mobility. The goal of rehabilitation team is to provide the patient with independence for as long as possible by focusing on movement.
   Developing large muscle groups to make the body stronger and give it more endurance (with assistance of KAFO /long leg brace).
6. Respiratory Muscle Strengthening for which following exercises are given:
   a) Spirometer exercises
   b) Blowing Whistle
   c) Blowing bubbles with straw in a bottle filled with water approximately 1-2 litres
   d) Sucking through straw etc.
7. 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.

5. Stroke:

Examination

1. Patient History.
2. Levels of Consciousness.
3. Communication.
4. Cognitive, Emotional and Behavioral States.
5. Cranial Nerve Testing.
6. Sensory Integrity.
7. Perception.
8. Joint Integrity and Mobility.
9. Tone/Reflexes.
10. Strength.
11. Postural Control and Balance.
12. Ambulation and Functional Mobility
13. Functional Status.

Specific Measures for Stroke

1. Fugl- Meyer Assessment of physical Performance (FMA).
2. Stroke Rehabilitation Assessment of Movement (STREAM).
3. Motor Assessment Scale.

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:

Commonly observed deficit:

1. Loss of trunk and postural control.
2. Poor sitting balance.
3. Poor standing balance.
4. Cognitive - perceptual impairment.
5. Impaired hand functions.

6. Speech.
7. Activities of Daily Living.

1. Strategies to improve Sensory Function:

Sensory stimulation is important for recovery by focusing on restoring sensitivity of more affected extremities and requires some residual sensory function with sufficient intensity to engage the system but not so strong to produce adverse effects like withdrawal.

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. Alterations in identity and personality may also result from the interaction of fluctuating emotional, cognitive, and physical abilities as well as from changes in social context and family dynamics.

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 councelling is a major factor for psychological rehabilitation in stroke.

6. Motor Neuron Disease:

Examination:

1. Muscle Peformance: can be measured by Manual Muscle Testing (MMT), isokinetic muscle strength testing or hand -held dynamometer. Muscle strength also can be assessed by Maximum Voluntary Isometric Contraction (MVIC).
2. Motor Function: Due to Spasticity, and weakness of muscles there could be many manifestations like Impairments in dexterity, incoordination of both gross and fine movements as well as loss of motor control. Therefore Functional assessment of both Upper and lower extremities should be done. Functional ability of hands should be done in detail.
3. Tone and Reflexes: Tone can be assessed by Modified Ashworth Scale and reflexes by deep tendon reflexes.
4. Joint Integrity, Range of Motion and Muscle length: should be examined using standard methods.
5. Cranial Nerve involvement should be assessed. Pseudo Bulbar and Progressive Bulbar varieties of MND only show involvement of cranial nerves.
6. Postural mal alignment and imbalance are seen which can be assessed by Tests like Tinetti Performance Oriented Mobility Assessment(POMA), Berg Balance Scale, Timed Up andGo Test and Functional Reach Test
7. Gait: Deviations due to muscle imbalance should be assessed, so also endurance.
8. Respiratory Function: There could be involvement of respiratory muscles resulting into breathlessness, Low vital capacity and lack of cough effectiveness. Therefore Respiratory Function evaluation should be done in detail by using a hand-held spirometer. Aerobic capacity and cardiovascular pulmonary endurance should also be tested to evaluate aerobic conditioning.
9. Because of being in bed for long time without mobility there are chances of getting trophic ulcers: periodic skin inspection should be done.
10. Functional Status: Functional Independence Measure (FIM) can be used to document functional status.
11. Environment Barriers: should be considered for easy accessibility and safety.
12. Fatigue: Fatigue Severity Scale to be used.
13. Cognition: Impairments such as executive functioning, language comprehension, memory and abstract reasoning should be examined.Mini - Mental State examination can be used.
15. Pyschosocial Function: Can be assessed by Beck's Depression Inventory, Hospital Anxiety and Depression Scale (HADS)

16. Pain: seen in ALS and can be assessed by Visual Analog Scale.

Specific Measures for MND:

ALS Functional Rating Scale-Revised (ALSFRS-R): The functional status of ALS patients can be rated by ALS Functional Rating Scale (ALSFRS) and revised version ALSFRS-R. It correlates with muscle strength of both upper and lower limbs. ALSFRS-R includes respiratory muscles measures of upper and lower extremity muscle strength.

