

CELL / STEM CELL THERAPY IN NEUROLOGICAL DISORDERS

FIFTH EDITION



Dr. Alok Sharma

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A NeuroGen Publication

Cell / Stem Cell Therapy In Neurological Disorders

Fifth Edition

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Cell / 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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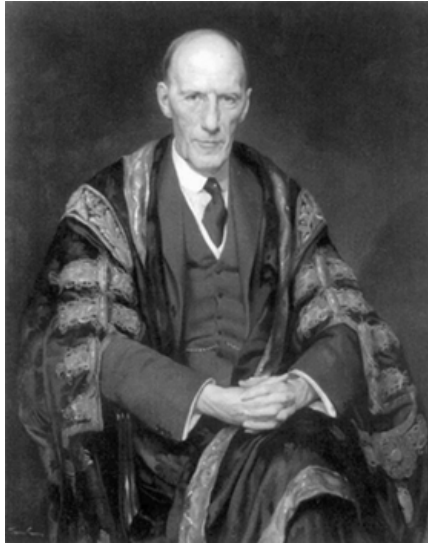
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**This Book is Dedicated to
all our Patients**



A Prayer

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

- Sir Robert Hutchison

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

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

- George Bernard Shaw

PREFACE

“Stem cell Therapy - An idea whose time has come”

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

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

Another important facet of the debate on the use of stem cells is based on the principles and practice of “evidence based medicine”. Whereas there is no denying the fact that evidence based medicine is the bedrock on which more recent practices are based, it is also a fact that the principles of evidence based medicine, as we now practice are a creation and evolution of the past few decades. The notion of evidence based medicine did not exist from the 1800’s to the 1970’s, a period in which almost all of the modern aspects of medicine we now practice were discovered. In fact, it would not be an exaggeration to say that none of the discoveries and innovations of medicine in the 20th century would have happened if the present day yardsticks of evidence based medicine had been in place then. A realization that the systems we created to protect ourselves from the exploitation of commercial agencies is now hampering the very growth and development of medicine has led to us now turning to the concept of “practice based evidence”.

Clinical trials are expensive. Geron spent US\$ 56 million before it could embark on its historic embryonic stem cell study this year. Outside of the pharmaceutical and biotechnology companies these sort of resources are almost unavailable. It is time, therefore, that we relooked at “evidence based medicine” and turned to “practice based evidence” so that the individual practitioner of medicine could be a part of the newer developments and evaluation of the systems of medicine. Ninety percent of current neurosurgical practice is not supported by prospective randomized double blind clinical trials. The same is true for many other surgical branches too. Progress in medicine has come when individual physicians pioneered newer form of therapy that they believed in. Day to day decisions made in clinical practice specially in intensive care setups and operating rooms are made empirically based on the treating physicians experiences and approach and the clinical circumstances at hand. Life is not a randomized trial and all decisions in medicine cannot be based on randomized clinical trials. Evidence generated from the individual physicians practice needs to be respected too. Thus “practice based evidence” needs to be looked at in a way similar to “evidence based medicine.”

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

There is a very thin line that separates “helping someone” and “taking advantage of someone’s helplessness”. It is important that we never cross this line. There are two sides to the ethical debate on basing our treatment options on evidence based medicine. [1] One side of the debate is “Is it ethical for doctors to offer to patients treatment options that have not become a standard of care as yet?.” [2] The other side of the debate is “Is it ethical to deny patients suffering from disabling diseases, treatments options that are safe and available, whilst we wait many years for the results of multicentric international trial to prove that these treatments work?” Both these questions are answered differently by different people depending on what is at stake for them.

Another question that remains unanswered is when does a treatment that is “unproven or experimental” become a treatment that is “proven or established”. How many publications documenting safety and efficacy will it take to make that shift? Is a single publication enough, or are 10, 50 or 100 ok, or are multicentric international trials the only basis to make any treatment option an excepted form

of treatment. Is it necessary to go on reinventing the wheel just to satisfy our intellectual considerations whilst millions of patients continue to suffer? Our own belief is, that based on the already published work and our own clinical experience, this form of treatment is no more experimental since the safety and efficacy of stem cell treatment in many of the neurological disorders has been established and documented in several published articles from several countries. However getting a consensus on these issues is not easy. The role of regulatory bodies in this field also needs to be relooked. Whereas there is no denying the importance of regulation in all aspects of medical care and research, it is also important for the regulatory bodies all over the world to ensure that regulations do not hinder or slow down the evolution of newer forms of treatment. They also need to realize that in this field that is evolving at a breathtaking speed, regulations made several years ago may no longer be valid in the present. That the regulations need to be modified as more evidence pours in from all over the world. That the regulations need to adapt and evolve as the research and clinical results are evolving. That individual doctors, medical institutions and medical associations need to be trusted and given the responsibility to both develop and implement these newer forms of therapy as well as monitor and prevent its misuse.

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

This book attempts to put together information to help answer some of these difficult issues and questions. Whereas there exists a wealth of published information on the basic science work and animal experimental work to show the efficacy of stem cells in neurological disorders, in this book we focus on trials and clinical

treatments done in human patients. The book has been created for those medical practitioners, who are keen to start using stem cell therapy for their patients with incurable neurological disorders, to understand some of the fundamental principles as well as practical aspects that are involved in this line of therapy as well as get informed about all the current clinical data from all over the world that is already published. Our own clinical experiences and techniques have also been incorporated. We believe that this therapy should be available conveniently in all the cities and towns at an affordable cost. This will not only make a big difference to the lives of millions of patients suffering from incurable neurological disorders, but will also further the cause of medicine and science. This book we hope is one small step in that direction. Yes we believe that “Stem cell therapy is an idea whose time has come.”

Dr. Alok Sharma

PREFACE TO THE SECOND EDITION

“Two sides of the Coin”

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

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

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

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

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

We also believe that the centers / practitioners working with the following principles should be looked upon in a more permissive manner :- [a] Those who strictly treat patients in accordance with the Helsinki Declaration. That means they do not treat patients where other more established treatment forms are available and the patients have not already taken them. [b] The medical practitioners practicing this are working within the general broad specialty of their qualifications and are dealing with diseases anatomically and physiologically that concern their broad specialty and that they have received specialized training in cell therapy or done some basic research work in their fields.[c] Whilst doing this treatment they are also making this an object of their research and evaluating its safety and efficacy.[d] They are publishing the results and outcomes of their clinical work, including their negative results

and complications if any.[e] They are taking special informed consent [f] There is a honesty and transparency to their work as shown by the fact that their clinical results are in the public domain and they present their results in national and international scientific conferences.[g] They have Institutional Committees that monitor the ethical, scientific and medical aspects of the work.[h] That quality standards are maintained that is they have GMP facilities, follow GCP standards &/or have other accreditations such as NABH/JCI/ISO etc.

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

Dr. Alok Sharma

PREFACE TO THE THIRD EDITION

The very fact that we have had to bring out a 3rd edition of this book within 6 years of writing the first edition is evidence of the fast moving pace of research and clinical applications of stem cell therapy. The last two years have seen a quantum increase in the number of publications highlighting the clinical efficacy of stem cell therapy in various disorders. The public opinion too is changing in a major way towards making stem cell therapy more available to the patient population. This has resulted in governments all over the world making serious efforts to draft new regulations for stem cell therapy. The lead in this was taken by Japan which has formulated an excellent set of regulations which simultaneously make the more low risk types of stem cell therapy more easily available and have stricter regulations for the high risk types of therapies. Korea is another country which has come up with a progressive set of regulations. In India the scenario has shifted with the Drug Controller General of India (DGCI) taking over the regulations from the Indian council of medical research (ICMR). A key change in the regulatory environment in the country has been the fact that unlike in the past, the present regulators (DGCI) are far more open to considering the views of stem cell therapists as well as patients.

This progressive approach is likely to result in India coming out with a set of regulations which might be better than that of Japan and Korea. Thus the overall change in the public perception, medical opinion as well as regulatory bodies as well as the large evidence that is now available in published literature has resulted in a new found acceptance of this new form of therapy. When we wrote the first edition of this book we had no publications, when we wrote the 2nd edition of this book we had 28 publications and when we are now publishing our 3rd edition two year after the second we have 41 publications. This itself tells the whole story of rapid pace at which stem cell therapy is evolving. We believe that in another 5 years stem cell therapy will become a standard of care for many incurable neurological conditions.

Dr. Alok Sharma

PREFACE TO THE FOURTH EDITION

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

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

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

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

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

Dr. Alok Sharma

PREFACE TO THE FIFTH EDITION

In 2010 when we published the 1st edition of this book, we had treated 400 patients. By the 4th edition in the year 2018 we had treated 6500 patients and at the time of publication of this 5th edition we have treated over 16000 patients. More interestingly patients have come to us from 103 different countries from all the six continents of the planet. The fact that families travel 15 to 20 hours in flights across continents with their special needs children to come to us for treatment is the evidence of their hope, faith and trust in our treatment. In addition, our clinical results have been published in 108 peer reviewed scientific papers in International medical journals. The world's first paper on Cell therapy for Autism was published by us in the journal "Stem Cell International" in 2013. A larger study was published in the "American Journal of Stem Cells" in 2023 and recently the world's largest study of 1011 patients has been accepted for publication. There are several other landmark papers on Intellectual Disability, Cerebral Palsy, Muscular Dystrophy, Spinal Cord Injury, ALS etc. that have been published by us over these years. We have also published 26 books on various aspects of regenerative medicine and management of neurological disabilities. Worldwide too, there has been an increase in the number of papers published highlighting the safety and benefits of cell therapy in various neurological conditions. These include 47 papers in autism, 62 papers in cerebral palsy, 40 papers in muscular dystrophy, 86 papers in spinal cord injury, 54 papers in ALS etc. There are now even published Meta-Analysis & Systematic Reviews documenting the safety & benefits of cell/stem cells in various neurological conditions.

Whereas thousands of patients from across the world are safely receiving the benefits of cell therapy and there are publications all over the world showing safety and efficacy, the medical community in general refuses to accept the beneficial effects of regenerative medicine. Their conversation about Cell /Stem cell therapy has not changed from 2010 till now. What they are demanding over the last 15 years is for prospective randomized double blind placebo control clinical trials to prove the efficacy. There is an important point of distinction that the critics do not recognise despite several government documents as well as court judgements that make this important separation. Fundamentally there are two types of treatments: 1) Drugs and Products that are manufactured by the companies and sold to doctors and hospitals. 2) Procedures done by doctors in their own hospitals. Stem cell therapy is also divided into these two categories of products and procedures. There are several companies that manufacture stem cell derived products and sell them to doctors. These products as per Indian law require approval from CDSCO which is the Indian equivalent of the US FDA. The CDSCO, the US FDA and FDAs of all other countries only regulate products and do not give approval to procedures. Several government regulatory documents state this and court orders within India and the United States, state the same thing. So, when critics say

the procedure is not FDA approved what they don't realise is that FDA does not approve procedures of any kind including stem cell procedure. They only approve stem cell derived products. Our requests to all our medical practitioner colleagues is that instead of blindly opposing this new and exciting field that has brought hope and health to thousands of children and adults, they should read our peer reviewed published papers and most importantly talk to the patient families of children with Autism, Cerebral palsy and Muscular dystrophy etc. and hear directly from them how their children have benefited from this treatment. When a child who has been nonverbal for 6-7 years suddenly starts talking after cell therapy there is no question of whether it is working or not, when a totally wheelchair bound child starts walking on his own feet after cell therapy there is no debate as to whether the cell therapy has worked or not.

In the preface to the first edition, I had quoted Victor Hugo who had used the term "An idea whose time has come". In 2010 my preface started with "*Stem cell Therapy - An idea whose time has come*" 16 years and 16000 patients later, I am having to repeat the same statement this time only wishing that all my medical colleagues join us on this amazing journey of giving mental and physical health as well as life to the millions of patients across the world who we have shown can be helped in Cell/Stem Cell Therapy.

Dr. Alok Sharma

“Primum non nocere”
First do no harm!

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

A) AUTISM:

1. Sharma A, Gokulchandran N, Sane H, Kulkarni P, Kannan K, Shaikh Z, Biju H, Paranjape A, D'sa M, Badhe P. Autologous bone marrow mononuclear cell administration in a large cohort of 1011 autism spectrum disorder patients. *Clinical Transplantation and Research*.
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5. Sharma A, et al., The baseline pattern and age related developmental metabolic changes in the brain of children with autism as measured on positron emission tomography/computed tomography scan. *World J Nucl Med* 2018;17
6. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Pooja Kulkarni, Sarita Kalburgi, Ridhima Sharma, Prerna Badhe, Samson Nivins. PET CT Scan Brain As A Monitoring Tool To Study The Effects Of Autologous Bone Marrow Mononuclear Cell Transplantation In Autism Spectrum Disorder. *International Journal of Current Advanced Research*. Sep 2017
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11. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Avantika Patil, Akshata Shetty, Hema Biju, Pooja Kulkarni, Prerna Badhe. Amelioration of Autism by Autologous Bone Marrow Mononuclear Cells and Neurorehabilitation: A Case Report. American Journal of Medical Case Reports, 2015, Vol. 3, No. 10, 304-309
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B) CEREBRAL PALSY:

1. Alok Sharma, Nandini Gokulchandran, Pooja Kulkarni, Sandhya Kiran Mulangi, Khushboo Bhagawanani, Vishal Ganar, Hemangi Sane, Prerna Badhe. Multiple Cellular Therapies Along with Neurorehabilitation in Spastic Diplegic Cerebral Palsy: A Case Report. Innov Clin Neurosci. 2020;17(10-12)
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7. Dr. Alok Sharma, Dr. Nandini Gokulchandran, Mrs. Suhasini Pai, Ms. Pooja Kulkarni , Dr. Hemangi Sane , Dr. Khushboo Bhagwanani ,Dr. Prerna Badhe. Diplegic dystonic Cerebral Palsy treated with cellular therapy: a case report. Journal- International Journal of Case Studies. 2017
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Duchenne Muscular Dystrophy-A Case Report. Indian journal of Clinical Practice. 2012; 23 (3): 169-72.

D) SPINAL CORD INJURY:

1. Alok Sharma, Hemangi Sane, Nandini Gokulchandran, Pooja Kulkarni, Alitta Jose, Vivek Nair, Rohit Das, Vaibhav Lakhanpal, and Prerna Badhe, Intrathecal transplantation of autologous bone marrow mononuclear cells in patients with sub-acute and chronic spinal cord injury: An open-label study, International Journal of Health Sciences 2020, 14(2):1-9.
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E) STROKE:

1. Sharma A, Sane H, Paranjape A, Jose A, Mehta D, Inamdar S, Badhe P, Gokulchandran N. Cellular therapy improves brain metabolism in a case of chronic ischemic Stroke. International Journal of Science and Healthcare Research. 2019; vol 4 (1): 2455-7587.
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F) ALS / MND

1. Sharma A., Sane H., Paranjape A., Pradhan R., Varghese R., Biju H., Nair V., Sawant D., Gokulchandran N., Badhe P. Role of reproductive hormonal status on the outcome of autologous bone marrow mononuclear cells intra-

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G) INTELLECTUAL DISABILITY

1. Sharma, A., Gokulchandran, N., Varghese, R., Sane, H., Ganar, V., Kulkarni, P., Nivins, S., & Badhe, P. (2019). Improved brain function in an adult case of Intellectual Disability with Autism Spectrum Disorder following Cell Therapy. *International Journal of Medical Science and Clinical Invention*, 6(2), 4294-4301. <https://doi.org/10.18535/ijmsci/v6i2.03>
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H) MISCELLANEOUS:

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 4. Sharma A, Gokulchandran N, Sane H, Nivins S, Kulkarni P, Mane V, Maheshwari M, Badhe P. Efficacy of Autologous Bone Marrow Derived Mononuclear Cells in the treatment of neurodeficits in Down's Syndrome: A case report. *British Journal of BioMedical Research*. 2018, 2(2): 281-288.
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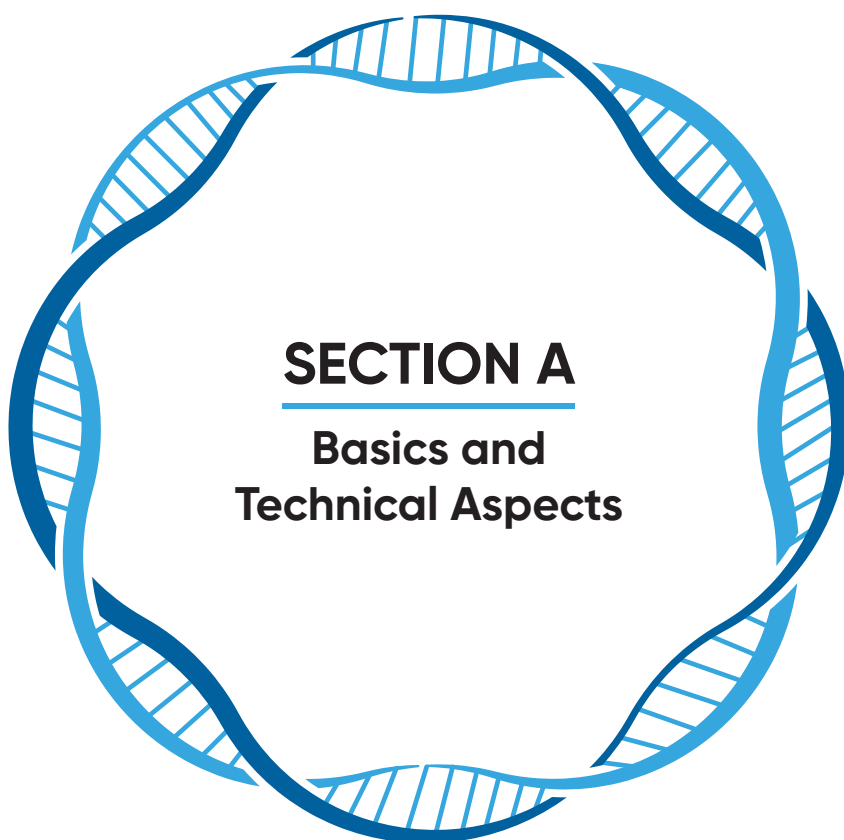
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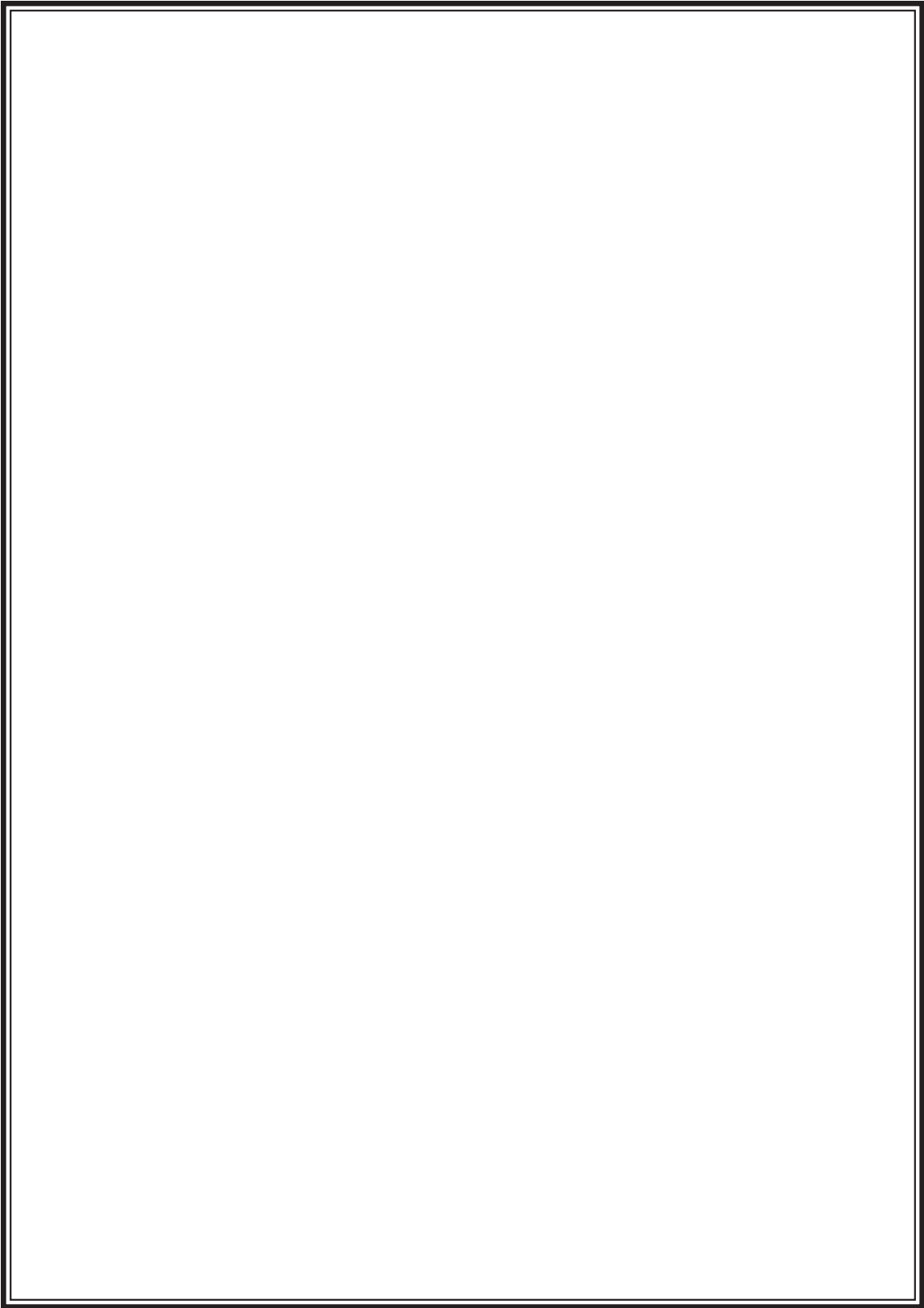
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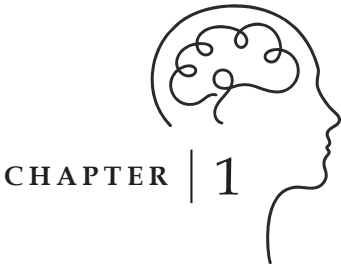


"I would go anywhere in the world for a therapy that is safe and that could accomplish the goal of recovery"

- Christopher Reeve







Introduction : Neuroregenerative and Neurorestorative Medicine

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

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

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

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

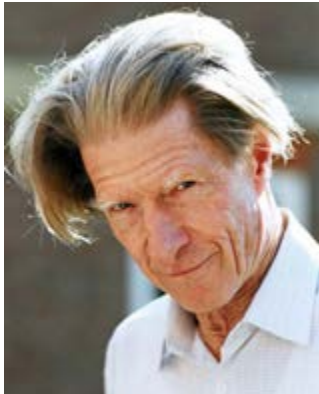
Neuroregeneration is a modern concept which uses neuroplasticity, neurorestoration and neurogenesis to develop novel therapeutic strategies. Neurorestoration is a subdiscipline of neuroscience which studies neural regeneration, neural structural repair or replacement, neuroplasticity and neuromodulation.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

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

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

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

Nobel Prize Winners in Sem Cell Research 2012



John B. Gurdon



Shinya Yamanaka

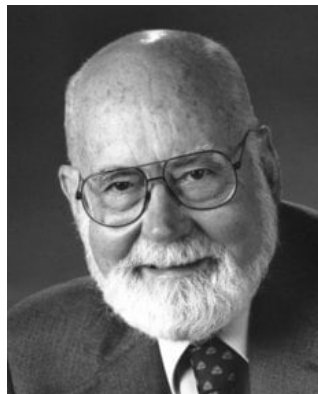


2007

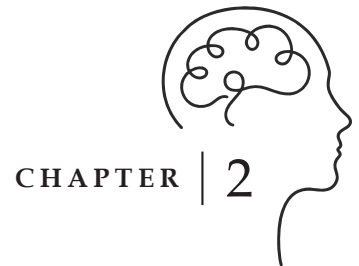
1990



Sir Martin Evans



Dr. E. Thomas



Historical Review: Evolution of Stem Cell Therapy

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

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One remarkable but overlooked feature of biology is the capacity of animals to restore the lost portions. For decades, efforts have been made to find the sources of fresh cells for these regenerative occurrences. Humans share some of the aforementioned qualities because our bodies are continuously rebuilding blood, skin, and other tissues, even though we are unable to restore a missing finger or limb.