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: (20)

1. The rate of progress of the disease
2. Presence of spasticity, bulbar involvement, respiratory involvement causing hypoxia and fatigue.
3. Phase of Disease. Exercises are to prescribed according to level impairment, functional limitation and level of disability

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.

In case of decreased independence in ADLs like climbing, overhead activities and difficulty in buttoning etc, strengthening exercises to be prescribed avoiding fatigue. In case of difficulty in Ambulation, Orthotic devices like AFO, hand splints to be considered.

Stage 3: In case of fatigability in long distance ambulation, deep breathing exercises to 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 upto 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. In case of severe upper extremity weakness, consider 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.

The research evidence suggests:

1. Overuse weakness does not occur in muscles with MMT grade 3(fair) or greater out of 5(normal).
2. Moderate resistance exercises can increase strength in muscles with a MMT grade3 or greater out of 5.
3. Strength gains are proportional to initial muscle strength.
4. Heavy eccentric exercise should be avoided.
5. Exercises may produce functional benefits.
6. Psychological benefits have yet to be determined.

Patients with severe respiratory and bulbar complications may not benefit from active exercise programs.The goal in end stage is to optimize health and increase QOL.

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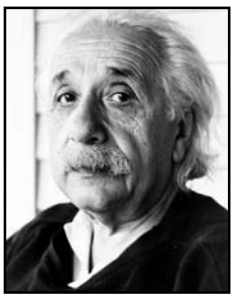

Every error is an opportunity to learn, just don't commit the same mistake again. That is stupidity. But commit as many new mistakes as you are capable of. Don't be afraid, because its the only way nature allows you to learn."

–Albert Einstein

19

Complications of Stem Cell Therapy

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) Tumorogenecity/ 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 allogenic 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.

So far, we have not come across any reported complication, such as tumorogenecity, 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 [3]. 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 [4].

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 [5,6,7]. Also these disorders present with seizures as a co-morbidity [8,9]. 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 in 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	**On the day of transplantation** Previous medication with same dosage, IV, twice a day at an interval of 12 hours **From the next day of transplantation** Previous medication with the same dosage as before, oral	**On the day of transplantation** Previous medication with same dosage, IV, twice a day at an interval of 12 hours **From the next day of transplantation** Previous medication with the same dosage as before, oral
	Patients not taking any antiepileptic medication	**On the day of transplantation** Levetiracetam 10mg/kg body weight, IV, twice a day at an interval of 12 hours **From the next day of transplantation** Levetiracetam 10mg/kg body weight, oral for 3 months	**On the day of transplantation** Levetiracetam 10mg/kg body weight, IV, twice a day at an interval of 12 hours **From the next day of transplantation** Levetiracetam 10mg/kg body weight, oral for 3 months
Patients without history of seizures	Patients not taking any antiepileptic medication	**On the day of transplantation** Levetiracetam 10 mg/kg body weight, IV, twice a day at an interval of 12 hours **From the next day of transplantation** Levetiracetam 10mg/kg body weight, oral for 3 months	Antiepileptic prophylaxis was not given to any petient

Table 2

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.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 morbidly or mortality.