This chapter covers the history of stem cells, from the term's initial coining over a century ago to the most current advancements in the last ten years, when stem cells are being studied and used to treat a wide range of diseases. The finding gave rise to optimism regarding the medical potential of regeneration as a potential cure for numerous diseases that were previously thought to be incurable. By using stem cells' remarkable ability to develop into specialized cells, damaged tissue might be repaired with a fresh supply of healthy cells for the first time in human history.

Introduction to the Concept of Stem Cells

Research on stem cells commenced because scientists wanted to gain insight into how tissues are preserved in adulthood, not how distinct cell types develop in the

embryo. It has long been understood that there is cellular heterogeneity within a particular tissue: differentiated cells in certain tissues, such as the skin, blood, and intestinal epithelium, have a short lifespan and cannot self-renew. The idea that stem cells, which are cells with a high capacity for renewal and the ability to produce daughter cells that go through additional differentiation, are responsible for maintaining such tissues resulted from this. They are called multipotent or unipotent cells because they only produce the differentiated lineages that are suitable for the tissue in which they live.

The primary characteristic of stem cells is their ability to self-renew and differentiate into other types of cells. The ability of stem cells to proliferate and differentiate makes them very promising for the creation of innovative cell-based treatments. Furthermore, new research points to a possible link between some cancers and the deregulation of stem cell characteristics.

Historical Review And Evolution of Stem Cell Therapy

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

Year	Events
1868	Coining of the term Stammzelle by Ernst Haeckel, a German biologist
1892	Boveri proposed that cells along the germ line lineage between the fertilized egg and committed germ cells can be called stem cells
1896	Pappenheim used stem cell to describe a precursor cell capable of giving rise to both red and white blood cells.
Early 1900(s)	Physicians administered bone marrow cells by mouth to patients with anemia and leukemia
Early 1950(s)	Identity of cells that could regenerate into tissues was first revealed; experiments with bone marrow established the existence of stem cells
1956	Dr. E Donall Thomas, a bone marrow transplant specialist, administered donor adult stem cells to leukemia patients who went into complete remission
1958	Jean Dausset, a French medical researcher identified that the bone marrow transplant between identical twins guaranteed complete HLA compatibility between donor and recipient.
1963	Mc Culloch and Till started series of experiments involving administration of bone marrow cells into irradiated mice.

1964	Researchers isolated cells from teratocarcinoma and replicated in cell culture as stem cells. These were termed as Embryonic Carcinoma Cells(ECs)
1973	First unrelated bone marrow transplant was performed
1980(s)	James Thomson et al. at the University of Wisconsin extracted the first line of embryonic cells (ESCs) from mice
1981	Embryonic stem cells were derived from mouse embryo by 2 groups. The term Embryonic Stem Cells was coined; it was shown that these cells could be cultured in vitro.
1984	Congress passed National Organ Transplant Act which included the evaluation of unrelated marrow transplantation and feasibility of establishing a national donor registry.
1985	Yamamura detected the presence of stem cells in the pulp tissue
1988	Cord blood stem cells were used in treatment of blood cancer and Fanconi Anemia.
1990	Dr E Thomas and Joseph E Murray were awarded Nobel prize in Physiology for discoveries concerning cell and organ transplantation in treatment of human diseases.
1996	Dolly - the sheep. First mammal cloned from an adult somatic cell, using the process of nuclear transfer by Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute.
1998	Scientist isolated the first human embryonic stem cells
1999	Mc Donald J W et al. published a paper showing differentiation of transplanted embryonic stem cells into the injured rat spinal cord. The cells were witnessed to home at the site of injury and differentiate into astrocytes, oligodendrocytes and neurons.
2001	George W Bush announced his decision to allow federal funding of research only on existing human embryonic stem cells putting a halt on any further derivation of human stem cells and research.
2002,2004	CD34+ stem cells from bone marrow and umbilical cord blood were investigated and found to differentiate into different cell types.
2006	Generation of induced pluripotent stem cells (iPSCs) from the laboratory of Shinya Yamanaka which demonstrated the reprogramming of mouse somatic cells to pluripotency.
2007	Researchers in China reported that cell population derived from human fetal bone marrow had osteogenic, adipogenic, neural and erythroid lineage at a single cell level.

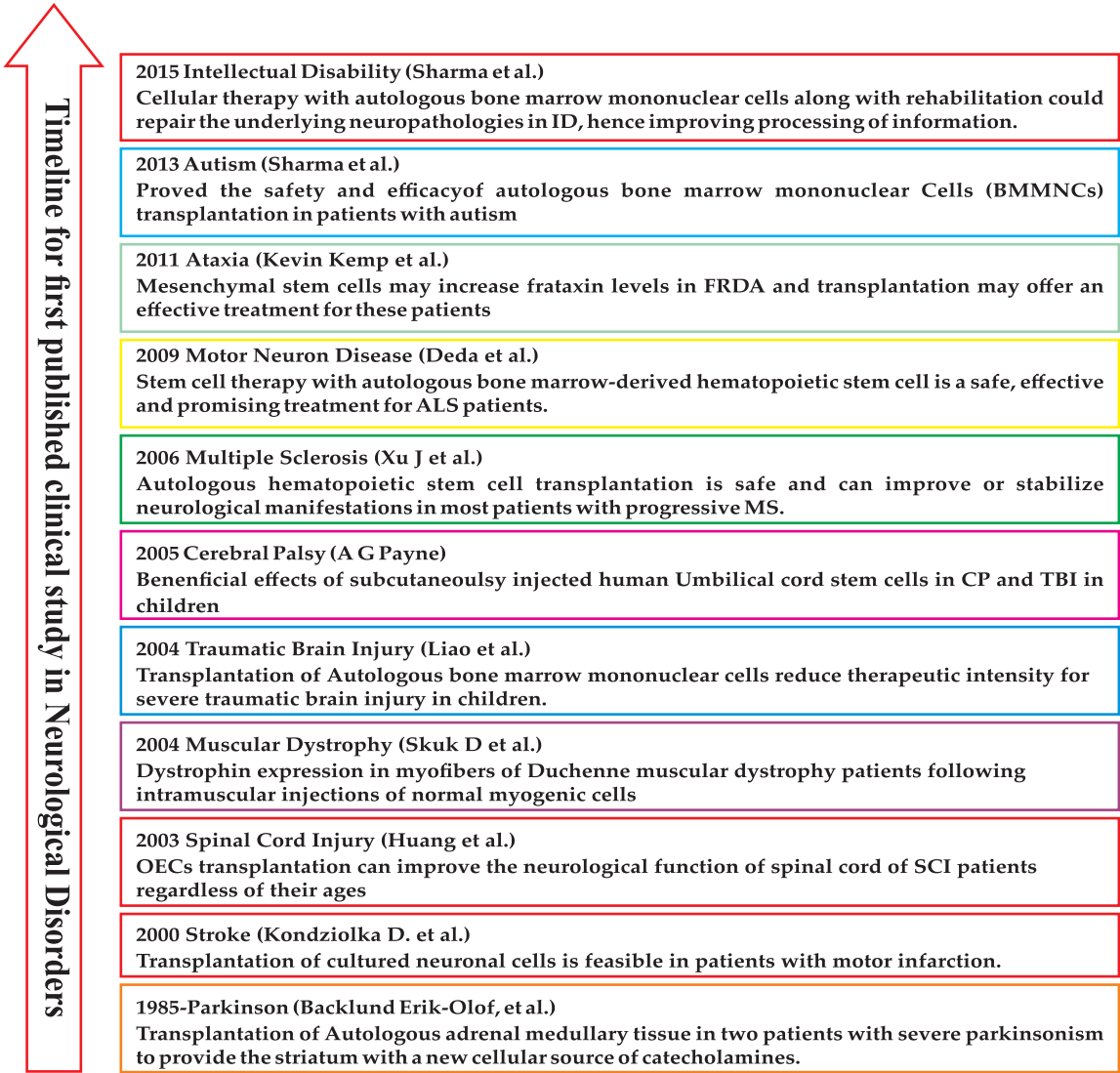
2009	President B Obama reversed the decision clearing the way again for stem cell research to progress in US
2014	Teams led by Dieter Egli of the New York Stem Cell Foundation and Young Gie Chung from CHA University in Seoul, South Korea, independently produced human embryonic stem cells from adult cells, using therapeutic cloning
2014	Masayo Takahashi started world’s first trial of a therapy based on induced pluripotent stem cells, to treat a form of age-related blindness.

What is in store for the future?

Since 1868, when the phrase “stem cells” was first used, the field of stem cell research and therapy has rapidly advanced. The discovery of embryonic stem cells ushered in a new age of stem cell use. However, a dispute involving the use of human embryonic stem cells for research engulfed stem cell research. Since then, researchers and medical professionals have been investigating alternate stem cell sources, such as the bone or the umbilical cord. Numerous stem cell types are being investigated for the treatment of incurable diseases of organs other than hematopoietic, including the heart, liver, brain, muscles, and others. There will be a lot more in the years to come

Clinical research on stem cells has now begun. Around the world, a large number of clinical investigations are being carried out employing various cell types and methods. The safety and viability of adult stem cells have put them at the forefront of clinical research. Stem cell therapy is currently seen as the key to the future of individualized medicine and healthcare.

It’s interesting to note how succinctly and plainly the two US presidents’ remarks capture the entire worldwide ethical discussion around stem cell research. These are two opposing sides of the same coin that have been replicated here.



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

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

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

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

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

Thank you all for coming. God bless."

President Obama Speech on Stem Cell Policy Change

March 9, 2009



(Source: White House Press Office)

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

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

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

But in recent years, when it comes to stem cell research, rather than furthering discovery, our government has forced what I believe is a false choice between sound science and moral values. In this case, I believe the two are not inconsistent. As a person of faith, I believe we are called to care for each other and work to ease human suffering. I believe we have been given the capacity and will to pursue this research - and the humanity and conscience to do so responsibly.

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

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

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

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

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

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

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

That is why today, I am also signing a Presidential Memorandum directing the head of the White House Office of Science and Technology Policy to develop a strategy for restoring scientific integrity to government decision making. To ensure that in this new Administration, we base our public policies on the soundest science; that we appoint scientific advisors based on their credentials and experience, not their politics or ideology; and that we are

open and honest with the American people about the science behind our decisions. That is how we will harness the power of science to achieve our goals - to preserve our environment and protect our national security; to create the jobs of the future, and live longer, healthier lives.

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

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

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

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

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

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

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

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

Sincerely,
Gavin Nore

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

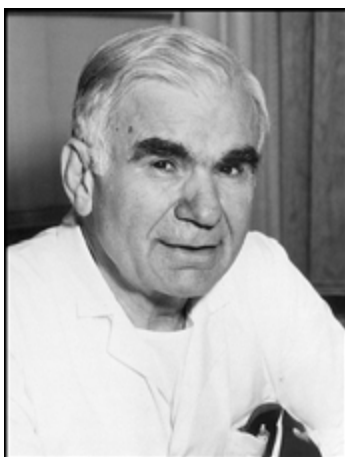
Courtesy: letterstopresidentobama.tumblr.com

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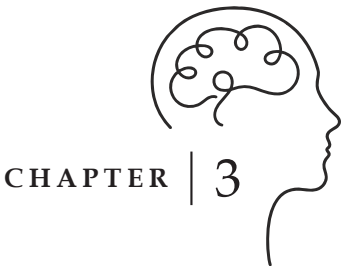
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"Our enduring hope is invested in Biological research"



M. Gazi Yasargil
(Neurosurgeon of The Millenium)



Basics of Cell / Stem Cells: Types and Sources

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

The field of stem cell therapy has advanced with time to such an extent that it has percolated in every branch of medicine. Laboratory investigations and advanced biotechnology have led to an exponential increase in our understanding of stem cells. Clinical studies are now being conducted to try to improve a variety of illnesses based on this foundational knowledge. The medical community now needs to understand the fundamentals of these stem cells. Here we make an effort to simplify the complex scientific information regarding stem cells.

The structure and functions of the human body are complex. It consists of a variety of cell types, each having its own cytoskeleton, genetic composition, and cellular roles and processes. Despite this complexity, each of these cells originates from a pool of early embryonic stem cells. The specialized or differentiated cells that comprise the human body are produced by these stem cells both early in development and later in life. Scientists have been working to figure out how unspecialized stem cells differentiate into several specialized stem cell types for the past 20 years. Stem cells have the ability to self-renew or differentiate into specific cell types. They are special for creating medicinal treatments that can replace lost or injured cells because of their differentiation and transdifferentiation properties. We shall

examine a few of the key characteristics of stem cells in this chapter.

What Are Stem Cells?

The ability to self-renew and differentiate into multiple cell types are two unique characteristics that distinguish a stem cell. A colony of identical cells can be created by the endless division of these cells. On cue, stem cells can differentiate into two distinct cell lines through asymmetric division. One remains a contributing member of the original stem cell line and is identical to the parent. In contrast to its parent cell, the other cell has a different set of genetic instructions and is distinguished by a lower capacity for proliferation and a more limited capacity for development. A stem cell eventually develops into a “progenitor” or “precursor” cell, dedicated to creating one or a small number of terminally differentiated cells, including muscle cells, neurons, 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 : Both embryonic and extra-embryonic tissue are produced by totipotent stem cells. A clone produced by somatic cell nuclear transfer (SCNT) to an enucleated oocyte is the artificial counterpart of the physiological totipotent stem cell, which is a fertilized oocyte (zygote) or first blastomere, which consists of the 8-cell stage.
2. Pluripotent stem cells possess : the ability to produce cells from the endoderm, mesoderm, and ectoderm of the three germ layers of the embryo.
3. Multipotent stem cells : Only one of the germ cell layers, the ecto, meso, or endoderm, is produced by multipotent stem cells. Adult bone marrow and 8-day-old embryos are among the sources.
4. Monopotent/unipotent stem cells : are tissue-committed stem cells that differentiate into cells of a single lineage, such as neural, intestinal, hepatic, skeletal muscle, hematopoietic, or epidermal stem cells. (2)

Classification of Stem Cells

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

- Embryonic Stem Cells
- Fetal Stem Cells

- Umbilical Cord Stem Cells
- Adult Stem Cells
- Induced Pluripotent Stem Cells

A. Embryonic Stem Cells:

Pluripotent embryonic stem cells are produced from the inner cell mass (ICM) of 5-7 day blastocysts that are acquired from IVF clinics. (3) In the end, the ICM produces the three germ layers and then the entire embryo. Due to the embryonic stem cell's ability to build "germ layers", self-renew endlessly, and form any type of cell in the body, this field has become much more accessible, but it has also generated ethical and legal problems.

As with mES cells, hES cell lines have recently been grown on human feeder cells to prevent xenogenic effects (8) and without feeder cells in serum-free conditions (9). 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 the future.

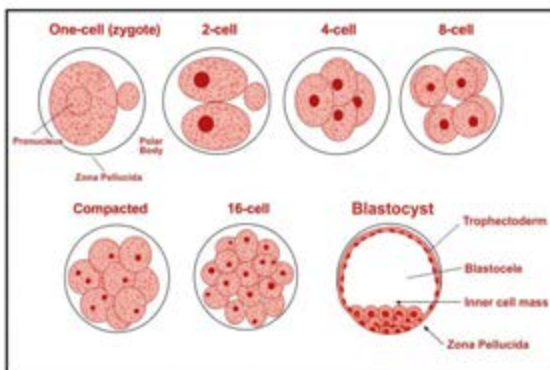
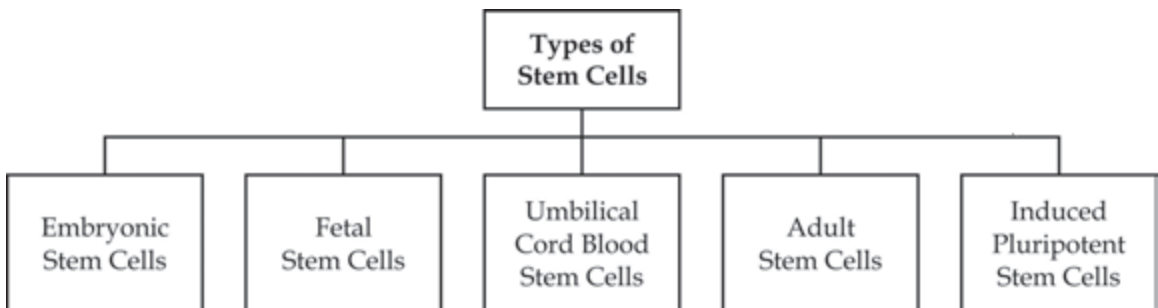


Figure 1: Development of a zygote to a blastocyst (from where embryonic stem cells are derived)

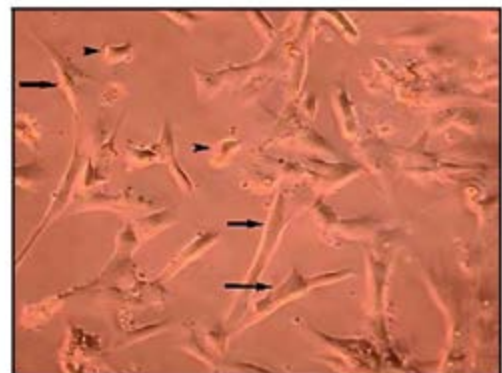


Figure 2: Mesenchymal stem cells

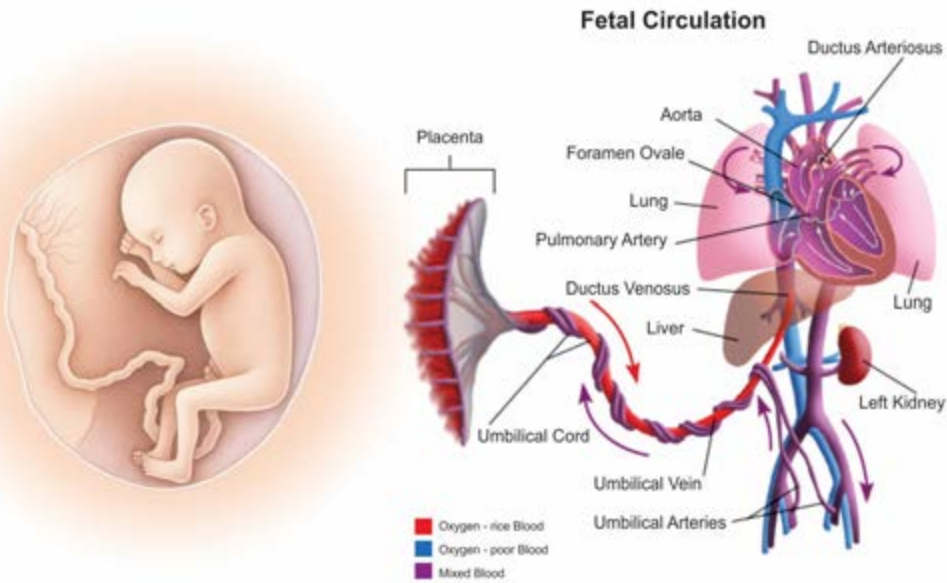


Figure 3: The umbilical cord and placenta: a rich source of stem cells

B. Fetal Stem Cells:

With their distinct and intriguing characteristics, fetal stem cells (FSCs) are a relatively recent addition to the community of various stem cell sources (15). FSCs can be isolated from a range of somatic organs, amniotic fluid, and the placenta during the course of pregnancy, in addition to the fetal blood and hemopoietic organs in the early stages of pregnancy (16). They can also be obtained from extra-embryonic sources (17). A rich source of hemopoietic stem cells (HSCs) is fetal blood. Other stem cell types derived from fetal blood include mesenchymal stem cells, endothelial stem cells, epithelial stem cells, and neural stem cells (18). These cells multiply more rapidly than those in adult bone marrow or cord blood. In terms of plasticity characteristics and rates of proliferation, these cells are in between adult and embryonic stem cells.

C. Umbilical Cord Stem Cells

Immediately after birth, umbilical cord blood stem cells can be extracted from the cord. Umbilical cord blood is a rich source of hematopoietic stem cells, just like bone marrow is. It has been effectively utilized as an alternate allogeneic donor source to treat a range of genetic, hematologic, immunologic, and oncologic illnesses. The blood that remains in the umbilical vein after birth includes a rich reservoir of hematopoietic stem and progenitor cells. Non-hematopoietic stem cells can also be obtained from fresh cord blood, which shows promise. Endothelial cells, MSCs, and unrestrained somatic stem cells (USSC) are among the compo-

nents it comprises. Compared to adult or paediatric bone marrow stem cells, these hematopoietic stem cells are less developed.

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

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

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

However, there are a few disadvantages (20):

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

In order to treat leukemia and other blood disorders, cord blood has lately become a viable alternative source of hematopoietic stem cells.

Numerous cord blood banks have been set up around the globe to store umbilical cord stem cells. These are often either privatized or pure public banks. Some financial institutions provide both forms of banking (mixed type). The kind of biological material that is stored in umbilical cord stem cell banks varies as well. Only the cord blood (from the umbilical vein), which mostly contains hematopoietic stem cells, is kept in certain banks. Parts of the placenta and cord, which are abundant in mesenchymal stem cells, are increasingly being kept in banks.

D. Adult Stem Cells

Adult stem cells are pluripotent and self-renewing and can differentiate into the mature cells that inhabit their surroundings and may also be capable of transdifferentiation. These adult stem cells' main function is to start the organ's healing process after damage or injury. There is practical difficulty in obtaining these cells due to the following reasons:

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

Compared to the use of embryonic stem cells, these cells are the favored option because their use does not raise any moral, ethical, or legal concerns. Their pluripotency is still up for question, though, and the idea of adult stem cell plasticity has been very fluid.

Numerous organs and tissues, including bone marrow, the central nervous system, the nose, peripheral blood, blood vessels, skeletal muscle, skin, teeth, the heart, the stomach, the liver, the ovarian epithelium, and the testis.

Bone Marrow-Derived Cells

Bone marrow is the most widely accessible and deeply researched source of adult stem cells. It has been discovered that the bone marrow contains a variety of stem cell types, each with a unique capacity to develop and produce cells from one or more germ layers.

At first, it was believed that bone marrow only contained hematopoietic stem cells. However, increasing evidence suggests the presence of a heterogeneous population of cells with varying plasticity.

Potential pluripotent stem cell candidates identified in bone marrow are:

1. Mononuclear Cells:

The diverse population of bone marrow mononuclear cells consists of mesenchymal stromal cells, endothelial progenitor cells (EPCs), very small embryonic-like (VSELs) stem cells, and hematopoietic lineage cells like lymphocytes, monocytes, stem cells, and progenitor cells. Human adult bone marrow, peripheral blood, and umbilical cord are used to separate mononuclear cells. This cell combination has demonstrated encouraging therapeutic promise for a number of neurological disorders(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 cartilage, muscle, bone, tendon, ligament fat and a variety of other connective tissues. Since bone marrow-derived cells have a far greater range of differentiation potential than previously believed, they appear to retain a remarkable degree of plasticity. Angiogenesis, somatic muscle development, liver regeneration, and the creation of cell types in the central nervous system have all been linked to bone marrow cells. It is probable that other populations of primitive non-hematopoietic stem cells could infect MSC. Every time an MSC is shown to “transdifferentiate” into cells from other germ layers, this possibility should be taken into account. The International Society for Cellular Therapy has suggested that the term “MSC stem cells” be dropped in favor of “multipotent mesenchymal stromal cells” due to a number of discrepancies that have been discovered in the field of MSC research. (22)

3. Multipotent Adult Progenitor Cells (MAPC):

MAPC are isolated from BM as well as 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, when injected into a growing blastocyst, have been shown to contribute to all three germ layers, 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:

These cells were obtained from human adult BM by cultivating BM MNC on fibronectin under low oxygen tension. MIAMI cells were extracted from the bone marrow of individuals aged 3 to 72. MIAMI cell-derived colonies showed several markers for cells from all three germ layers, indicating that they had pluripotency, at least according to in vitro tests. The capacity of these cells to finish blastocyst development hasn't been examined yet, though. Although it's unclear how these cells relate to MSC and MAPC, it's likely that they are overlapping cell types that were distinguished by slightly different isolation/expansion techniques.

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/ex-

pansion strategies.

6. *Very Small Embryonic-Like (VSEL) Stem Cells:*

In murine BM, a homogeneous population of uncommon Sca-1+ lin-CD45-cells (~0.01% of BM MNC) was recently discovered. They express pluripotent stem cell markers like SSEA-1, Oct-4, Nanog, Rex-1, and Rif-1 telomerase protein, as identified by RQ-PCR and immunohistochemistry (24). VSELs (2-4 µm in diameter) exhibit a number of characteristics common to embryonic stem cells, including i) a large nucleus encircled by a narrow rim of cytoplasm and ii) open-type chromatin (euchromatin), according to a direct electron microscopy study. It's interesting to note that these cells have many mitochondria and diploid DNA despite their tiny size. However, VSEL are CD90-CD105-CD29- and do not express MHC-1 or HLA-DR antigens.

7. *Central Nervous system (CNS)*

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

8. *Nose:*

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

9. *Skin:*

A unique population of stem cells, including melanoblasts and epidermal stem cells, are found in the skin. Both neural and mesodermal cell types can be produced by these cells, and the majority of the neural cells they produce share traits with Schwann cells and peripheral neurons (35)

10. *Adipose tissue:*

Made up of resident monocytes/macrophages, lymphocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, and mature and preadipocytes, adipose tissue is a very complex tissue. Thus, pluripotent stromal cells originating from adipose tissue are abundant in this tissue compartment. It has been shown that

AT contains processed lipoaspirate (PLA) cells, which are stem cells that resemble BM-MSCs, expressing many proteins that are compatible with the neuronal phenotype and displaying a morphology similar to that of neurons.(36, 37)

11. Peripheral Nervous System (PNS):

Schwann cells are the supporting cells of the PNS. Like oligodendrocyte, Schwann cells wrap themselves around nerve axons, but the major difference is that a single Schwann cell makes up a single segment of an axon's myelin sheath. One of the biological elements that migrates to the site of tissue damage following spinal cord injury is Schwann cells, which originate from the dorsal and ventral roots. Numerous investigations have shown that Schwann cells are capable of remyelinating, and the role of this myelin in the transmission of brain impulses has been verified. (38, 39)

12. Eye stem cells

The adult mouse eye has been found to have stem cells. It has been demonstrated that single pigmented ciliary edge cells can clonally multiply in vitro to create spherical colonies of cells that can develop into rod photoreceptors, bipolar neurons, and Muller glia, among other cell types specific to the retina. Instead of being found in the central and peripheral retinal pigmented epithelium, the adult retinal stem cells were found on the pigmentary ciliary edge. (40)

13. Dental Stem Cells:

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

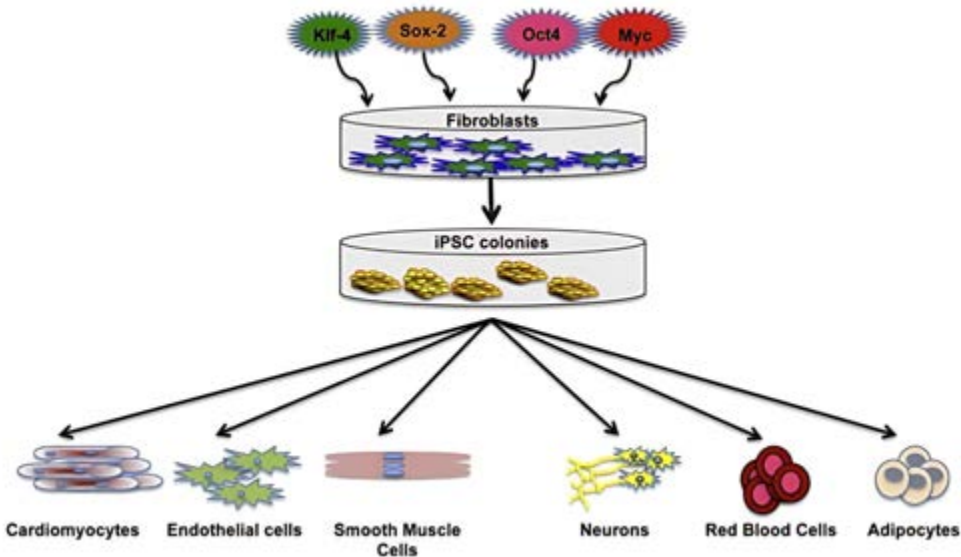
14. Muscular Stem Cells:

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 the repair of regular wear and tear of muscle fibres. These cells help to regenerate the damaged muscles.

E. Induced Pluripotent Stem Cells:

Recent advances in stem cell research have shown two major approaches to generating mature neurons. The first involves reprogramming adult somatic cells into induced pluripotent stem cells (iPSCs), which resemble embryonic stem cells in their karyotype, telomerase activity, surface markers, and ability to differenti-

ate into derivatives of all three germ layers, while also bypassing the ethical and immunological concerns of embryonic stem cells (48–52). The second approach, supported by recent studies, demonstrates that adult somatic cells such as fibroblasts can be directly reprogrammed into subtype-specific neurons, including motor neurons, through transcription factor-mediated conversion, without passing through the intermediate iPSC or progenitor stage (41–47).



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.

Figure 4: Induced pluripotent stem cells for regenerative and biomedical discovery.

Conclusion

Stem cells have received much attention for their potential use in cell-based therapies for various human diseases. Understanding different types of stem cells and their niches is essential for future clinical applications. All the above-mentioned stem cells have distinct differential potentials which need to be explored and manipulated to optimise their therapeutic potential. Until now, adult bone marrow stem cells have been the most studied type of cells.

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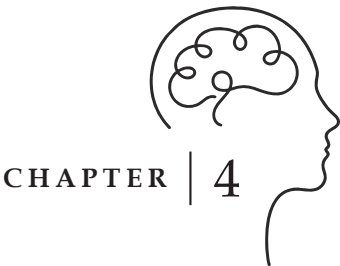
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School yourself to demureness and patience and learn to inure yourself to drudgery in science. Perfect as the wing is of the bird, it would never raise the bird up without resting on air. Facts are the air of the scientist. Without them your theories 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



Mechanism of Action

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

The naturally occurring stem cells in the organs constantly repair the daily wear and tear of tissues through multitudes of mechanisms. In various disease models, the mechanism of action of stem cells has been studied in great depth. The pathways through which they act have also been studied in vitro. The microcellular environment plays a crucial role in deciding the fate of stem cells. Stem cells carry out the repair process by neuromodulation, neuroprotection, axon sprouting, neural circuit reconstruction, neurogenesis, neuroregeneration, neurorepair, neuroreplacement, and muscle regeneration. The mechanism

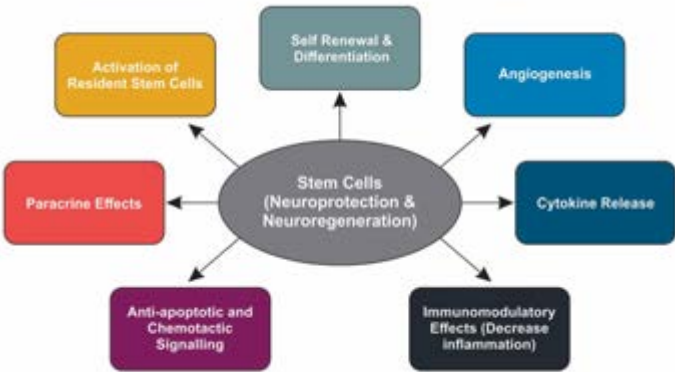


Figure 1: Mechanism of Action

of stem cells is divided into two categories: direct cell replacement and indirect repair via paracrine mechanisms.

Figure 1: Mechanism of Action

1. Direct Cell Replacement:

Homing of the stem cells

During transplantation, stem cells are known to move to damaged or injured areas. This characteristic is ascribed to the surface expression of chemokines, growth factors, and extracellular matrix receptors. There is also proof that stem cells, both exogenous and endogenous, can move from the injection or infusion site into the injured area. The bone marrow contains MSCs that can be activated, target infarcted regions, and develop into the desired tissue type. Granulocyte colony-stimulating factor (G-CSF), which has been extensively researched, encourages the mobilization of stem cells produced from bone marrow in the context of acute injury. (1) Stromal cell-derived factor 1 (SDF-1), monocyte chemoattractant protein-3 (MCP-3), stem cell factor (SCF), and/or IL-8 expression may also be necessary for this homing process.

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

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

Stem Cell Plasticity

Stem cell plasticity refers to the capacity of the tissue-derived stem cells to give rise to cell types other than that of the resident tissues. "Cell fusion" is one of the processes that explains stem cell plasticity. (3) Direct fusion between an undifferentiated stem cell and a more developed cell produces a single cell that exhibits characteristics of both cells. However, as there has been no proof of cell fusion in experimental experiments, this mechanism has been left out of many papers. The processes of "transdifferentiation" or "dedifferentiation" (4), in which cells can either differentiate into a different cell type within the same tissue or into a completely different tissue without acquiring an intermediate, identifiable, undifferentiated progenitor state, provide additional evidence for the plasticity of stem cells. The most plausible explanation for the adaptability of stem cells is the potential

that real pluripotent stem cells exist in different body tissues.

Adult stem cells like bone marrow cells have been reported to generate a wide spectrum of different cell types, including hepatocytes, endothelial, myocardial, neuronal, and glial cells. When transplanted into injured mouse muscle, cells originating from mouse bone marrow produce skeletal muscle cells, according to a 1998 study by Ferrari et al. (5) Hematopoietic cells can develop into functioning hepatocytes, endothelial cells, liver, gut, lung, and skin epithelial cells, as well as cardiac myocytes. (6–12) Brain astrocytes can be produced by bone marrow mesenchymal stromal cells (MSC). It has been demonstrated that enriched stem cells from adult mouse skeletal muscle can generate blood cells. (13–15) Genetically modified cells from one adult mouse organ appear to have produced cell type traits of other organs after transplantation in the majority of these plasticity investigations, indicating that even cell types have the capacity to develop in different ways.

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

2. The Paracrine Effect

In order to orchestrate the stem cell-driven repair process through neuroprotection, increasing angiogenesis, decreasing inflammation, preventing apoptosis, releasing chemotactic factors, aiding in extracellular matrix tissue remodeling, and activating resident/satellite cells, stem cells transplanted into injured tissue express paracrine signaling factors, such as cytokines and other growth factors. These factors are covered in more detail below. (18)

Neuroprotection

Stem cells release a wide range of neuroprotective growth factors, such as insulin-like growth factor type 1, glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor-2, neurotrophin-3 (NT-3), nerve growth factor (NGF), and BDNF. These growth factors aid in cell survival by activating several signaling pathways. One of the most crucial development factors is brain-derived neurotrophic factor (BDNF). It has been demonstrated to improve neuronal survival, development, and function maintenance. (19)

Increased Angiogenesis

Stem cells produce local signaling molecules like hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (FGF2) that enhance angiogenesis & may improve perfusion to chronically isch-

emic tissue. (20, 21)

Chen et al. have recently shown that treatment with bone marrow stromal cells enhances angiogenesis by increasing endogenous levels of VEGF and VEGFR2.(22) They previously showed that giving mice recombinant human VEGF₁₆₅ 48 hours after a stroke greatly enhanced penumbra angiogenesis and enhanced functional recovery. Hepatic Growth Factor (HGF) exhibits favourable effects on neovascularization and tissue remodeling, while FGF2 is connected closely with endothelial cell proliferation and may be a more potent angiogenic factor than VEGF. (23)

In cell culture and in vivo, mesenchymal stem cells (MSCs) dramatically increase the secretion of VEGF, HGF, and FGF2 in response to injury or stress. This may enhance regional blood flow and encourage autocrine self-survival. Improved end-organ function has also been linked to increased perfusion brought on by the synthesis of stem cell angiogenic growth factor. Therefore, it is possible that VEGF, HGF, and FGF2 are significant paracrine signaling molecules in stem cell-mediated angiogenesis, protection, and survival. (24)

Immunomodulation

Stem cells appear to attenuate infarct size and injury by modulating local inflammation. When transplanted into injured tissue, the stem cell faces a hostile, nutrient-deficient, inflammatory environment and may release substances which limit local inflammation in order to enhance its survival. Modulation of local tissue levels of pro-inflammatory cytokines by anti-inflammatory paracrine factors released by stem cells (such as IL-10 and TGF- β) is important in conferring improved outcomes after stem cell therapy. (25)

Anti-Apoptosis

At the infarct border zone, stem cells in a third pathway help salvage fragile or dysfunctional cell types. After 10 weeks, the cardiac scar breadth is decreased when MSC is injected into a cryo-induced infarct. The anti-apoptosis signaling mechanism that MSCs seem to activate successfully shields ischemia-threatened cell types from apoptosis. Additionally, genes linked to improved DNA repair, elevated antioxidant enzymes, and expanded detoxifier systems are expressed characteristically in adult progenitor cells, according to expression profiling. HGF has been observed to improve cell growth and to reduce cell apoptosis. (26)

Beneficial Remodeling of the Extracellular Matrix

Stem cell transplantation alters extracellular matrix, resulting in post-infarct remodeling, strengthening of the infarct scar, and prevention of deterioration in organ function. MSCs improved this function by increasing the cellularity and decreasing the production of extracellular matrix proteins such as collagen type I,

collagen type III, and TIMP-1, which results 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, livers, brains, and kidneys. It's possible that these local stem cells have growth factor receptors that can be triggered to cause them to migrate and proliferate, which will aid in the repair of injured tissue as well as the restoration of dead tissue. In reaction to damage, mesenchymal stem cells have also released HGF and IGF-1, which may stimulate the local cardiac stem cells when implanted into ischemic myocardial tissue. (27)

In conclusion, stem cells mediate enhanced angiogenesis, suppression of inflammation, and improved function via paracrine actions on injured cells, neighbouring resident stem cells, the extracellular matrix, and the infarct zone, even though the precise mechanisms for protection via stem cells are still unknown. A better comprehension of these paracrine processes could enable earlier and more successful clinical treatments.

Remyelination

Remyelination is the phenomenon by which new myelin sheaths are generated around axons in the adult central nervous system. Prior attempts to regenerate myelin-forming cells have been successful, but they have been constrained by the lesions' multifocal character and the incapacity to generate a significant number of myelin-producing cells in culture. These drawbacks can be somewhat addressed by stem cell-based therapy, which could be helpful in the future for the treatment of demyelinating disorders.

According to recent research, remyelination can be achieved by introducing cells such as Schwann cells, oligodendrocyte lineage cell lines, olfactory ensheathing cells (OECs), neural stem cells, embryonic stem cells, adult bone marrow-derived stem cells, and Schwann cells into demyelinated areas. These cells may have a remyelinating effect by one or more processes, such as the following: The stem cells differentiate to do direct cell replacement and function as an immunomodulator by generating soluble substances into neural and glial cells in the lesion, and they promote differentiation of endogenous cells. Interactions with viable axons and supportive astrocytic responses are required for endogenous immature cells to fulfil their potential remyelinating capacity. (28,29)

Contrary to the general expectations that stem cells would primarily contribute to the 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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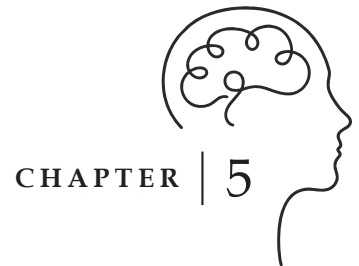
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"We are what we repeatedly do.

Excellence is therefore not an act but a habit"

-Aristotle



Laboratory Aspects of Cell / Stem Cell Therapy

“This field [stem cell research] isn’t growing; it’s EXPLODING.”

– Barth Green

A crucial and initial step in the entire stem cell therapy process is stem cell harvesting. Several techniques exist for obtaining, cultivating, distinguishing, and conserving. Each of these has unique heterogeneous protocols that various scientists adhere to. Since these cells are being injected into humans for clinical purposes, strict aseptic measures must be taken. Prior to transplantation, the safety of the cells must be guaranteed. It is also necessary to determine the viability of the cells in order to correlate them with efficacy. Cell markers must also be used to determine the type of stem cells. Good Clinical Laboratory Practices ought to be adhered to for each of these procedures.

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

Basic methodology

A diverse population of progenitor cells makes up the cells obtained from any source. This combination must be isolated from the cells of interest for clinical use. After that, they are either introduced without culturing or purified and cultivated before use.

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

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

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

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

Bone marrow-derived stem cell separation

Open Method

Patients were administered Granulocyte Colony Stimulating Factor (GCSF) subcutaneously, 48 hours and 24 hours prior to BMMNCs aspiration. Bone marrow was aspirated under general anesthesia with sedation in the operation theatre under aseptic conditions. 80-100 ml of bone marrow (depending on the age and body weight of the patient) was aspirated from the anterior superior iliac bone using a bone marrow aspiration needle and collected in heparinized tubes. Heparin (Gland Pharma) diluted in NS (250 IU/ml) is added to sterile 50 ml BD Falcon tubes in which bone marrow is collected. The bone marrow was diluted in the ratio of 1:1 with normal saline and subjected to centrifugation at $440\times g$ rpm for 35 minutes in a swinging bucket rotor without brake at 20°C through a density gradient method. MNCs were then obtained as a buffy coat, which was collected using a 3 ml BD pipette. The MNCs were washed thrice with normal saline by centrifuging at $300\times g$ for 15 minutes in a swinging bucket rotor without brake at 20°C and finally resuspended in 1 ml of normal saline. Viability of the isolated MNCs was checked using trypan blue vital dye which was mixed in 1:1 proportion and

loaded onto the haemocytometer for the total cell count and viability. More than 1×10^8 MNCs are injected with viability of more than 98%. CD34+ analysis of the samples was also done using the Invitrogen Attune NXT flow cytometer.

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 Smart-PreP System (Harvest Technologies, Plymouth, MA) to yield 40 mL of treating volume.

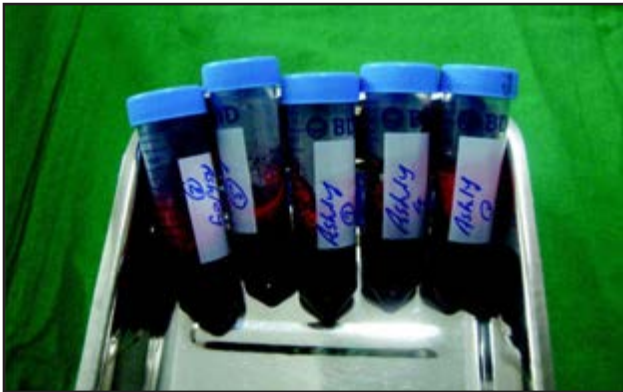


Figure 1: Aspirated bone marrow in tubes. Each tube contains about 20 ml of bone marrow mixed with heparin.

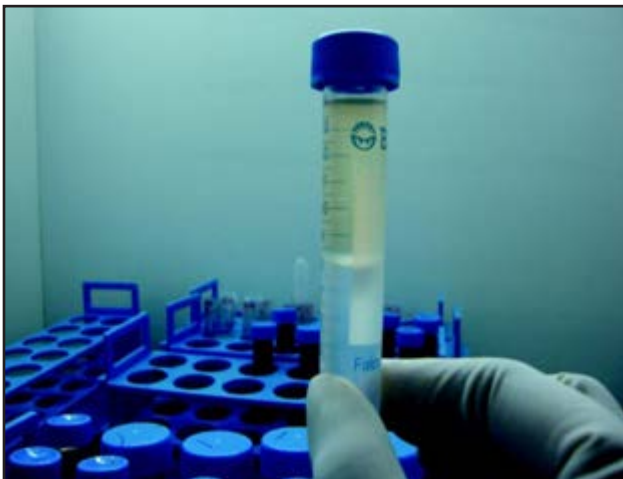


Figure 2: Buffy coat containing separated fraction of mononuclear concentrate

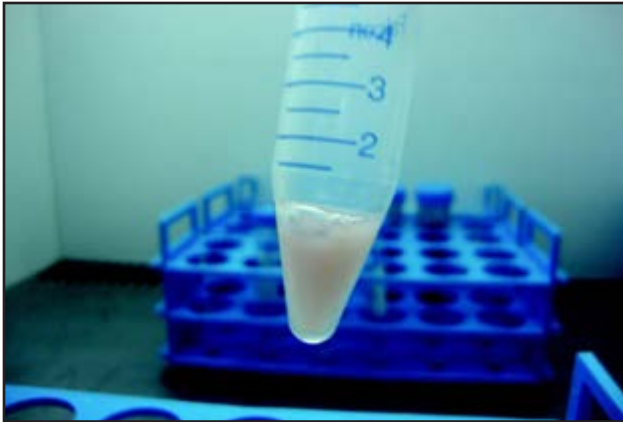


Figure 3: Purified concentrate of mononuclear cells in solution (heterogenous mixture of stem cells – mainly hematopoietic)

Stem cell isolation from Peripheral blood

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

The process of mobilizing hematopoietic stem cells (HSCs) from peripheral blood is one way of obtaining them. The administration of cytokines, such as granulocyte colony-stimulating factor (G-CSF), with or without chemotherapy, is required to mobilize HSCs and then harvest HSCs from the blood using apheresis because there are very few HSCs visible in the peripheral blood at the steady state.