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

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

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"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

In the recent years medicine has evolved and novel treatment options have emerged in the form of stem cell therapy. This evolution has brought forth mindboggling possibilities for finding treatments and cures for a variety of degenerative conditions. This sudden evolution of biological means for the treatment of incurable diseases has also raised multiple ethical and moral dilemmas. Therefore various regulatory medical bodies have felt an urgent need to monitor and regulate the research in the field of stem cell therapy. Divers opinions, cultural and religious views have made it challenging to device a single policy to govern stem cell research. Rapid economic growth in many developing nations, especially, in Asia has also experienced a proportionate surge in IT sectors as well as biomedical research. Moreover, with restrictions on stem cell research imposed in the US, a shift of activity in this field has been seen, with the opportunity being explored to its earnest in countries, such as China, India, Japan and Korea. However, 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.

The research in the field of cellular therapies is increasing; however, the regulatory bodies should consider these results while drafting the regulations. The regulations for a 'new drug' follow the steps of evidence based medicine. Cellular therapy poses millions of possibilities to be tested due to variations in the source of cells, types of cells, dose of cells and different routes of administration. 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.

Research has progressed to show safety of cellular therapy in various incurable,

progressive and fatal diseases. Is it fair that on one hand we claim to protect patients from adverse effects of the therapy using evidence based medicine whereas on the other hand we let them die waiting for the treatment? The regulatory bodies should consider the concept of 'Practice Based Evidence' and not rely only on 'Evidence Based Medicine'. Where evidence based medicine disregards the evidence generated by individual practitioners, practice based evidence progressed forward through such evidence.

Medical era progressed in the time when the evidence based medicine was not implemented so rigorously. Progress of the medical field was through the individual clinical practitioners who 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. It is not possible to measure all the medical practices on the yardstick of evidence based medicine. Evidence based medicine fails to answer questions like what can be considered as a conclusive evidence for a particular form of treatment?

The more daunting fact of evidence based medicine is the funds required to gather enough information to support the medical practices. The lack of funds for a large scale research trial at the hands of individual medical practitioners has led pharmacological and other industries to drive medical research to suit their business objectives. Practice based evidence could therefore provide faster progress in medicine guided by individual medical experts. Newer treatments must be very carefully and rigorously monitored for its safety. But once the safety of these treatments has been established, regulatory bodies may consider use of these treatments in case of the disease that have no cure. Regulatory bodies while monitoring very closely may also promote such practices to generate larger practice based evidence. This would make such treatments easily available and more affordable and the medical innovation will be by the experts in field of medicine and not driven by commercial gain and exploitation.

Individual medical expertise and experiences are of importance but caution needs to be exercised to cause no harm. The purpose and aim of regularizing and monitoring the medical practices is to protect patients from harm and exploitation. But regulations should also safeguard medical progress. The challenge in front of the regulatory bodies is to protect the vulnerable patients with incurable disorders from the false promises of new experimental therapies but at the same time make such treatments available to them. There is a very little difference between 'Helping the patients with incurable diseases with novel treatments' and 'Exploiting the helplessness of the patients' suffering from incurable diseases' and that is the distinction regulators all over the world need to make while designing the regulatory guidelines.

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].

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]

Japanese diet passed and implemented 'Regenerative medicine promotion law' in the last year that revolutionalised the regenerative medicine in Japan. As per the suggestions of this law, The Pharmaceuticals and medical devices agency partially amended Pharmaceuticals, Medical Devices, and Other Therapeutic Products Act (PMD act) to create a separate approval system from that of drugs and Ministry of health, labor and welfare (MHLW) passes an act on safety of regenerative medicine was devised to promote marketing of safe regenerative medicine practices.

The Pharmaceuticals and medical devices agency, partial amendment of Pharmaceuticals affairs law, renamed as Pharmaceuticals, Medical Devices, and Other Therapeutic Products Act (PMD Act) [7]:

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 [8]. At the provisional approval stage the treatment could be approved for commercial use as well as national insurance coverage.

Ministry of health, labor and welfare (MHLW), Japan & Japanese Pharmaceuticals and Medical devices agency (PMDA), act on safety of regenerative medicine [9]:

Although there was a separate channel created for the approval of cell based therapies, the partial amendment of PMD act did not specify the route of approval. Therefore an act of safety of regenerative medicine was devised to make sure the safety of the treatments provided and to ensure that the efficacy was established in the due course. Regenerative medicine products and 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 has a separate approval channel and different approval procedure.