Hematopoietic Stem cell selection

Immune cells like lymphocytes and monocytes, rather than HSCs, make up the majority of mononuclear cells obtained by peripheral blood apheresis/leukaphereses. Though CD34 or CD133 are common cell markers of hematopoietic stem cells (HSC) and hematopoietic progenitor cells, little is known about the biology or phenotype of peripheral blood stem cells. The isolation of these cells is carried out either via positive selection, i.e., isolation according to the marker molecules they express or negative selection, which is depletion of all other cells according to the markers they express. The negative selection process is seen to be inferior to positive selection.

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

Isolation of Adipocyte cells

Adipose-derived stem cells are usually isolated from lipoaspirate waste from lipo-

suction. They are comprised of three distinct layers: 1) an upper layer of oil due to the lysis of mature adipocytes, 2) a middle layer of adipose tissue, and 3) a bottom, liquid infranatant containing saline and blood cells.

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

Standard Protocol for ASC Isolation:

Differential centrifugation and enzymatic digestion are used in the conventional procedure. (2) PBS is used to wash the lipoaspirate until the adipose layer turns yellow or gold. Collagenase is used to enzymatically break down the sample in the following step. Isolation was then carried out using control medium (CM), which contained antibiotics, fetal bovine serum, and DMEM. Collagenase inactivation is accomplished using CM. Additionally, CM is used to further culture the isolated ASCs.

A rapid procedure was created in order to quickly acquire a viable population of ASCs because the regular protocol takes a long time.

Rapid protocol for ASC isolation

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

Umbilical Cord blood processing

There are currently two methods used to extract cord blood cells: automated and manual.

Cells from the center of what has “settled” out from the cord blood are manually extracted after the blood has been left to sit for a while. For a long time, this was the only approach that was available. However, manual processing has two possible drawbacks. First, mononuclear cells were lost using manual procedures, which could only recover 40–80% of cells. Secondly, there is a higher chance of bacterial contamination. On the other hand, automated processing uses a fully closed system to prevent bacterial contamination and enables up to 99% recovery of the

cells needed for transplantation. Additionally, the possibility of human error is reduced. Unfortunately, the cost of using automated processing has increased due to these developments (4).

Endometrial cell processing and expansion

Harvesting

A class 100 biological safety cabinet in a class 10,000 clean room prepares a “collection tube” prior to the collection process. A 50 ml conical tube filled with 30 ml of GMP-grade phosphate buffered saline (PBS) is prepared by adding 0.2 ml amphotericin B (Sigma-Aldrich, St. Louis, MO), 0.2 ml penicillin/streptomycin (Sigma), and 0.1 ml EDTA- Na_2 (Sigma). Five millilitres of menstrual blood are collected using a modified version of the published protocol. The donor is in charge of collection. For 30 to 60 minutes, a sterilized Diva cup is placed inside the vagina. The Diva cup’s contents must be decanted into the collecting tube after removal. After being transported to the clean room, the collection tube is centrifuged for ten minutes at 600 g. The cells are centrifuged at 600 g for 10 minutes at room temperature after the collection tube has been taken to the Biological Safety Cabinet, where the supernatant is extracted and the tube is filled to 50 ml with PBS. Mononuclear cells are collected using a Ficoll-Paque density gradient (Fisher Scientific, Portsmouth, NH) after the cell pellet has been washed three times with fifty millilitres of PBS. 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 an endotoxin level of ≤ 10 EU/ml and a haemoglobin level of ≤ 25 clinical grade ciprofloxacin (5 mg/mL, Bayer A.G., Germany) and 4 mM L-glutamine (cDMEM).

The resultant cells are visually, morphologically, microscopically, mononuclear cells that are significantly devoid of erythrocytes and polymorphonuclear leukocytes. 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

After being plated in a T-75 flask with 15 ml of cDMEM, the cells are grown for 24 hours at 37°C with 5% CO_2 in a completely humidified environment. This facilitates the adhesion of the ERC precursors. The flask is gently rinsed with cDMEM to remove non-adherent cells. The next step is to separate adherent cells by rinsing them with PBS and adding 0.05% trypsin with EDTA (Gibco, Grand Island, NY, USA) for two minutes at 37°C with 5% CO_2 in a completely humidified environment. 30 millilitres of cDMEM are used to centrifuge, wash, and plate the cells in a T-175 flask. As a result, each starting T-175 flask produces about 10,000 ERC. The

flask is then cultured for 5 days, which yields approximately 1 million cells in the T-175 flask (Passage 1). The cells are then passaged in a T-175 flask at a density of about 200,000 cells. Between 100 and 200 million cells are collected at passages three and four.

Induced pluripotent cell processing

Reprogramming somatic cells to resemble embryonic stage cells produces induced pluripotent cells, or iPSCs. The somatic cells are cultivated in settings similar to those of embryonic stem cells after being introduced with a specific and constrained set of stimuli. (6) Yamanaka et al. introduced 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)—into mouse fibroblasts via retroviral means for the first time in order to generate iPSCs. (6,7) These iPSC cell lines express genes unique to ES cells and have morphological and growth characteristics comparable to those of ES cells. When iPSC cells were transplanted into immunocompromised mice, tissues from all three germ layers harbouring germ-cell tumors (teratomas) formed, demonstrating the pluripotent potential of iPSC cells. Since then, human somatic cells and many mice cell types have been treated using the same procedure. Following the introduction of factors, cells are cultivated to produce colonies that resemble pluripotent cells. Following that, these cells are separated according to their shape, surface markers, etc. It takes about one to two weeks for mouse cells to generate iPSCs and three to four weeks for human cells. In an effort to prevent the development of tumors caused by viruses, iPSCs are now produced free of viruses and vectors. For iPSC differentiation, growth factors and cytokines should undergo rigorous testing to guarantee high biological activity, high purity, freeze-thaw stability, and structural uniformity. Additionally, they must permit the best possible development, proliferation, and storage of differentiated cells. Reprogramming, culturing, engineering, differentiation, and cell analysis are the main processes involved in producing iPSCs. It is essential to validate their pluripotency and differentiation capacity into the desired cell lineage. (9)

Isolation of Neural Stem Cells

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

Their isolation procedures follow common steps, including tissue dissection, digestion, and cell enrichment. The origin of the tissue influences the type of isolated cells as well as their differentiation capacity & proliferation. (11) Brain tissue is dissected first. Enzymatic digestion separates NSCs from the rest of the tissue, which is an important step since it influences the survival rate of the cells. Some-

times enzymatic digestion is insufficient, so a mechanical technique, like trituration, is employed to separate the digested materials into a single-cell suspension following enzymatic digestion. Last but not least, the NSCs are enriched by a variety of techniques, including immunopanning, FACS, differential gradient centrifugation, and approaches based on adherent cell characteristics. These cells are kept and multiplied either as adherent cultures that form a monolayer on the plate surface or as free-floating cell clusters, or neurospheres. The neurosphere test, which is still in use with several changes, has been the most widely used technique to show that NSCs are present in culture. However, these cells are yet to be translated for therapeutic use.

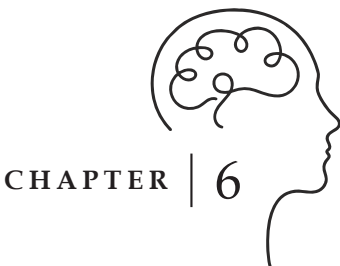
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"The best research questions come from the patient's bedside"



Prof. Harvey Cushing
Neurosurgeon of the Millenium



Surgical Aspects of Cell / Stem Cell Therapy: Routes of Administration

“This field [stem cell research] isn’t growing; it’s EXPLODING.”
– Barth Green

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, and/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

A number of variables, including the patient’s age, weight, marrow distribution, physical condition, and doctor’s experience, may influence the site selection. The pelvis is the most often used site, though. It is simple to aspirate from either the anterior or posterior iliac crests. Although the posterior superior iliac spine is easily visible and accessible, doing so requires turning the patient into a lateral or prone posture, which can be uncomfortable and difficult. The patient should lie comfortably in the supine posture in order to access the anterior superior iliac spine. The distribution of fat in obese persons may cause the landmarks to be completely ob-

sured. Normally, the anterior and posterior iliac spines do not sample differently.

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

A standard bone marrow aspiration needle is inserted through the skin till the bone is felt. Heparin is used to cleanse the needle before use. Before inserting the needle, some surgeons use a surgical blade to make a tiny incision that exposes bone, although in our experience, this is rarely necessary. With downward pressure, the needle that is securely fastened to the obturator is screwed within a clockwise and anticlockwise motion until it reaches the periosteum. The needle is inserted until it enters the cortex using a similar motion. At this stage, the needle feels firmly lodged in the bone after first experiencing an abrupt giving way of resistance as it enters the soft trabecular bone. The needle's entry angle is crucial because it must match the bone's curvature. 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 labelled. Under sterile conditions, collected bone marrow is transported to the laboratory in a special transporter. (1)

Transplantation of Stem Cells in neurological disorders

The delivery of the cells, which can be accomplished locally (via intrathecal or direct implantation into the brain or spinal cord) or systemically (by intravenous or intraarterial methods), is the other surgical component of stem cell therapy. There are currently no comparative studies that could tell us which is the preferable way, and different institutes are using different approaches to transplant the cells. Nonetheless, local distribution would seem to be a more sensible choice given the blood-brain barrier.

Intrathecal delivery

The patient is positioned in the "foetal ball" position, which is lateral decubitus. These two techniques aid in the spinous processes' opening. Occasionally, the patient is made to sit, leaning over a tabletop. The isolated BMMNCs were injected

immediately using a 25G spinal needle between the fourth and fifth lumbar vertebrae under general anaesthesia with sedation. Simultaneously, 20 mg/kg body weight of methylprednisolone in 100 ml Isolyte P or normal saline was given intravenously to reduce local inflammation and enhance the survival of transplanted BMMNCs. Local anaesthetic is typically used for this surgery. Children are put under general anaesthesia.



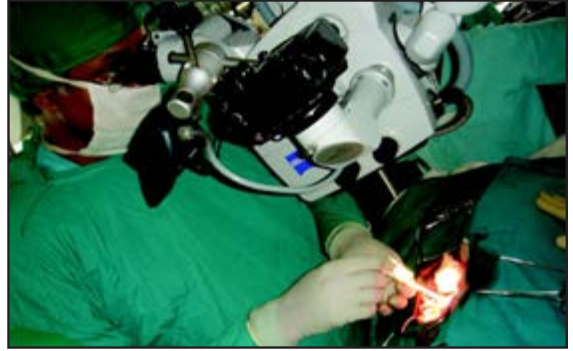
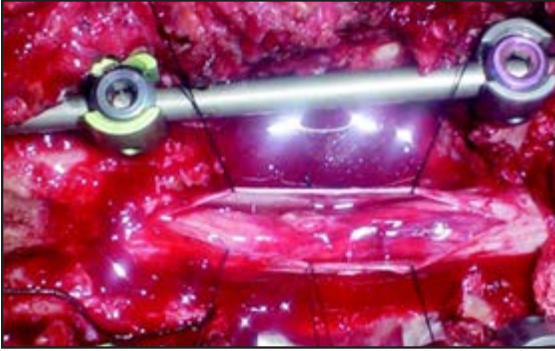
Figure 1: Spinal Needle for Intrathecal Injection



Figure 2: Bone marrow aspiration



Figure 3: Delivery of Stem cells



Figures 4 & 5: Intraspinal transplantation of stem cells in a case of thoracic spinal cord injury.



Figure 6: Intra-arterial direct injection of stem cells into the carotid artery following carotid endarterectomy



Figure 7: STA-MCA bypass



Figure 8: Leksell Stereotactic Frame for direct stem cell implantation into the brain.

In patients with cardiac issues, where excessive intravenous infusion is to be avoided; in patients taking anticoagulant or antiplatelet medications to prevent bleeding into the subarachnoid space; in cases where the spine is scoliotic, which frequently occurs in patients with muscular dystrophy; and in certain prior lumbar spine surgery cases, a spinal needle is preferred over a catheter.

It can be extremely challenging to insert a needle intrathecally in individuals with significant spinal abnormalities, such as scoliosis, and help may be required to precisely pinpoint the spot and direction of needle placement on the C-arm.

Callera et al. (2007) demonstrated for the first time that autologous bone marrow CD34+ cells labelled with magnetic nanoparticles delivered into the spinal cord via lumbar puncture (LP) technique migrate 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 arachnoid, which is then opened independently with microscissors, is spared when the dura is cut. High-power microscopic magnification is used to locate the dorsal surface of the contusion site. Following adequate surface exposure in the contusion site, six distinct locations around the contusion site's edge are injected with 300 μL aliquots of cell paste (a total volume of 1.8 mL). A 27-gauge needle linked to a 1-mL syringe is used to inject 2×10^8 cells at a rate of 30 $\mu\text{L}/\text{min}$ in order to prevent direct cord injury. The injection location is about 5 mm below the dorsal surface. After the injection is finished, the dura and arachnoid are closed, and the injection needle is left in place for five minutes to stop cell leakage through the injection track. The muscle and skin are closed in layers. (3)
- b. Following a minilaminectomy and spinal cord exposure. One of the wings is sliced to expose the dura and use a 27-gauge scalp vein. The opposite wing is placed into the dorsal root entry zone at a 45-degree angle while being supported by a hemostat. Its depth in the spinal cord is 3 mm. There are two injections administered above the damage site on each side, and two injections administered below the injury site. Surgeons in China are infusing 35 micro-

litres of stem cells. In the course of his planned studies, Wise Young plans to inject increasing doses of 4 μL , 8 μL , and 16 μL . 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 a superficial temporal artery to middle cerebral artery bypass or carotid endarterectomy, stem cells might be administered intraarterially as soon as the treatment was over. The benefit of this strategy is that the stem cells would reach the ischemic brain immediately, and since the artery is already open, the stem cell injection doesn't require a separate surgery. The endovascular interventional pathway is an alternative approach of direct intra-arterial injection. This is accomplished by puncturing the femoral artery and guiding a catheter to the brain's arteries. This has the advantage of being a comparatively non-invasive method and avoiding the drawbacks of intravenous injection.

Stereotactic implantation into the brain

Cell transplantation for neurological conditions started with Stereotactic implantation of fetal cells for Parkinson's disease. (4) However, this has since been abandoned when a randomized experiment by Freed et al. revealed that the clinical outcomes were not appreciably different from those of non-transplanted patients. (5) While there are other stereotactic systems available worldwide, the Leksell and CRW systems are the two most widely used among them. The Leksell system entails placing the frame on the patient's head and having the frame in place during an MRI. After identifying the region on the MRI scan where the tissue is to be transplanted, the X, Y, and Z coordinates are retrieved using the MRI software. 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 are inserted at the desired location using the X,Y and Z coordinates. Under local anesthetic, the entire process is carried out.

Cells are also transplanted into the muscle in cases of muscular dystrophy. The "motor points" are the locations where these must be injected; they are covered in full in chapter 7. Povidone iodine is used to clean the region at these motor locations. The 26G needle is inserted into the muscle at an angle (around 45 degrees) to inject the cells diluted in CSF. To make sure the needle is not inside a blood vessel, the syringe's piston or plunger is slightly removed. After injecting the cells, the needle is taken out, and the wound is promptly covered with a benzoin seal.

Intravenous injection

Intravenous injection (IV) is the most widely used route of administration for stem cells. It is safe and minimally invasive and has no ethical issues involved. In spite of these advantages, it is not the most efficient mode of transplantation. Studies have shown that on IV administration, the 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

Preoperative assessment: Depending on the type of mutation and the disease's stage, the heart is impacted to differing degrees. The myocardium is replaced by connective tissue or fat, which leads to dilated cardiomyopathy. Additionally, ventricular arrhythmias, T-wave abnormalities, and tachycardia could be present. This calls for a thorough pre-operative cardiac evaluation that includes an echocardiography and an ECG, as well as 24-hour Holter monitoring for arrhythmias. Another reason for concern is pulmonary insufficiency, which can be brought on by scoliosis, weak abdominal muscles, and other conditions, including changed lung and chest wall mechanics. Tests for pulmonary function are advised but are never practical. A reasonable estimate of respiratory reserve can be obtained from an arterial blood gas analysis.

Intraoperative and anaesthetic considerations: Anaesthetic and intraoperative issues include hypersomnolence, heightened sensitivity to anaesthetic drugs, and heightened risk of respiratory complications from hypotonia, persistent aspiration, and central and peripheral hypoventilation. hypotension brought on by a reduced cardiac reserve, lumbar puncture difficulties brought on by scoliosis, delayed stomach emptying because of GI tract hypomotility, and a higher risk of regurgitation and aspiration.

Multiple Sclerosis

Since this medical condition mainly affects the neurological system, the heart and lungs are usually spared any damage.

Anaesthesia considerations: it is recommended to take corticosteroid supplements throughout the perioperative phase. MS symptoms are known to worsen after surgery, particularly when fever and infection are present. However, general anaesthesia is often safe.

Cerebral Palsy

Intraoperative and anaesthesia considerations: Pre-operative Evaluation: In order to lessen spasticity, these kids are typically taking anticonvulsants and other med-

ications. They have higher salivation and are more likely to get respiratory tract infections.

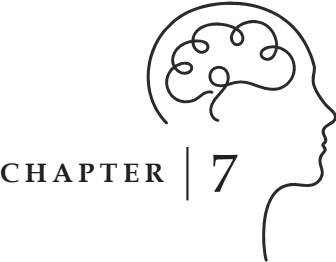
Considerations for Anaesthesia: higher risk of GER reflux. increased risk of aspiration from combined salivary secretions and regurgitant contents. Positioning may be hampered by skeletal and muscular spasticity that causes contractures and joint abnormalities. increased sensitivity to anaesthetic drugs, resulting in slow emergence.

Spinal Cord Injury

Alveolar ventilation impairment, particularly in cases of cervical cord injury, with impaired coughing and secretion clearance; cardiovascular instability characterized by autonomic hyperreflexia; chronic pulmonary and genitourinary infections; altered thermoregulation; decubitus ulcers; osteoporosis and skeletal muscle atrophy as a result of prolonged immobilization; increased susceptibility to deep vein thrombosis; positioning difficulties; and difficulty performing a lumbar puncture if the lumbar spine has undergone surgery or instrumentation.

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

- Goethe



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

There's always something new to learn.

Point is the point at which the innervating nerve enters the muscle or, in the case of deeply placed muscle, the point where the muscle emerges from the undercovers 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.

Although there are some exceptions, motor points are often located where the middle and upper thirds of the muscles' fleshy bellies meet, e.g., the motor point of the vastus medialis, whose nerve enters the lower part of the muscle, is situated a short distance above the knee joint. Deeply positioned muscles, such as the extensor hallucis longus in the lower leg, may be most effectively stimulated where they emerge from underneath the more superficial ones. A motor point is the location on the skin where an innervated muscle can be most easily stimulated with low-intensity percutaneous electrical stimulation. This location on the skin typically covers the muscle's band or zone of innervations as well as its neurovascular hilus. 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 localized in a narrow band of innervations where the other large-diameter nerves

and motor endplates are most concentrated. The exact location of the 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

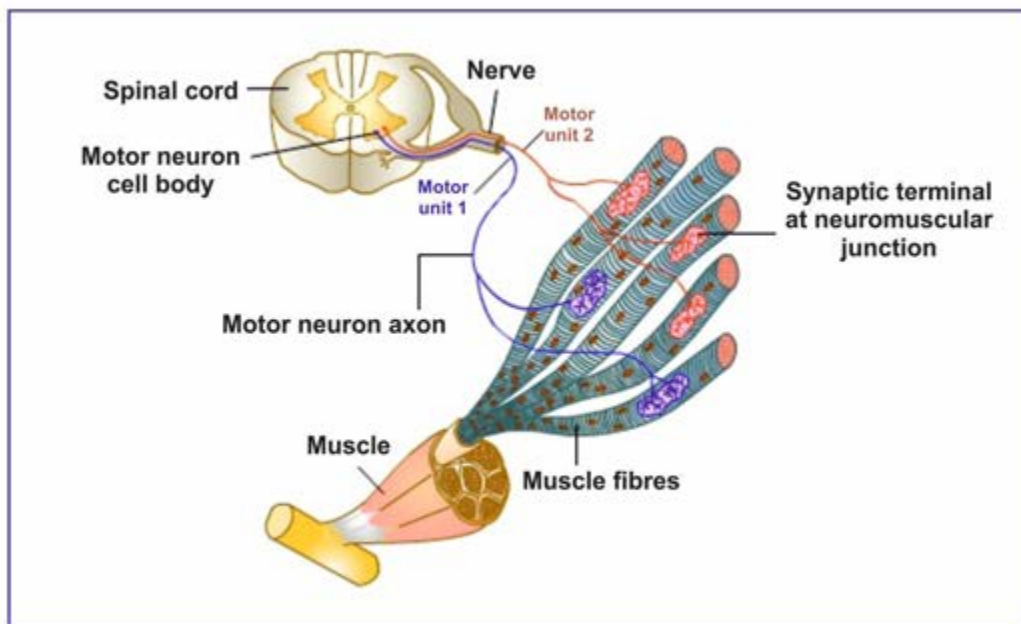


Figure 1: A Neuromuscular Junction

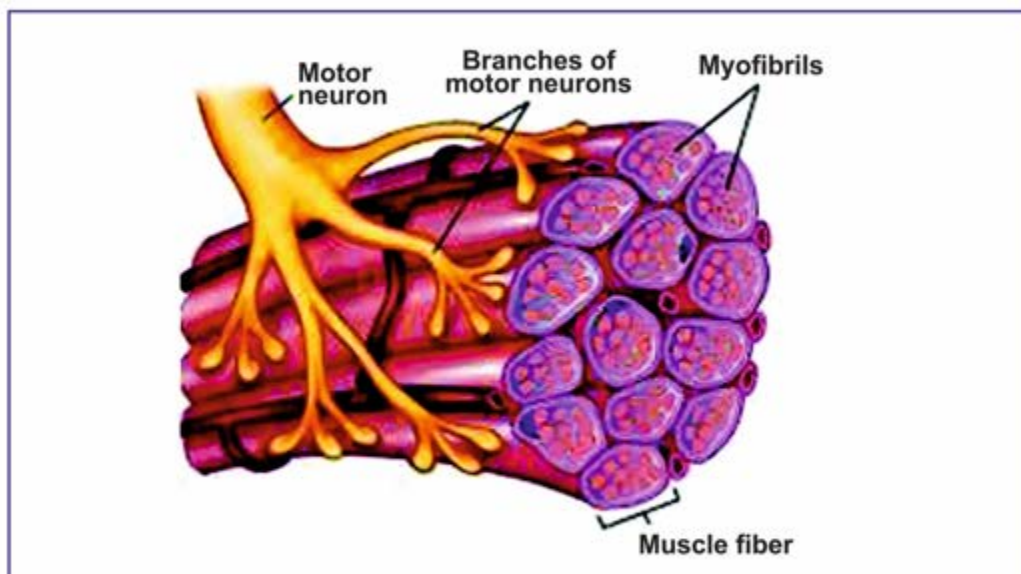


Figure 2 : The Motor Unit

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 a 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 an automatic surge is commonly used. A faradic current is a short-duration, interrupted direct current with a pulse dura-

tion of 0.1 - 1 ms & a frequency of 50 - 100 Hz. The 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: Plotted motor points



Figure 5: Injection of stem cells in tibialis anterior muscle motor point.



Figure 6: Injection of stem cells in the motor point.



Figure 7: Intramuscular injection.



Figure 8: 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 & the patient's complaint of weakness & difficulty in ADL. Post stem cell injection these muscles need specific training & individual muscle strengthening programmes so that the patient can gain efficiency & independence 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 the 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.

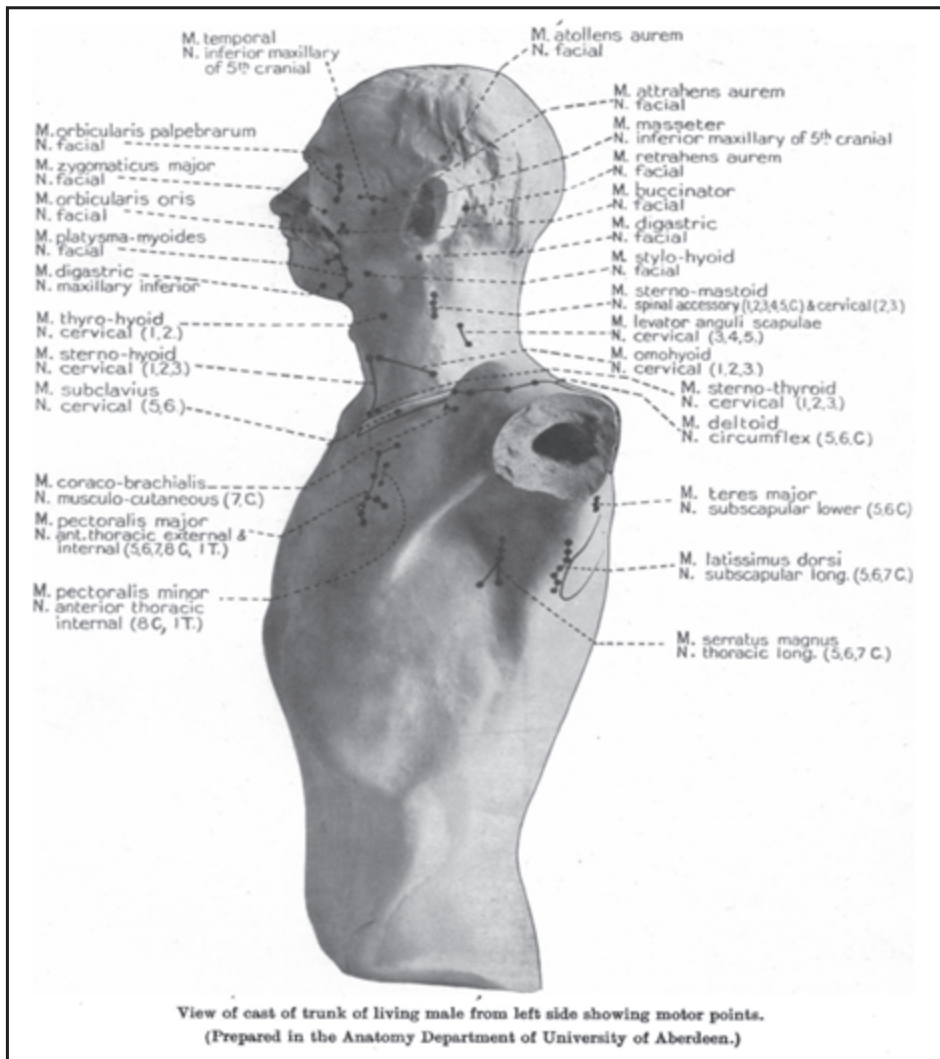
C) In trunk:

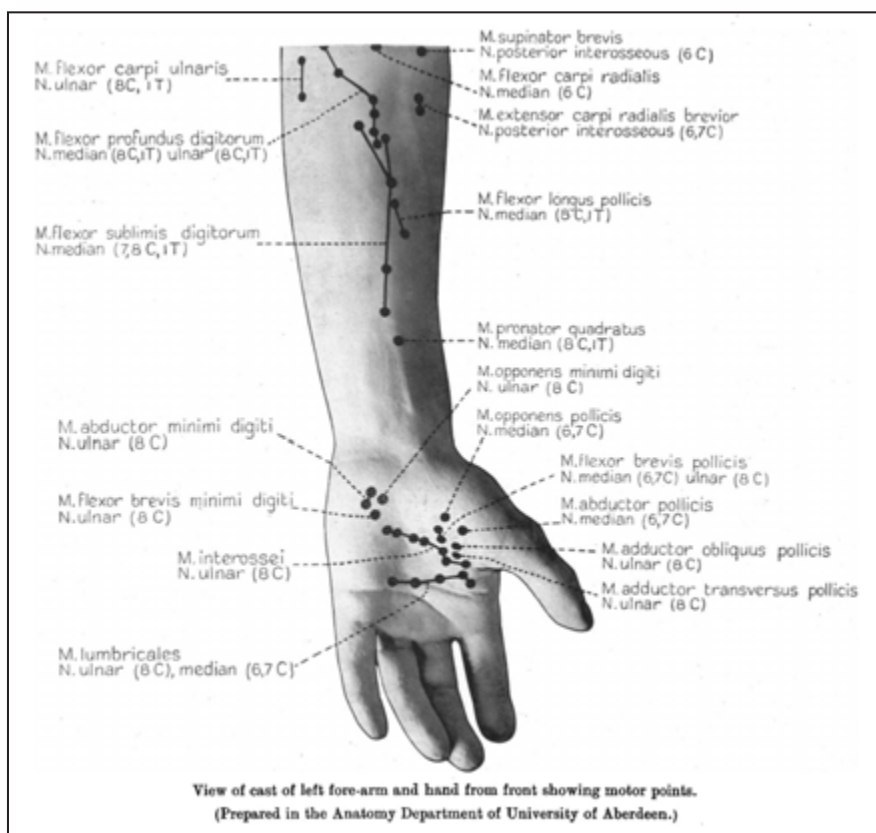
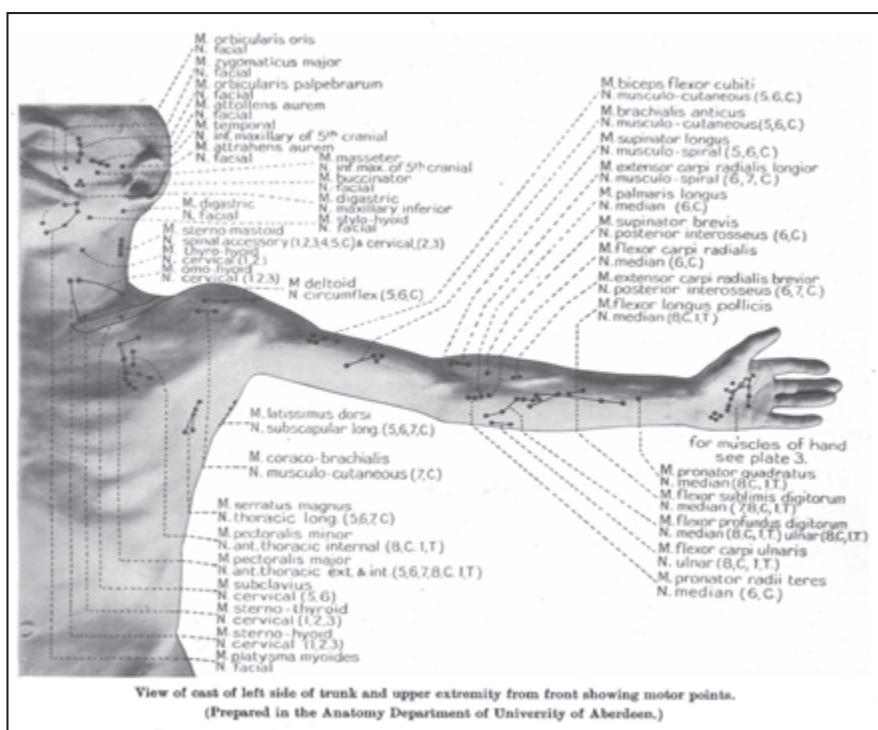
Abdomen & back extensors are considered, & in neck muscles, sternocleidomastoid.

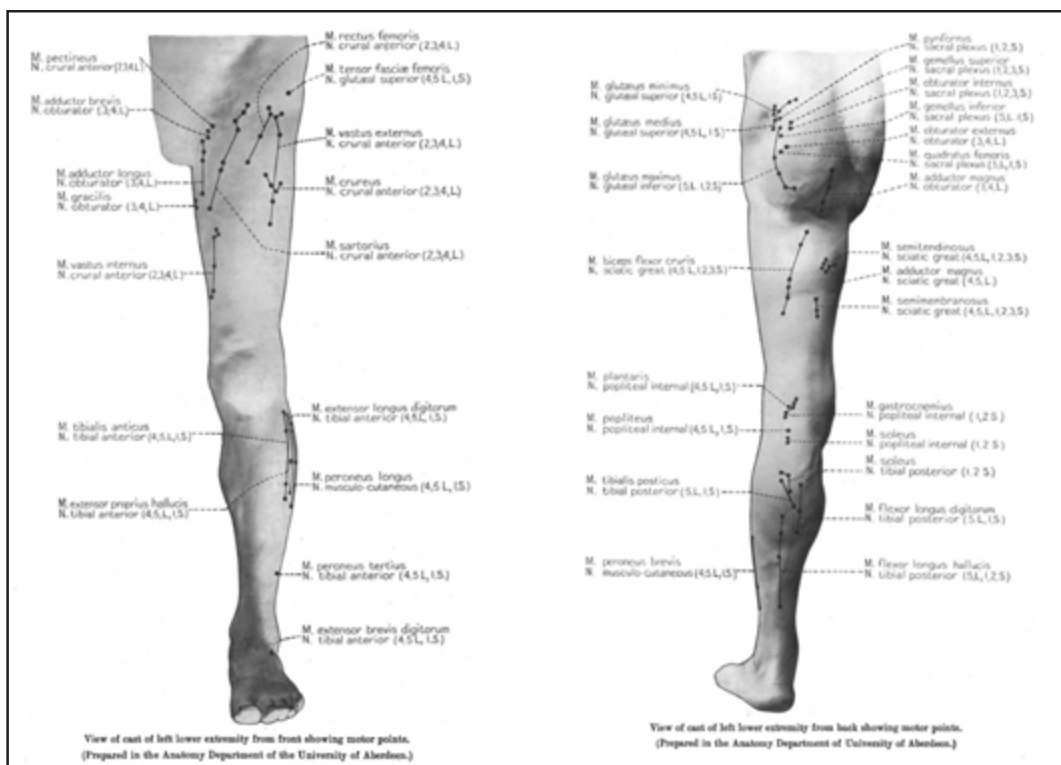
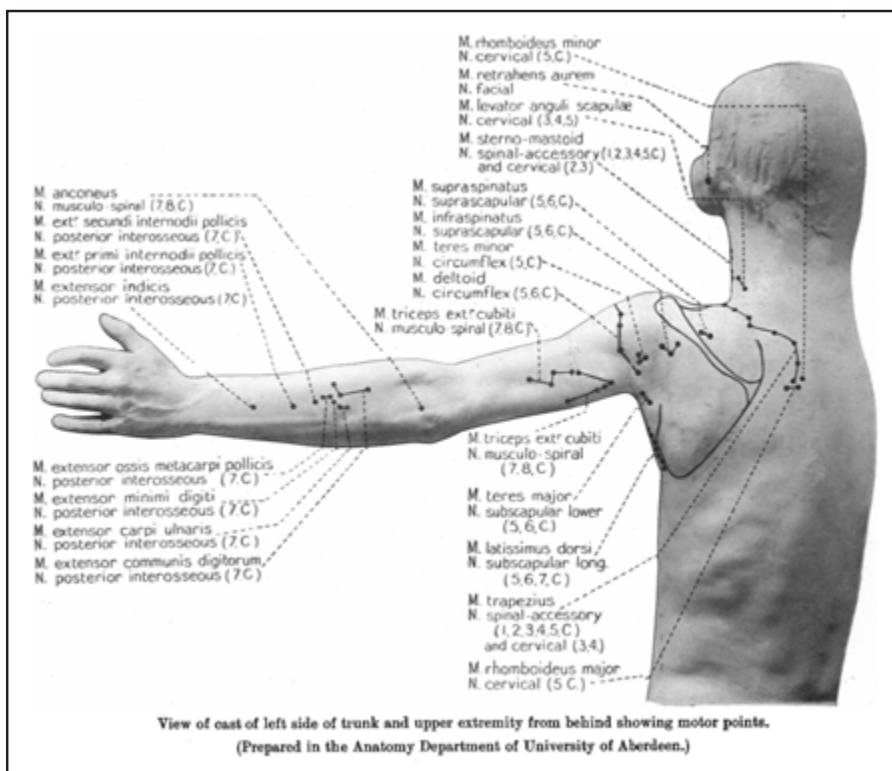
D) Facial Muscles:

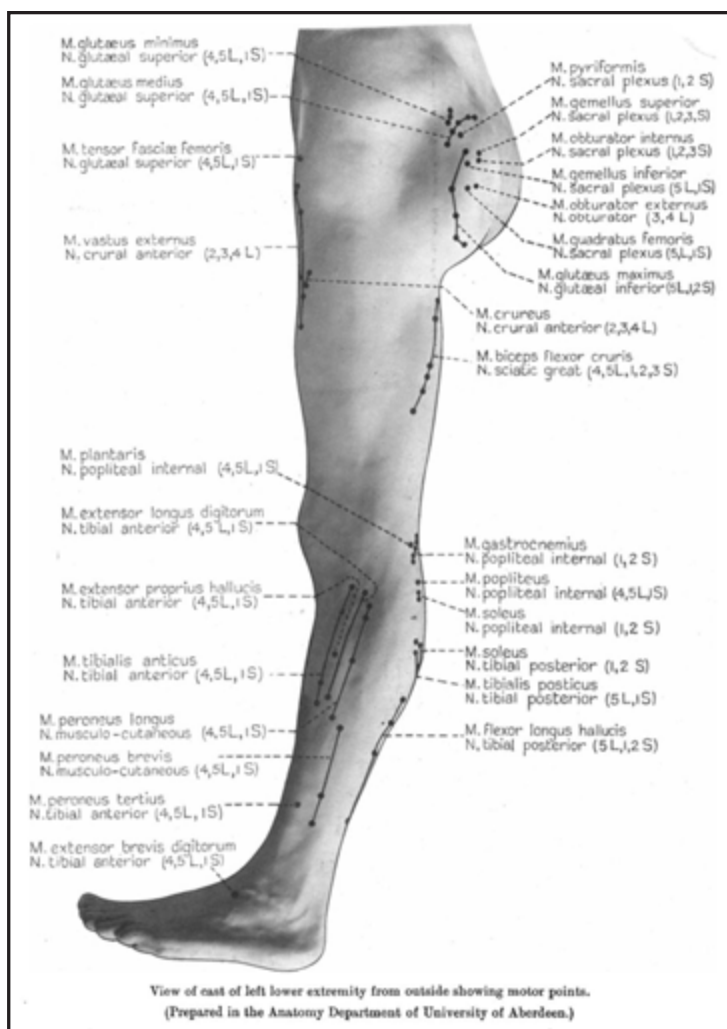
In case of facial muscle weakness in conditions like Motor Neuron Disease & a few muscular dystrophies, facial muscles motor points are also selected for intramuscular injections, e.g., orbicularis oris, orbicularis oculi, Buccinator, risorius, frontalis, mentalis, etc.

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



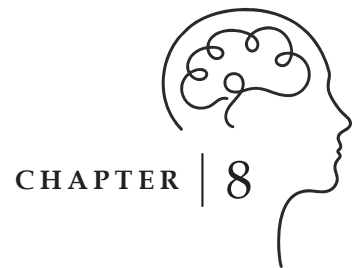






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Special Diagnostics

BIOCHEMICAL TESTS

Introduction:

This chapter describes special tests that help to improve the outcome of stem cell therapy. In many neurological conditions, including autism, ADHD, and Alzheimer's, abnormal DNA methylation patterns are regularly found. These illnesses cause dysregulation of gene expression, which interferes with regular cellular functions and contributes to the pathophysiology of the disease. In our experience, methylation pathways help in neurotransmitter production and DNA repair, & previous studies have also shown disruption in methylation pathways is related to neurological disorders. In addition to being vital for DNA repair, which preserves genomic stability and brain health, methylation processes are necessary for the conversion of tryptophan into neurotransmitters like serotonin. Biochemical tests that support stem cell therapy outcomes can be done using different body fluids, each giving unique information on muscle damage, renal function, immune status, and regenerative processes. Neurotransmitter (NT) levels may be assessed in blood, urine, or cerebrospinal fluid (CSF); the MTHFR gene test and Folate Re-

ceptor Antibody Test (FRAT) are performed on blood samples; S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) are measured in CSF and urine; gut microbiome analysis is conducted on stool samples; and CSF methylfolate testing is performed on cerebrospinal fluid.

Gut Microbiome Test (GMT):

The human gut is made up of millions of microorganisms, and it has been proposed that microbial homeostasis is maintained by a balanced gut microbial makeup [1]. Dysbiosis has been defined as an imbalance in bacterial composition, bacterial metabolic activity, or bacterial distribution in the gut [2]. Therefore, any imbalance in the amount and community of gut microbiota at a critical developmental stage of a child may affect the enteric nervous system (ENS) and central nervous system (CNS), which are components of the microbial gut-brain axis [3]. A bidirectional homeostatic pathway of communication between the gut microbiota, which is comprised of trillions of microorganisms inhabiting the gastrointestinal tract lumen, which houses the enteric nervous system (ENS) and the central nervous system (CNS) via immunological, metabolic, and neuronal signalling pathways, is known as the microbiota-gut-brain axis [4]. Gut microbiota influence the central nervous system, the immune system, and the nervous system through various mechanisms, including the secretion of metabolites. This axis shows how the gut flora communicates with the brain and how they interact [5]. Attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), cerebral palsy and epilepsy, schizophrenia, neuropsychiatric disorders, emotional dysregulation, anxiety disorders, aggression, and depressive states are among the mental and behavioural outcomes that children may experience as a result of perinatal GM dysbiosis, according to an abundance of clinical and preclinical studies. This process may be mediated through the GM-brain axis [6].

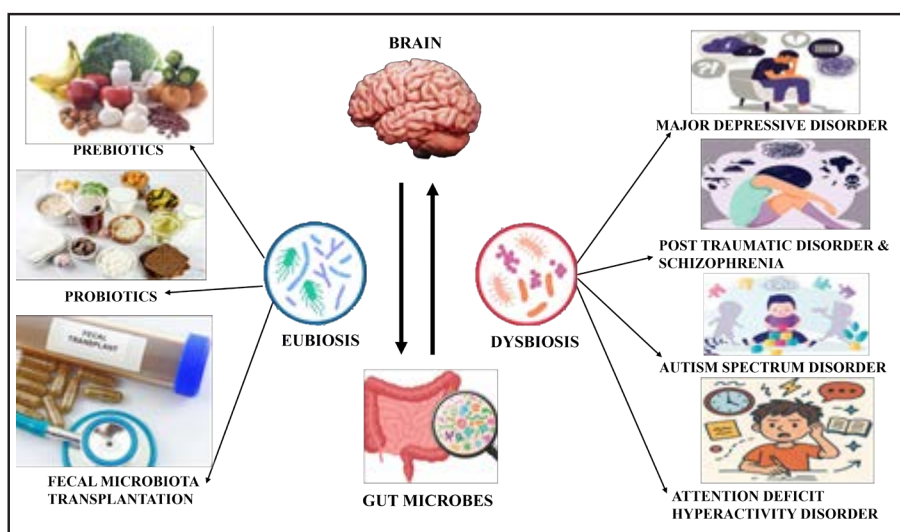


Figure 1: Influence of Gut Microbiota Homeostasis on Neurological Function

The common samples for gut testing widely used are stool, saliva, blood, and biopsies from intestinal lining. Each sample provides unique insights into gut health. But stool samples reflect a more accurate picture of the gut profile, as they contain a wide range of microorganisms from the intestine. Collecting stool samples is an easier and non-invasive method, and it allows the assessment of taxonomic diversity [7, 8]. Novau-Ferré et al. suggested that responses in ASD and ADHD may be influenced by differences in gut microbiome. The composition of the gut microbiota was positively changed by probiotic administration, providing information for potential future treatment approaches that target the microbiome in neurodevelopmental diseases [9]. Research by Fattorusso et al. indicates that children with Autism Spectrum Disorder (ASD) are commonly found to have gastrointestinal disorders compared to healthy kids. Many children with ASD have gastrointestinal (GI) symptoms such as diarrhoea and constipation, suggesting a link between gut health and neurobehavioural symptoms [10]. It seems that there could potentially be a connection between ADHD and decreased microbial variety, as studies have revealed that patients with ADHD frequently have lower gut microbiota diversity than healthy controls. People with ADHD have been found to have a unique metabolic profile, which includes noticeably reduced amounts of important Short-Chain Fatty Acids (SCFAs), such as propionic and acetic acid [11]. Peng Y et al. articulated that when compared to healthy individuals, children with cerebral palsy showed a markedly greater microbial diversity and distinct bacterial profiles in their gut microbiota [12].