Low risk regenerative medicine products (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.

This committee is similar to the IC-SCR suggested in the Indian guidelines. However, this committee has an authority to conditionally approve the treatment and marketing using the cell based products; unlike IC-SCR which is only restricted for the research in cell based products and therapies.

Medium risk regenerative medicine products (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.

This committee is similar to the IC-SCR suggested in the Indian guidelines but is formed of people outside of the institute but is not a committee on a national level like that of the NAC-SCR. 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 products (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. 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.

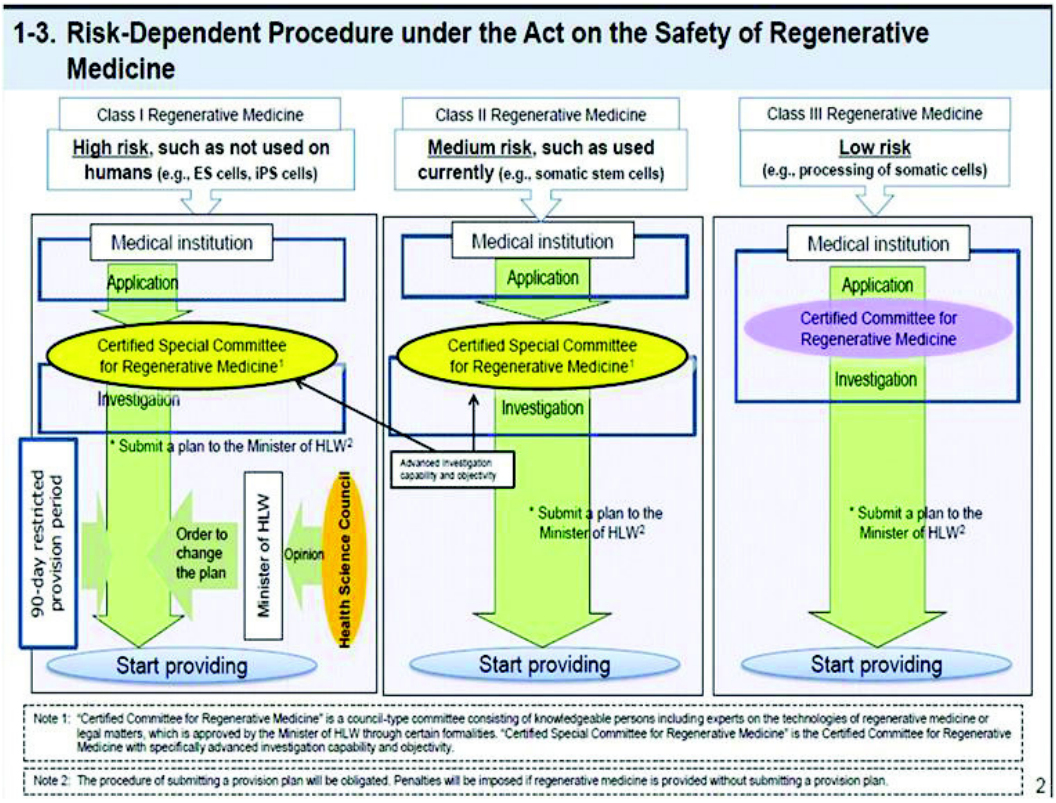

*Figure 1: Categorization of regenerative medicine * Diagram available online at http://www.mhlw.go.jp/english/policy/health-medical/medical-care/dl/150407-01.pdf*

The publication of the human iPS cell paper by Japanese researchers has renewed the vigour with regards to stem cell research in Japan. The governmental committee revised the guideline for human ES cell research in August 2009. The original guideline was split into two separate ones: one about derivation of human ES cells and the other about use of human ES cells. The renewed two-level review was abolished and now a protocol only needs an approval of the institutional ethics review committee. Another change in policies in Japan, recently, is pertaining to research that aims to produce germ lineage, which was prohibited till this year.