Wu, H. et al. proposed that, in addition to gastrointestinal issues including constipation, recurrent gastrointestinal bleeding, and gastroesophageal reflux symptoms like bloating and vomiting, changes in the gut microbiota in children with cerebral palsy (CP) may also have an impact on brain issues like epilepsy and cognitive decline. Chronic inflammation may be exacerbated by dysbiosis, which can involve changes in the abundance of some opportunistic and helpful bacteria [13]. Research by Ansari F et al. has demonstrated that the use of synbiotic products, which are a combination of probiotics like *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* and prebiotics like resistant starch and inulin, results in the production of high levels of neurotransmitters and neuropeptides, like GABA and brain-derived neurotrophic factor, improving central nervous system function and reducing the symptoms of mental illnesses like anxiety, depression, stress, and memory loss [14]. Barandouz et al. put forward that serotonin may be secreted by *Candida*, *Escherichia coli*, *Streptococcus*, and *Enterococcus*, while norepinephrine can be produced by *Bacillus*, *Saccharomyces* and *Escherichia coli*. The development of pathogenic bacteria and an imbalance in neurotransmitter levels might result from the generation of neurotransmitters by different microbiota. For instance, *Escherichia coli* may grow more rapidly when norepinephrine is present. Furthermore, research indicates that norepinephrine exposure may promote the growth of *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Shigella son-*

nei, and *Staphylococcus aureus* in vitro [15]. Studies by Ullah H et al. & Ashique S et al. recommended that by altering the gut microbiota, or dysbiosis, through the production of neuroactive compounds, immune system modulation through cytokines, and interference with nervous system pathways, gut pathogens may contribute to neurological disorders.

These modifications alter the microbiota-gut-brain axis [16, 17]. He Y et al. suggested that altered gut microbiota composition, including an overabundance of gut pathogens like *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* or *Clostridium bolteae*, is linked to various neurological disorders through the gut-brain axis [18]. Dinan K et al. urged that the gut-brain axis and neurological disorders may be affected by changes in the gut microbiota, such as those brought on by antibiotics, which can also change immunological responses in the gut [19]. Having a detailed individual gut profile of a patient is more beneficial to treat and work on it. As it can guide us in the selection of particular probiotics required for an individual and enables a more comprehensive personalized treatment approach to address both gut and behavioural issues [10]. Probiotic supplementation may aid in the restoration of dysbiosis in GM, which would lower inflammation, control immunological balance, and alleviate patients' clinical symptoms [13].

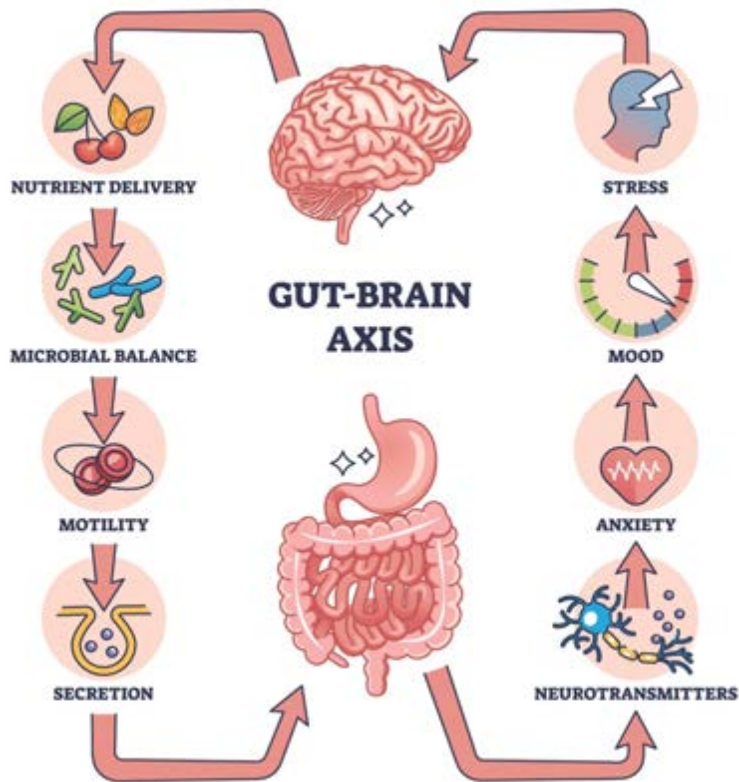


Figure 2: Bidirectional Communication of the Gut-Brain Axis

2. MTHFR Gene Test:

In humans, the MTHFR (methylenetetrahydrofolate reductase) is located on chromosome 1 at position p36.3. [20] The MTHFR test determines if a mutation is heterozygous or homozygous. A crucial enzyme in the folate metabolism pathway, MTHFR is in charge of converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the active form of folate needed for homocysteine methylation to methionine. The latter, as a cofactor, is essential for controlling the blood's folic acid and homocysteine levels. [20,21] There are 14 common or uncommon single nucleotide polymorphisms in the MTHFR gene that have been linked to enzyme deficiencies; the most prevalent ones are C677T and A1298C, which may lower MTHFR activity to varying degrees [20]. The MTHFR C677T polymorphism tends to decrease the efficiency of the one-carbon (C1) metabolic pathway and methyl group synthesis, which may have negative downstream consequences on gene expression [22]. The C677T polymorphism causes a 20% increase in serum homocysteine levels and is linked to a 60% decrease in enzyme function. [21,23] Numerous neuropsychiatric conditions, including autism, schizophrenia, depression, and attention deficit hyperactivity disorder, are linked to homocysteine and oxidative stress. [24] Folate is regarded as a crucial component of neurodevelopment, and neural tube abnormalities (NTD) are linked to its deficiency [25].

Li, Y. et al. described a number of mental illnesses, including schizophrenia, bipolar disorder, depression, autism, and ADHD, have been linked to the development of MTHFR activity reduction or folate insufficiency [26]. Rai, V. demonstrated that the pathogenesis of numerous neuropsychiatric illnesses, including autism, has typically been linked to elevated oxidative stress and elevated plasma homocysteine levels [23], suggesting that Methylenetetrahydrofolate reductase (MTHFR) plays a role in ASD. Therefore, MTHFR has been the focal point of investigation on ASD [27]. Studies of Wan, L. et al. and Gokcen, C. et al. have demonstrated that the A1298C genotype appeared to be the predominant linkage to the inattentive symptoms, leading to an increase in ADHD; compared with the C677T genotype, individuals with ADHD seem to carry significantly more mutants [28, 29]. It has been proposed by Li, C. X. et al. that in order to maintain the equilibrium of homocysteine in vivo in cases with MTHFR gene mutation, enough quantities of vitamin B12 and folate are required to control its metabolism [24].

Suman, P. et al. suggested that detecting MTHFR polymorphisms enables personalized treatment with folic acid supplementation in varying dosages, improving neurological and developmental results [30]. A study by Tisato, V. et al. put forward that additionally, it can assist us in comprehending how epigenetics affect neurodevelopment as The folate cycle, methionine cycle, and transsulfuration pathway are the three primary pathways that make up one-carbon metabolism, an integrated complex system which is essential for epigenetic regulation during

embryonic development [25]. Li, C. X. et al. encouraged that increased folic acid & homocysteine levels, which are linked to neuroinflammation and cognitive decline, can result from MTHFR mutations due to 5-MTHF. It could also be advantageous to monitor and control homocysteine levels with the right measures [24].

When the intake of inactive folic acid, especially from fortified foods or supplements, exceeds the metabolic capacity of the body, it can lead to unmetabolized folic acid (UMFA) buildup and cause negative effects. Many individuals with ASD have genetic variations in the MTHFR gene that can affect their ability to convert inactive folic acid to 5-MTHF, potentially leading to higher UMFA levels.



Figure 3: MTHFR Gene

3. SAM AND SAH Test:

One of the most prevalent and dynamic chemical changes in eukaryotes is methylation, which is an alkylation process in which a methyl group replaces a hydrogen atom [31]. It has significant effects on gene expression, RNA processing, and protein behaviour in addition to being engaged in a number of physiological processes. Methylation has also been identified as a crucial mechanism behind epigenetic controls, which include DNA methylation [31, 32]. Furthermore, metabolic research on autistic children has revealed that methylation capacity deficiencies may play a role in the onset and manifestation of autism symptoms. [33,34] The Met cycle produces the metabolites methionine (Met), S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH). [35] SAM is the universal methyl donor for a number of methyl-transfer processes, such as the methylation of DNA, RNA, proteins, phospholipids, and neurotransmitters, in every living organism [36].

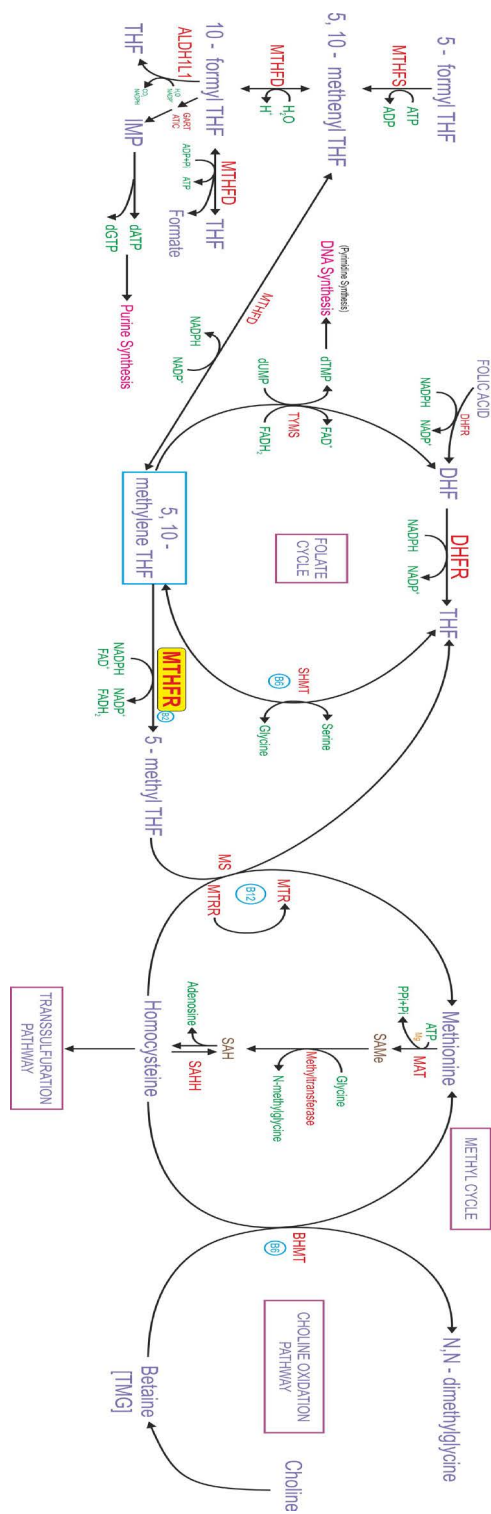


Figure 4: The folate-methionine cycle shows how folic acid is converted to 5-MTHF via MTHFR

S-adenosylhomocysteine (SAH), a strong inhibitor of the transmethylation processes associated with SAM, is the result of the irreversible conversion of SAM following methyl group transfer [33, 36]. By influencing gene transcription, protein localisation, small molecule degradation, and genome stability, several SAM-dependent processes play regulatory roles [37]. Transmethylation reactions are strongly suppressed by SAH, and it has been observed that transmethylation processes are hampered by either an increase in SAH levels or a decrease in SAM levels or the SAM/SAH ratio. SAH hydrolase facilitates the reversible conversion of SAH into homocysteine and adenosine [38]. When SAM concentrations are high, MTHFR is suppressed, which lowers 5-methylTHF synthesis and, consequently, homocysteine remethylation. On the other hand, remethylation of homocysteine is preferred when SAM concentrations are low.

The common genetic variation 677C/T can also alter MTHFR function, which lowers enzyme activity and in turn 5-methylTHF production [37]. In a study by Polat, T. et al., a common pathological mechanism in the folate-methionine pathway has been found in people with ASD. Decreased SAH levels could indicate less SAH catabolic activity, while decreased methionine and SAM levels could indicate remethylation pathway dysfunction [39].

Elshorbagy, A. K. et al. & Caudill, M. A. et al. suggested one potential link between the many risk factors and metabolic profiles associated with ASD is thought to be the reduced SAM/SAH ratio. Numerous mechanisms that result in abnormal folate-methionine metabolism have been linked to the range of clinical, metabolic, and immunophenotypic alters observed in ASD. Moreover, the SAM/SAH ratio, which is the ratio of SAM to SAH, has been identified as a measure of the methylation capacity of cells [35, 40]. Polat, T. et al. & Guo, B. Q. et al. proposed that the most fundamental and predictive biomarkers for assessing methylation ability in cells are these indices: Met (substrate of SAM), SAM (methyl donor), SAH (product of SAM and methylation inhibitor), and the SAM/SAH ratio (indicator of methylation capacity) [36, 39].

CSF Methylfolate test:

The immune system produces antibodies that mistakenly target the body's own folate receptors. These antibodies are known as folate receptor autoantibodies (FRAA). Which interferes with the transport of active folate into the brain, resulting in cerebral folate deficiency (CFD) syndrome, leading to limited speech development and behavioural and mood changes. About 70% of children diagnosed with ASD have autoantibodies against the folate receptor (FR α), resulting in low levels of folate within the brain despite normal levels in the blood.

Folate, a water-soluble vitamin belonging to the B complex group, is necessary for growth, development, and good health. Although the terms "folic acid" and

“folate” are frequently used interchangeably, folic acid is the synthetic version of the vitamin that is used in nutritional supplements and food fortification, while folates are the naturally occurring form. All biological events involving folate take place in the framework of methyl transferase-catalyzed, one-carbon-donor reactions (methyl, methylene, and formyl groups). Folate is an essential biological component of many physiologic processes as a cofactor or coenzyme. [41]

A study by Main, P. A. et al. suggested that many autistic people have been shown to have a defective folate-methionine pathway. Methylenetetrahydrofolate reductase (MTHFR) converts the majority of 5,10-MTHF to 5-MTHF, the primary form of folate in the blood and the sole form utilised in the central nervous system (CNS). Thymidine synthase transforms the remaining 5,10-MTHF into dihydrofolate (DHF) during the production of thymidylate, which is necessary for DNA replication and A family of folate receptor (FR) proteins primarily mediate folate transport through the choroid plexus. The reduced folate carrier 1 (RFC-1) FR proteins on the plasma side of the choroid plexus bind and endocytose folate into the intracellular compartment where it is concentrated. The choroid plexus's cerebrospinal fluid (CSF) side contains the RFC-1, which helps move the concentrated folate into the CSF. It is plausible that the folate-methionine pathway plays a role in autism since cerebral folate deficiency (CFD), a disorder linked to developmental delays (with or without autistic characteristics), is connected to defective folate transport into the central nervous system. [42] Hyland, K. et al. demonstrated that CFD is becoming increasingly recognized as a cause of neurological disease. There is growing recognition that CFD is a contributing factor to neurological disorders. [43] Folate receptor autoantibodies (FRAs) that obstruct folate transport across the blood–brain barrier are usually the cause of cerebral folate deficit (CFD) syndrome, a neurodevelopmental disease. [42] Knowing that a patient has low CSF 5-MTHF levels may assist direct treatment approaches, including taking supplements of folinic acid, which has shown promise in improving communication and reducing behaviours linked to ASD. [44] A thorough knowledge of the abnormalities in folate metabolism in ASD can be obtained by testing for FRAAs in addition to CSF 5-MTHF. [45]

Ramaekers, V. T. et al. recommended that by identifying a subgroup of ASD patients with particular folate metabolism problems, CSF 5-MTHF testing can enable more specialised and successful therapies. Periodically measuring CSF 5-MTHF levels in individuals with ASD receiving folate-based medications can act as a biomarker to track treatment response and modify dosages as necessary to achieve the best possible therapeutic results. 5-MTHF affects gene expression through epigenetic processes and is an essential methyl donor in the methylation cycle. ASD has been linked to altered methylation patterns; measuring CSF 5-MTHF levels can provide insight into these epigenetic changes and possibly identify new treatment targets. When conventional therapies fail, it is used to detect CFD, a disorder

that may be causing mental symptoms, including those observed in cases of severe ADHD [46]. Pan LA et al. advocated that Adequate folate levels are essential for neurotransmitter synthesis, and deficiency, particularly CFD, may contribute to neurobehavioural disorders like ADHD, including severe, treatment-resistant cases. [47]

Folate Receptor Autoantibody Test (FRAAT):

FRAAT is a diagnostic blood test that screens for autoantibodies (folate receptor autoantibodies) against folate receptors in the blood, enabling early detection and intervention. A blocking assay and a binding assay are the two components of FRAAT. The FRAAT assays measure the quantity of binding and blocking antibodies that are present [48]. FRAAT test positive means there are antibodies to the folate receptor which are preventing active folate from entering the brain. The assays indirectly tell us whether folate (and its active form, 5-MTHF) is present in the brain [49]. Children who tested positive for binding FRAA had higher serum B12 levels than those who tested negative for binding FRAAs, and children who tested positive for blocking FRAA had comparatively better redox metabolism and inflammation markers than those who tested negative for blocking. Neurodevelopmental, neuropsychiatric, and neurodegenerative disorders like ASD, neural tube defects, schizophrenia, and depression have been connected to folate receptor autoantibodies [50]. The study by Frye RE et al. & Phunsawat P et al. highlights that FRAAs are associated with specific physiological and behavioural characteristics in children with ASD and that FRAA status is associated with poorer adaptive functioning [49 , 51]. Treatment typically involves folinic acid (Leucovorin) sup-

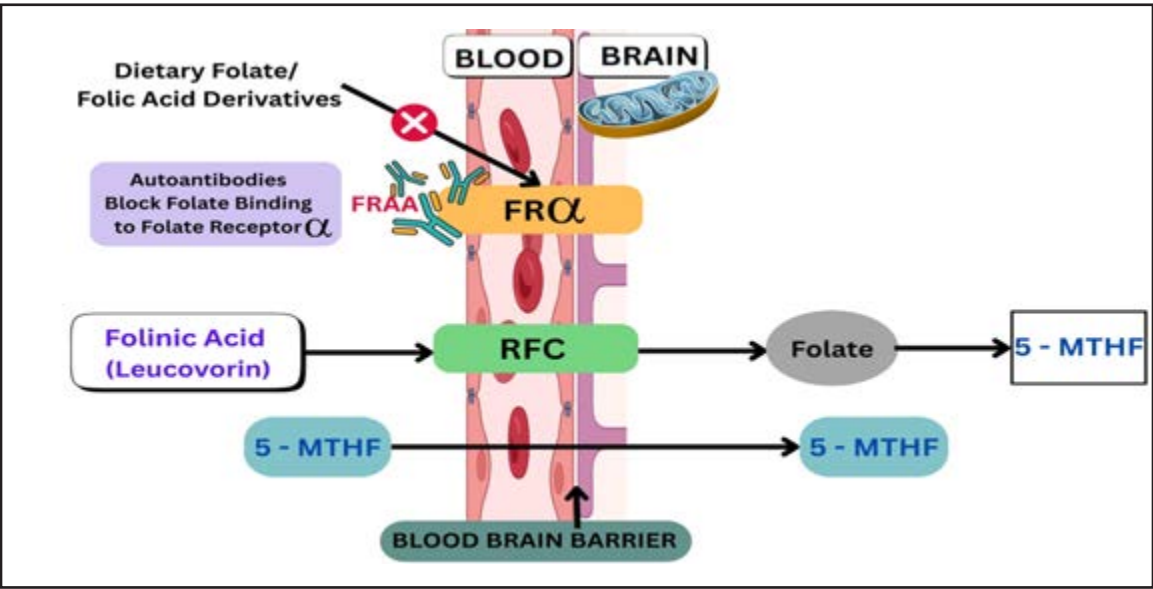


Figure 5: Cerebral Folate Transportation in Children with Autism

plementation that can bypass the blocked receptors and reach the brain & correct the folate[52]

Leucovorin can restore folate levels and neural functioning in the brain. The study by Frye RE et al. suggested that certain symptoms in children with ASD, particularly those who test positive for folate receptor alpha autoantibodies, can be significantly alleviated by leucovorin, a reduced folate that can avoid the blockage at the folate receptor alpha by using the reduced folate carrier, an alternative channel [53]. Studies by Rossignol DA et al. have presented that leucovorin has shown evidence, particularly for improving speech, social interaction, attention, stereotypes and communication in children with language delays, repetitive behaviour, irritability and hyperactivity [54].

6. Neurotransmitter Levels:

Neurodevelopmental diseases such as attention-deficit/hyperactivity disorder (ADHD), schizophrenia, and autism spectrum disorder (ASD) are linked to abnormalities in neurotransmitter production, release, or function [55]. Neurodevelopmental diseases can result from disruptions in these vital developmental processes caused by changes in neurotransmitter levels or activity, or malfunctions in their signaling pathways [56,57]. Since the majority of neurotransmitters and their systems exist before synaptogenesis, they are essential for brain development and are implicated in the etiology of numerous neurodevelopmental disorders. A number of neurological and neuropsychiatric conditions have been linked to disruptions in the dopamine (DA) and dopaminergic (DAergic) systems. Using DA as a neurotransmitter, the DAergic system regulates important cognitive and behavioural abilities, such as emotional and motivated behaviour. The etiology and evolution of key illnesses of the developing brain, such as autism, schizophrenia, attention deficit hyperactivity disorder, Down syndrome, and fragile X syndrome, are influenced by the DAergic system and have an impact on neurodevelopment [57].

Research by Marotta, R. et al. suggests that autism is influenced by a number of factors, including genetic abnormalities, anatomical abnormalities of the brain, and neurochemical dysfunctions of different neurotransmitters and neuropeptides, such as GABA and glutamate, serotonin, dopamine, and oxytocin. melatonin, and acetylcholine. Imbalanced levels of neurotransmitters in the blood and urine of an autistic kid may lead to different issues, like elevated serotonin levels that may affect sleep, anxiety, mood regulation, and learning ability. Altered gamma-aminobutyric acid (GABA) levels are mostly associated with sensory issues. Imbalance in dopamine and acetylcholine levels plays a major role in social behaviour, cognition, and repetitive behaviour. And dysregulation in glutamate is mainly associated with hyperactivity and impulsivity in autism [58].

Studies by Faraone, S.V. et al. have demonstrated abnormalities or dysfunction in

neurotransmitters such as dopamine and norepinephrine, especially in the frontal regions of the brain that are involved in attention, focus, and reward, and these neurotransmitters are significantly associated with ADHD. Dopamine plays a critical role in controlling motor control, reward, attention, and cognition. ADHD-related dopamine dysregulation is linked to issues with motivation, focus, and the operation of the reward system [59]. da Silva BS et al. proposed that working memory processes have been demonstrated to be modulated by norepinephrine's binding to adrenergic receptors; moderate or high levels of this neurotransmitter exhibit varying binding affinities to each type of receptor and, as a result, distinct physiological effects on working memory [60]. A study by Prince J. explained the primary symptoms of ADHD, such as impulsivity, hyperactivity, and inattention, are believed to be exacerbated by abnormally low levels or ineffective usage of dopamine and norepinephrine [61]. Studies by da Silva BS et al. linking altered levels of serotonergic, glutamatergic, and GABAergic neurotransmitters to ADHD have also implicated these systems, albeit not as thoroughly as the dopaminergic and noradrenergic systems [60].

Understanding neurochemical change will be aided by testing for neurotransmitter levels in blood and urine samples from individuals with autism. And this helps in targeting specific treatments and improving intervention strategies in ASD, ADHD, and schizophrenia based on an individual's neurochemical profile.

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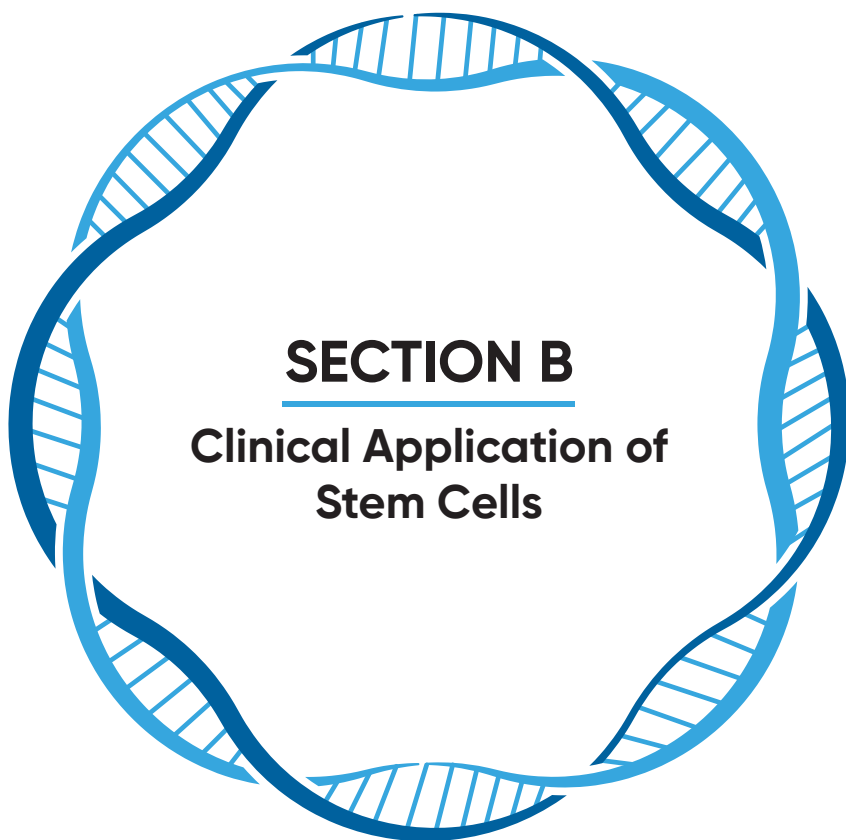
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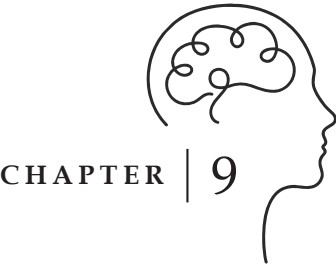
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"Things don't change.

You change your way of looking at them"

- Carlos Castaneda



Role of Cell / Stem Cells in Autism Spectrum Disorders

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

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

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

Over the past few decades, the prevalence of ASD has sharply grown for unknown causes. Compared to girls, boys are three to four times more likely to have it. Autism needs to be managed for the rest of one’s life, just like other neurodevelopmental illnesses. It affects people with disorders’ quality of life, social integration, economic well-being, and health, as well as those of their family and maybe the general public. To better understand the causes of autism and eventually provide

a treatment, a lot of research is being done worldwide. Newer treatments like stem cell therapy have shown immense potential as a treatment strategy for ASD. In-depth knowledge of how stem cell therapy functions in ASD is the primary emphasis of this chapter. It has been backed up by sufficient scientific evidence in the form of an international evaluation of clinical research and its findings.

Pathophysiology of Autism

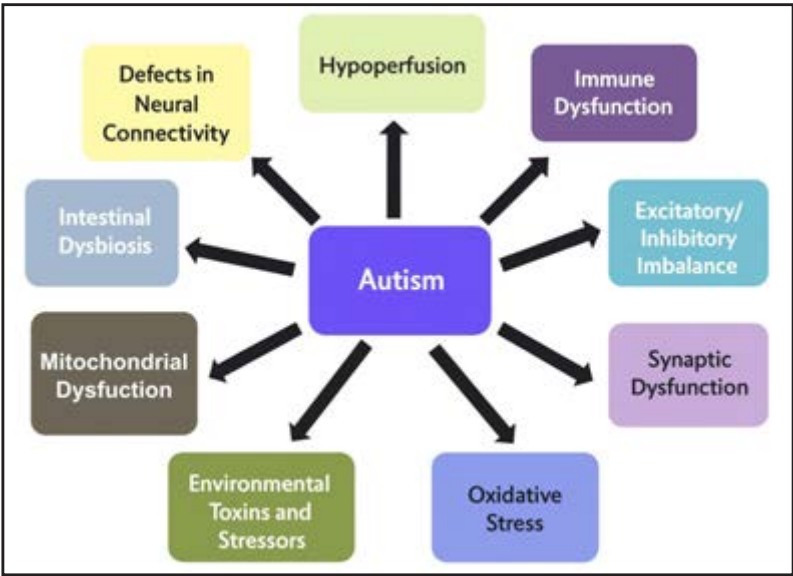


Figure 1: Pathophysiology of ASDs

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

creased connectivity in short pathways. This causes an imbalance in the connectivity of the brain of autism. (5)

Unmet medical needs

Since ASD is thought to represent the last common pathway of several etiological and neuropathological causes, it is challenging to find biomarkers unique to autism. (6) As a result, diagnosis depends on identifying a variety of behavioural symptoms that differ from one case to the next and overlap with other neuropsychiatric illnesses in children.

Additionally, it is challenging to design a standard treatment for ASD because of its heterogeneous nature. Autism can currently be treated with behavioural, dietary, medicinal, and rehabilitative approaches. These just treat the symptoms and related illnesses; they don't deal with the underlying pathophysiology of autism. The medications do not permanently cure the symptoms; they just temporarily reduce them. Therefore, it is urgent to develop a plan to address the underlying abnormal neural connections that will have a lasting impact.

Stem cell therapy in autism

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

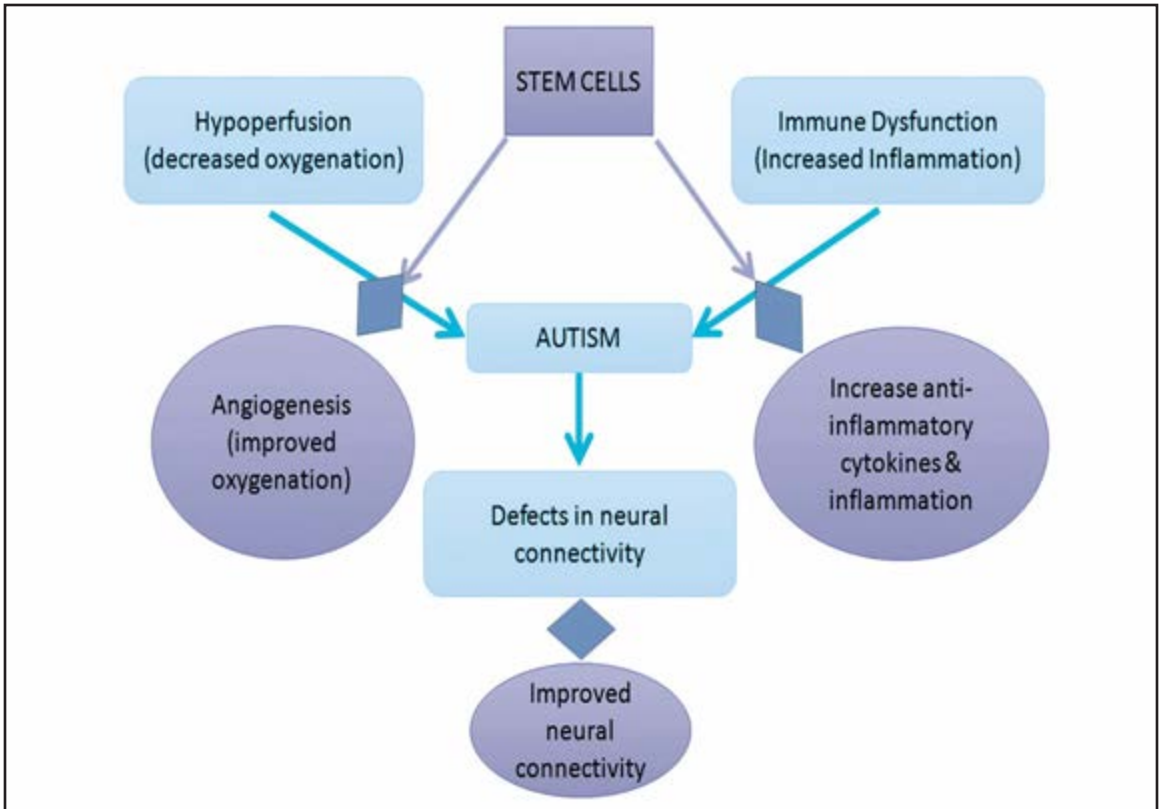


Figure 2: Stem Cell Therapy in Autism

Pre-clinical Studies

Certain neurodevelopmental conditions, such as autism, are exclusively found in humans. Autism is typically only diagnosed based on behavioural criteria. Because of this, it is challenging to reproduce autism in animal models that exhibit all of its clinical traits. Studying the impact of any intervention on these animal models is also made difficult by this. A popular animal model for autism is the BTBR inbred mouse strain, which exhibits severe behavioural abnormalities. MSC transplantation in BTBR mice reduced stereotyped behaviours, decreased cognitive stiffness, and improved social behaviour, according to a recent study by H Segal-Gavish et al. Comparing MSC-transplanted mice to sham-treated mice, tissue analysis showed higher BDNF protein levels in the hippocampus along with greater hippocampal neurogenesis. (15)

38 studies have been published, including 19 clinical studies and 19 case reports demonstrating the effect of stem cell therapy in ASD. Different types of cells were used, namely autologous bone marrow mononuclear cells, fetal stem cells and umbilical cord blood cells.

Sharma et al. (16) published the first clinical, which was an open-label proof of concept study in 32 patients with autism. They administered autologous bone marrow mononuclear cells intrathecally. The results of their trial demonstrated the safety and efficacy of stem cell therapy for autism. The results of this study have been described in detail below.

The next clinical study was published by Yong-Tao Lv et al., where they studied the use of human cord blood MNCs and MSCs in 37 patients. (17) They used CARS, CGI and ABC scales to assess the therapeutic efficacy in this study. A positive outcome was obtained in the treatment group. In 2014, Bradstreet et al. published their study using fetal stem cells. (18) The study was carried out on 45 children with autism. On follow-up after 6 months and 12 months, there was a significant change in Autism Treatment Evaluation Checklist (ATEC) test and Aberrant Behaviour Checklist (ABC) scores. Improvement was also seen in behaviour, eye contact, appetite, etc. In 2017, Dawson et al. published their Phase 1 safety and tolerability study wherein they administered a single intravenous infusion of autologous cord blood cells in 25 children with ASD. (19) The infusions were safe, with no serious adverse events and occasional allergic reactions and irritability reported. Improvements in ASD symptoms were observed on caregiver-completed measures (Vineland Adaptive Behavior Scales Second Edition (VABS-II, see figure 1) and Pervasive Developmental Disorder Behavior Inventory (PDDBI)), clinician assessment (CGI-I, figure 1), and computerized eye tracking assessments. Positive changes, including increased social communication skills and receptive/expressive language and decreased repetitive behavior and sensory sensitivities were observed six months post infusion and maintained at 12 months. A Phase 2 randomized study is underway to evaluate the efficacy of autologous or allogeneic CB therapy versus placebo in children with ASD.

In 2017, a case series from India was published by Shroff G wherein 3 cases of ASD were administered with embryonic stem cells. (20) The patients showed improvements in eye coordination, writing, balancing, cognition, and speech and showed reduced hypersensitivity to noises and smells. These patients also showed improvement in SPECT scans.

Nguyen et al. published a clinical trial, which was an open-label study in 54 patients of autism. They administered autologous bone marrow mononuclear cells intrathecally. Efficacy outcomes were evaluated at baseline, two, six, and twelve months based on (1) improvements in social interaction, adaptive behaviour, and daily living skills measured by the Vineland Adaptive Behaviour Scales (VABS-II) and Clinical Global Impression-Improvement (CGI-I) scale scores; and (2) changes in ASD severity evaluated by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), the Childhood Autism Rating Scale (CARS), and the Clinical Global Impression-Severity (CGI-S) scale scores. There was a significant difference in the CARS scores between the CT group and the control group. Like-

wise, the CT group's CGI-S scores improved more than the control group's. The CT group's VABS-II scores rose by 8.5 points, while the control group's climbed by 1.4 points. Lastly, the CT group's CGI-I scores increased while the control group's scores declined. The results of this study stated that intrathecal BMMNC combined with an educational intervention improved disease severity and adaptability more than education alone in children with ASD.

A study by Wong et al. aimed to document the safety and efficacy of a single infusion of autologous umbilical cord blood (UCB) in 20 autistic children. The Vineland Adaptive Behaviour Scale, Stanford Binet Intelligence Scale (SB-5), Expressive One-Word Picture Vocabulary Test, Pervasive Developmental Disorder-Behaviour Inventory, Repetitive Behaviour Scale-Revised, Sensory Experience Questionnaire (SEQ-2.1), Child Behaviour Checklist, Clinical Global Impression-Severity and Improvement (CGI-I) Scales, and eye-gaze tracking were among the comprehensive and structured safety evaluations that were administered to participants at T = 0, 6, 12, and 18 months. None experienced severe adverse reactions. Vineland-3, SB-5, BOSCC, and SEQ-2.1 scores at T = 12 and T = 18 months did not change significantly. Seven subjects had T = 18 CGI-I scores of 4 (no change), while twelve people had scores of 2-3 (minimally to considerably improved). Autism stem cell treatments should only be used in rigorous clinical trials with explicit risk assessments.

Our Published data

1. In 2025 Sharma et al. published an observational study in "Clinical Transplantation and Research" conducted retrospectively of autologous bone marrow mononuclear cells (BMMNCs) in 1011 patients with autism spectrum disorder (ASD) followed by multidisciplinary therapies. All patients were followed up at a mean follow-up of 19.3 months and changes in symptoms along with outcome measures, the Indian Scale of Autism Assessment (ISAA) and Childhood Autism Rating Scale (CARS), were recorded. A brain PET CT scan was used to objectively evaluate changes in brain metabolism. Overall, 90.6% (916 out of 1011) patients improved on at least one outcome measure after cell therapy. The scores on ISAA and CARS showed positive change after cell transplantation. It was found that 85% of patients showed improvement in ISAA scores, while 85.8% of patients showed improvement in CARS scores. Symptomatic improvements were observed in attention and concentration where 827 patients showed improvement out of 1006 patients (82.2%), In domain command following 803 out of 1000 (80.3%) showed improvement, under eye contact there was an improvement observed in 766 out of 978 patients (78.3), sitting tolerance of 679 out of 959 (70.8) improved, social interaction was improved in 658 out of 1011 patients, hyperactivity was reduced in 603 out of 927 patients (65%), communication & speech was improved in 605 out of 988 (61.2), stereotypical behavior showed improvement in 518 out of 973 (53.3%) patients, aggressiveness significantly decreased in 422 out of 829 patients, and

self-injurious behavior was reduced in 239 out of 483 patients(49.5%). On statistically analyzing the pre- and post-data using Wilcoxon’s Signed-Rank Test with a level of significance as $P<0.05$, it was found that the improvements on ISAA and CARS were statistically significant. A comparative PET CT scan of 401 patients showed improved metabolism in the amygdala, hippocampus, parahippocampal gyrus, caudate nucleus, cerebellum, mesial temporal lobe, thalamus, and superior and middle temporal poles of the brain which correlated with symptomatic improvements. No major adverse events were reported.

Table 1: Wilcoxon’s Signed-Rank Test (Level of significance $P<0.05$)

Scales	Pre-mean score	Post-mean score	Significance ($p<0.05$)
ISAA	116.93	107.17	Significant
CARS	38.24	35.73	Significant

Table 2: Wilcoxon’s Signed-Rank Test for ISAA and Domains (Level of significance $P<0.05$)

		Pre-Mean score	Post Mean score	Significance ($P<0.05$)
Scales	ISAA	116.93	107.17	Significant
Category	Social relationships and Reciprocity	35.28	32.74	Significant
	Emotional Responsiveness	14.03	12.43	Significant
	Speech, language and communication	23.57	22.19	Significant
	Behavior Patterns	18.55	16.6	Significant
	Sensory Aspects	15.09	13.72	Significant
	Cognitive Components	10.41	9.46	Significant

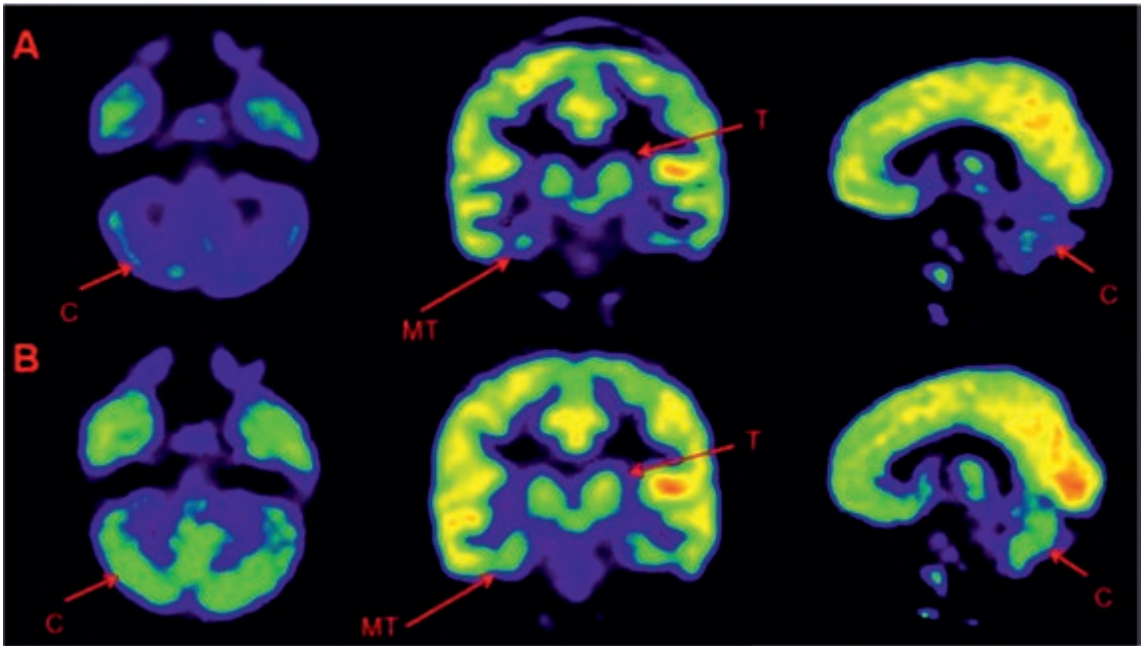


Figure 3: Representative images of comparative brain FDG PET scans of ASD patients performed before and after cell therapy. Top Row (A): Arrow-marked blue areas represent hypometabolism. Bottom Row (B): Arrow-marked green areas showing improved metabolism post-cell therapy. Cerebellum (C), medial temporal cortex (MT) and thalamus (T).

2. NeuroGen Brain and Spine Institute published the world's first study on stem cell therapy in autism in "Stem Cell International" in 2013. An open-label proof-of-concept study (Sharma et al.) of autologous bone marrow mononuclear cells (BMMNCs) intrathecal transplantation in 32 patients with autism followed by multidisciplinary therapies was performed. All patients were followed up for 26 months (Mean 12.7). Outcome measures used were ISAA, CGI and FIM/Wee-FIM scales. Positron Emission Tomography Computed Tomography (PET-CT) scan recorded objective changes. Out of 32 patients, a total of 29 (91%) patients improved on total ISAA scores and 20 patients (62%) showed decreased severity on CGI-I. In the domain of social relationships and reciprocity, 29 out of 32 (90.6%) patients showed improvement. Improved emotional responsiveness was observed in 18 out of 32 (56%) patients. Under the speech-language and communication domain, there was an improvement observed in 25 patients out of 32 (78%). Behaviour patterns of 21 out of 32 patients (66%) improved. Hyperactivity or restlessness (71%) and engaging in stereotypical and repetitive motor mannerisms (65%) decreased significantly. Sensory aspects improved in 14 out of 32 patients (44%). Cognitively, they showed improved consistency in attention, concentration and response time. 71% of patients showed better attention and concentration, and 45% of patients

showed a reduction in the delay in responding. The difference between pre- and post-scores was statistically significant ($p < 0.001$) on the Wilcoxon Matched-Pairs Signed Rank Test. On CGI-II 96% of patients showed global improvement. The efficacy was measured on the CGI-III efficacy index. Functional neuroimaging in the form of a PET-CT scan in eight patients documented changes in brain metabolism which correlated with clinical improvements. A few adverse 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.

Table 3: Change in the scores of CGI and ISAA before and after intervention.