In May 2010, a new guideline came into effect for germ cell research using human iPS cells and the two existing guidelines for human ES cell research were revised to allow germ cell research using human ES cells. Further guidelines for use of induced pluripotent stem cells and human embryonic stem cells have been drafted by the Ministry of Education, Culture, Sports, Science and Technology (MEXT),allows researchers to use human iPS cells and ES cells under the strict review system included in the original guideline, although the use of human ES cells is not possible until the derivation guideline (which is under control of the MEXT) is amended to enable researchers to establish clinical grade human ES cells [10].

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 [11]

In the US, the National Institutes of Health (NIH) is the central federal body governing stem cell research, but each US state can also decide on its own legislation. The US FDA is responsible for the regulation of cell therapy products. Products derived from stem cells are regulated as biologics under section 351 of the Public Health Act. To assist with regulatory compliance, the FDA has provided general guidance documents via the Centre for Biologics Evaluation and Research (CBER) section of its website (www.fda.gov/cber/guidelines.htm).

US FDA has also recognized the need to make distinction between various cells and cell therapy processes. The United States of America, department of health and human services, Code of federal regulations, food and drug administration, Part 1271: Regulations for Human cells, Tissues, and Cellular and Tissue based products makes a clear distinction about minimally manipulated cells and autologous transplantation from other cell types routed of transplantation. The article 1271 15B (Human cells, Tissues, and Cellular and Tissue based products), stated that 'You are not required to comply with the requirements of this part if you are an establishment that removes Human cells, Tissues and cellular and tissue-based products from an individual and implants such products into the same individual during the same surgical procedure'.

This article is suggestive of the distinction between the regulations and implies that the autologous and minimally manipulated cells should not be regulated by the same means as that of the other cell therapy products.

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].

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]
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.

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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)

21

Ethics

Consensus on the potential of stem cell therapy to address various incurable, debilitating disorders is unanimous. Stem Cell research and therapy is the frontline 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 pursing 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 point 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 left-over 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 in vitro fertilization (IVF) has determined they are no longer needed for reproductive purposes. However, federal funding may not be permitted for creation of embryos 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 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 four 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 the 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.

Our Views on the Ethical Basis Of Stem Cell 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 if offers hope of saving life, reestablishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published."

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 a 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 need 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 if 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 time side when there are treatment option 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 show 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 mobile, 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 musts 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 had 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.

(3) *That there is a 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.

(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.*

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 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?

So to go back to what we have mentioned in the preface that 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.

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NeuroGen Brain & Spine Institute

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 3000 patients from 36 different countries. The care offered here is very professional yet very caring. A separate pediatric neurorehabilitation facility makes it very child friendly. The institute is very scientific and academic in its approach and to date has published 40 scientific papers in international and national journals. 10 books have also been published and chapters contributed to organizations from America and other countries for research and treatment collaborations. The institute is very quality conscious and has several certifications including the ISO 9001:2008. NeuroGen doctors conduct conferences, workshops and CME's to impart knowledge to doctors, therapists as well as patient families. Cutting edge research, pioneering new treatments, state of the art surgical facilities and laboratories, multidisciplinary rehabilitation, the best medical professionals, comprehensive treatment facilities all under one roof, warm hospitality, pleasing and comfortable living facilities and a caring holistic approach make the NeuroGen Brain and Spine Institute a unique and special facility for patients with Neurological problems.






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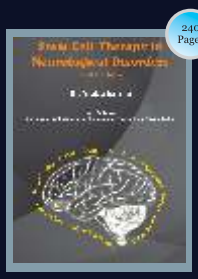

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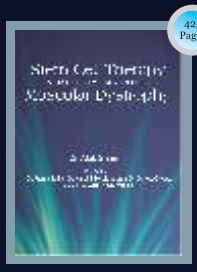

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