Scale	Median score before cellular therapy	Median score after 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 $P < 0.05$)

Table 4: Change in the ISAA scores of individual domains measured 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	35.23	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*
Behaviour 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 $P < 0.05$)

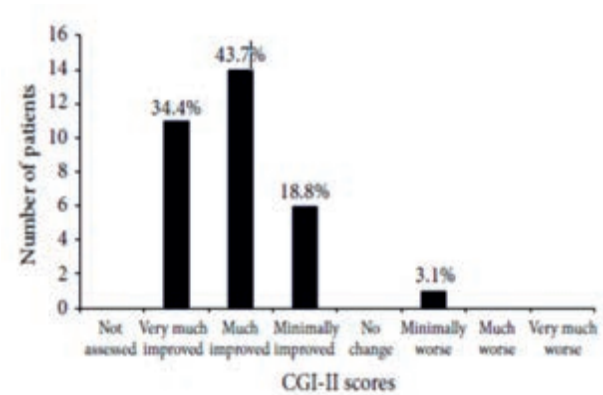


Figure 4: Frequency distribution of participants on CGI-II scale

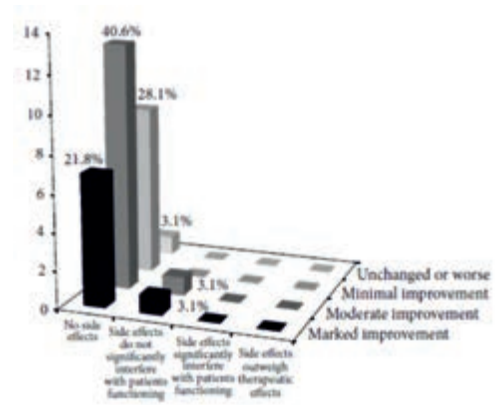


Figure 5: Frequency distribution of participants on CGI-III scale

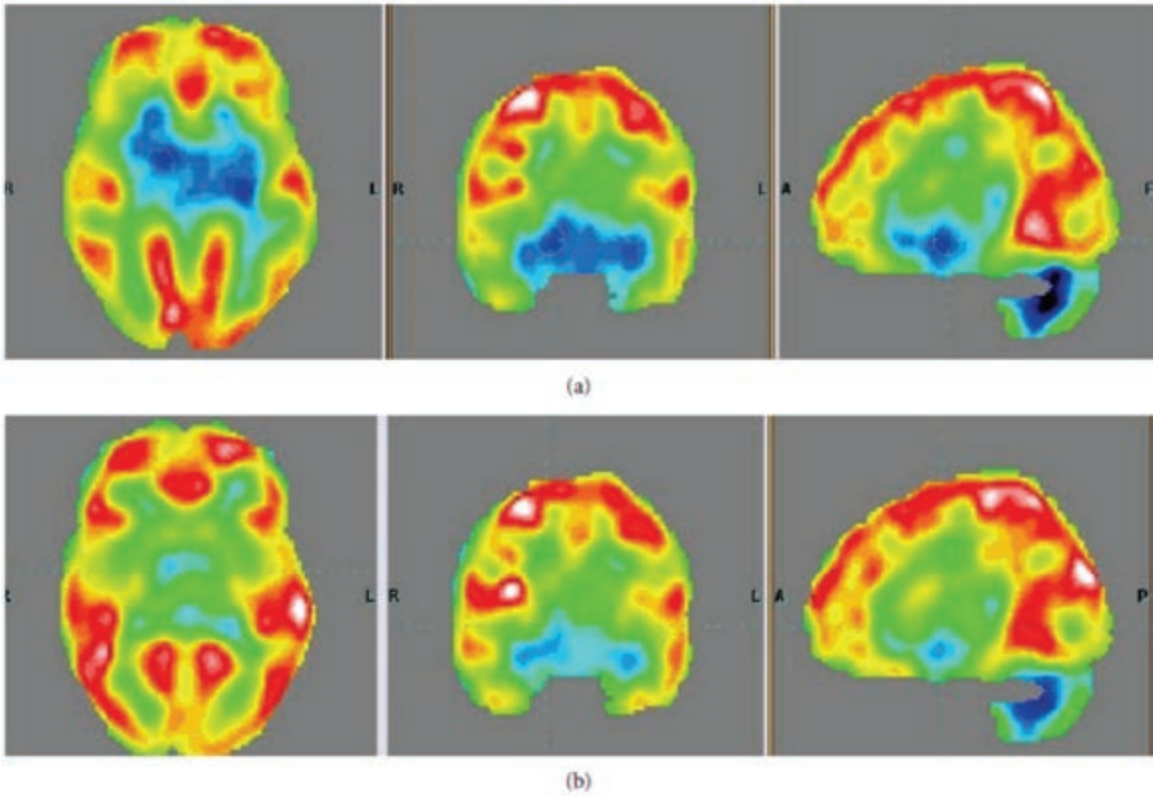


Figure 6: Findings in PET-CT scan before and after cellular therapy. (a) PET-CT scan before intervention showing reduced FDG uptake in the areas of the frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus, and mesial temporal lobe. (b) A PET-CT scan six months after the intervention comparison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

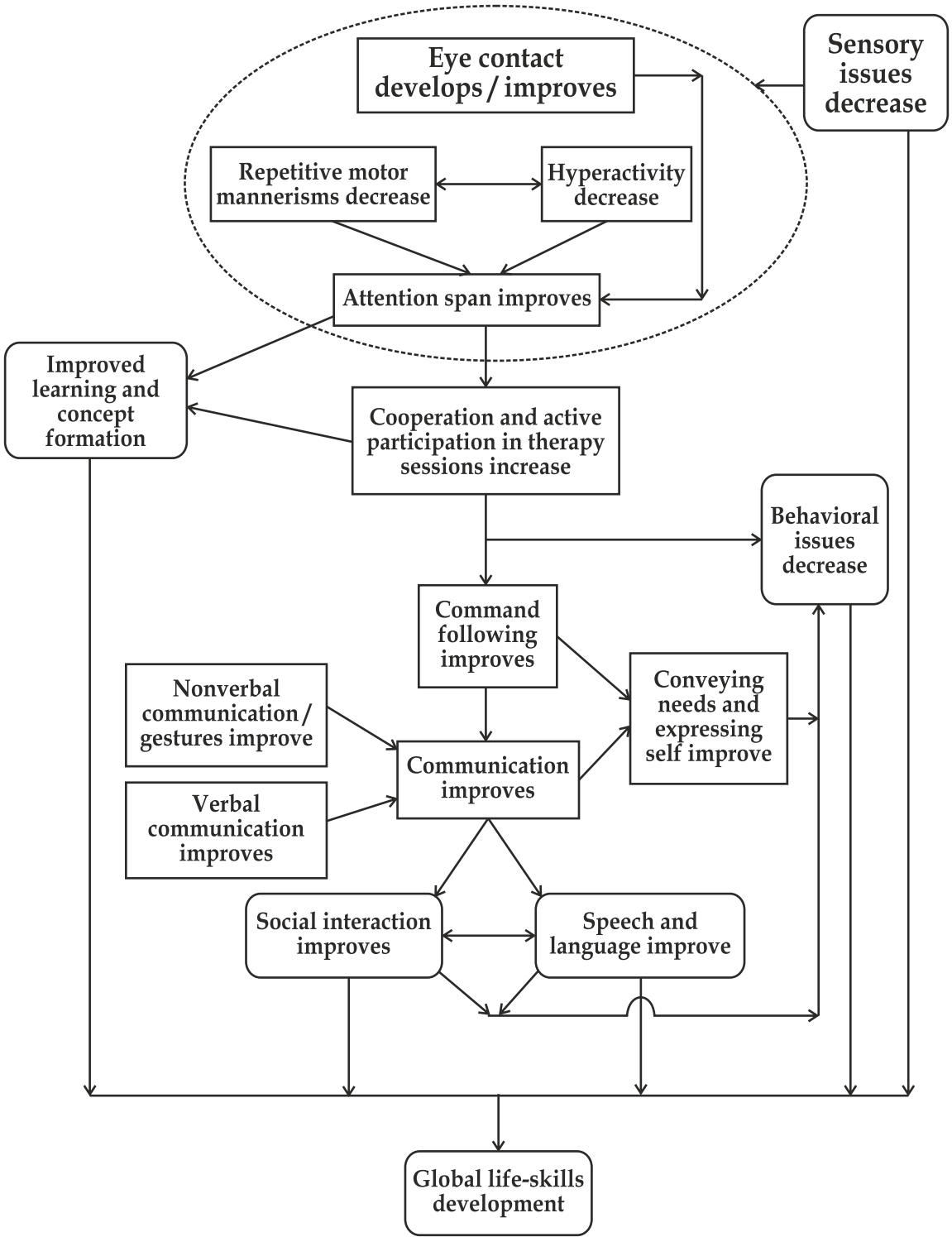


Figure 7: Schematic representation of clinical improvements after cellular therapy. This figure shows the proposed theoretical outline of observed changes after cellular therapy.

3. In continuation of the first study, another study was published in the “American Journal of Stem Cells” in 2020, which included a larger population of 254 patients with ASD showing the benefits of stem cell therapy. On mean follow-up of 7.50 months, improvements were observed in eye contact, attention and concentration, hyperactivity, sitting tolerance, social interaction, stereotypical behavior, aggressiveness, communication, speech, command following and self-stimulatory behavior. Statistically significant improvement was observed in scores of ISAA and CARS after intervention.

In subgroup analysis, a significantly better outcome of the intervention was found in patients of younger age and with a shorter duration of disease [<5 years from time of diagnosis]. 86 patients who underwent a repeat PET CT scan showed improved brain metabolism after intervention in areas which correlated to the symptomatic changes. No major procedure-related adverse events were recorded. However, 5 patients with a history of seizure and abnormal EEG, had an episode of seizure which was managed using medications. Outcome of intervention in these patients was not affected by seizures as improvements were observed in them.

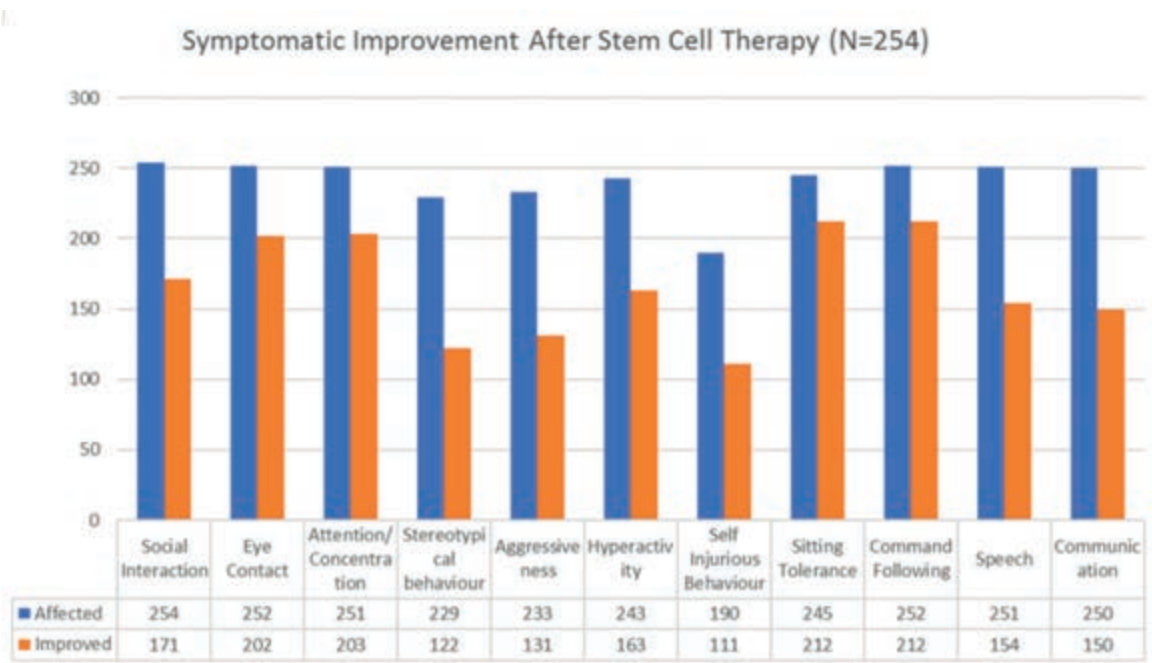


Figure 8: Graph representing improvements in symptoms after intrathecal transplantation of autologous BMMNCs.

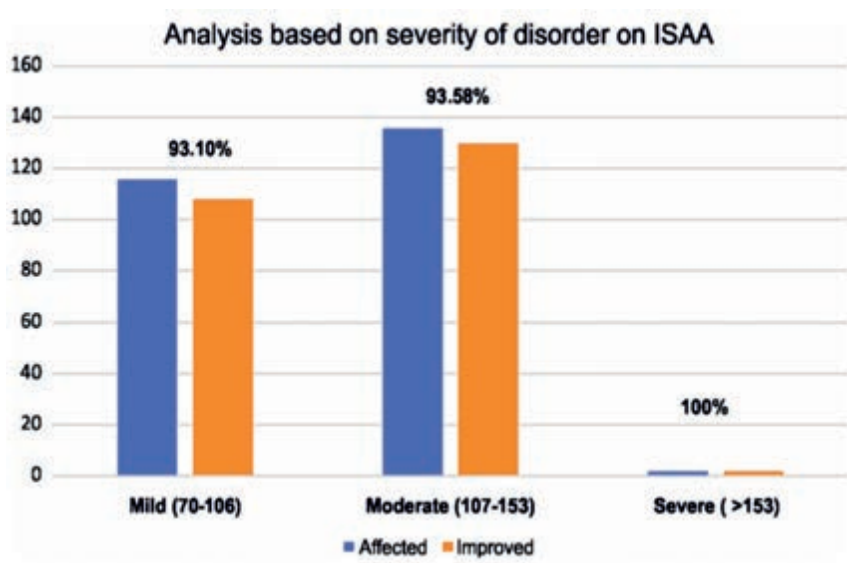


Figure 9: Graph representing results based on severity of disorder on ISAA.

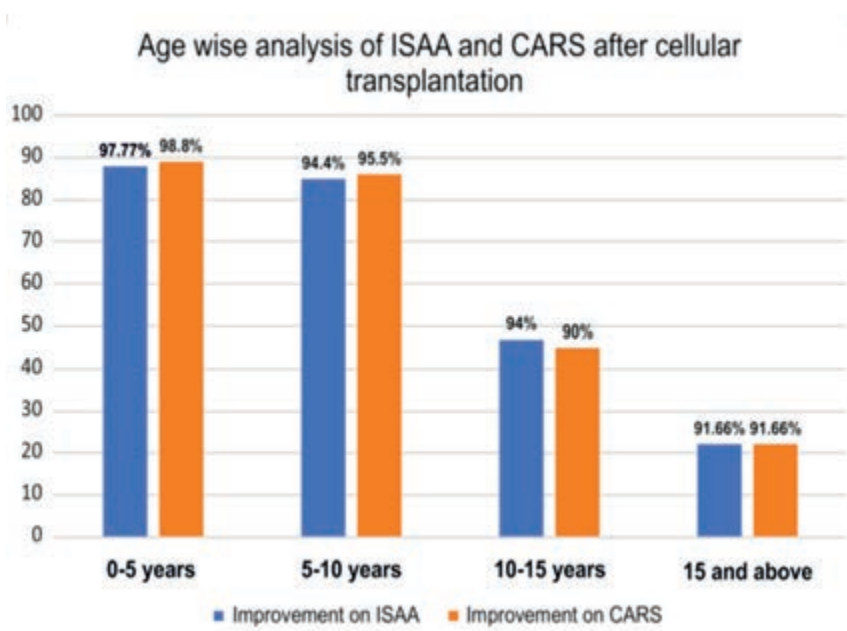


Figure 10: Graph representing post-stem cell therapy results of ISAA and CARS based on the age of patients.

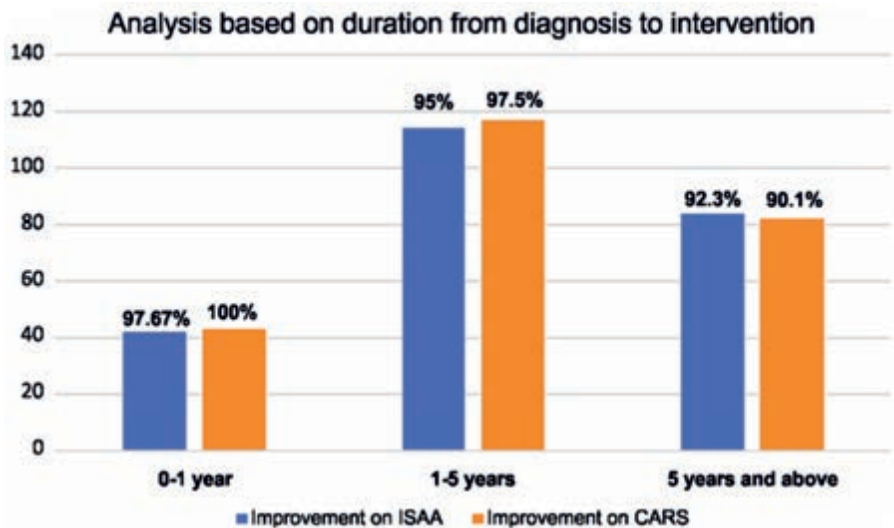


Figure 11: Graph representing post-stem cell therapy results of ISAA and CARS based on duration from diagnosis to intervention

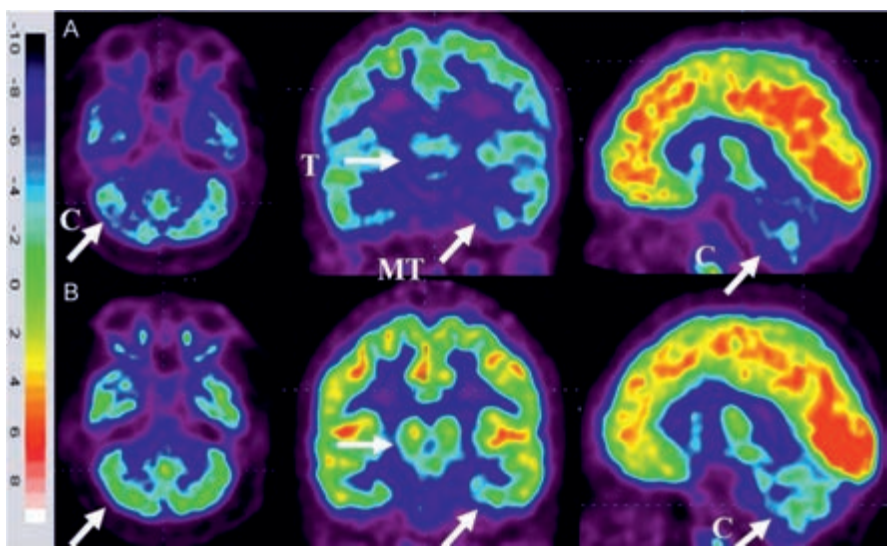


Figure 12: Representative images of FDG-PET/CT scan brain of ASD patients performed before and 6 months after stem cell therapy. A. Top Row: blue areas marked with arrows demonstrate hypometabolism. B. Below Row: green areas marked with arrows, they demonstrate improved metabolism following stem cell therapy. Thalamus [T], medial temporal cortex [MT] and cerebellum [C].

We have also published 12 case reports in various national and international peer-reviewed journals demonstrating the effect of stem cell therapy in autism cases. (21-32) All these cases were different with respect to age, severity of autism and symptoms presented. One of the patients also had comorbid ID. These cases underwent autologous bone marrow mononuclear cell transplantation along with personalized neurorehabilitation program which included applied behaviour analysis, psychological intervention, speech therapy, occupational therapy, art-based therapy, etc. These patients were followed up regularly to record changes in clinical symptoms and outcome measures such as CARS, CGI, ISAA and FIM were used to quantify the changes. On follow-up, improvements were observed in common symptoms such as speech, awareness, logical thinking, attention and concentration, eye contact, social interaction, emotional responses, command following, learning abilities, response time, sitting tolerance and ADLs. Hyperactivity, aggressive behaviour, stereotypical behaviour and self-stimulatory behaviour were also reduced. They all showed positive changes on outcome measures. The scores of the patient categorized as severe autism on CARS improved to no autism. Which indicates the patient required assistance but was out of the spectrum after the intervention. There was improvement in IQ as well of the patient with comorbid ID.

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

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

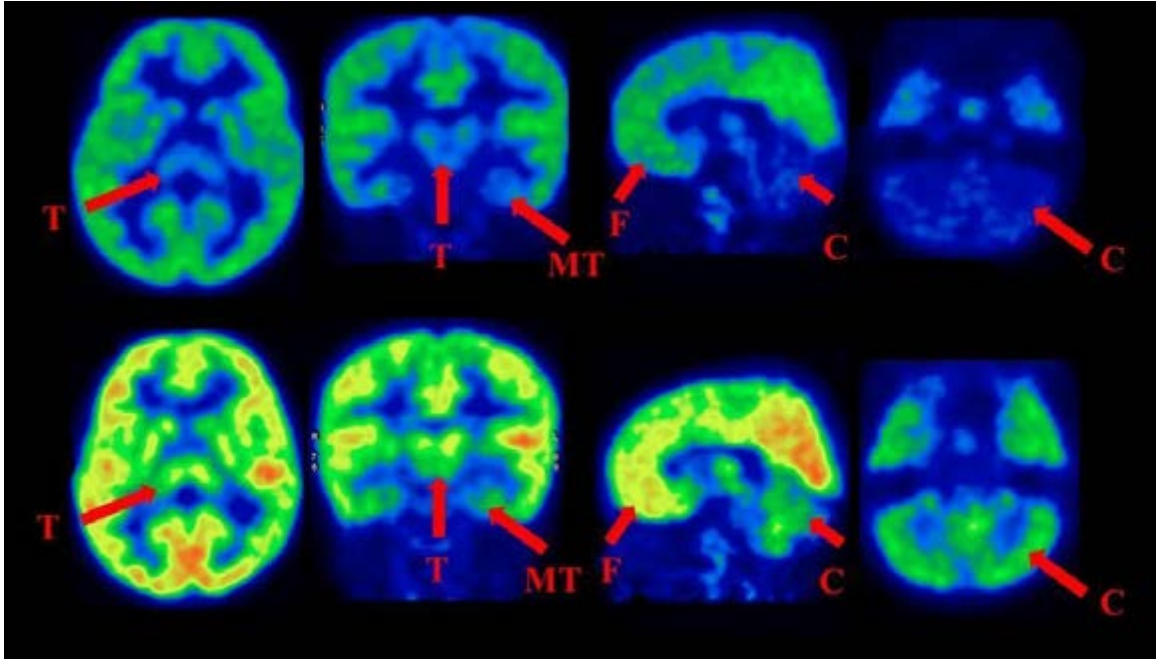


Figure 13: Comparative PET CT scans of an autism patient performed before and after cell therapy. Significant improvements was observed in the cerebellum (C), Thalamus (T), Frontal (F) and bilateral medial temporal cortices (MT)

Unpublished

Case Report

A 12-year-old male child was diagnosed with Autism Spectrum Disorder based on his clinical presentation. On assessment we observed that his attention and concentration were affected as he got easily distracted. His imitation skills were poor and inconsistent; he followed basic commands but he required 2 to 3 repetitions. His problem-solving and awareness were affected. There was the presence of laughing and crying without any reason. His sitting tolerance was poor, i.e., less than 5 seconds according to the parents, and his social interaction and social norms were also affected. There was the presence of motor mannerisms like hand flapping and rocking behaviour. He conveyed most of his basic needs non-verbally. His speech was affected and he could only make sounds. He was dependent on his caretakers for day-to-day activities. On FIM, he scored 62. On ISAA he scored 123, which indicated moderate autism and on CARS he scored 46.5, which indicated severe autism. This patient underwent 4 transplantations in a period of 3 years.

Improvements after 1st transplantation. Follow-up period – 9 months

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

Improvements after 2nd transplantation. Follow-up period – 12 months

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

Improvements after 3rd transplantation. Follow-up period – 12 months

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

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

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

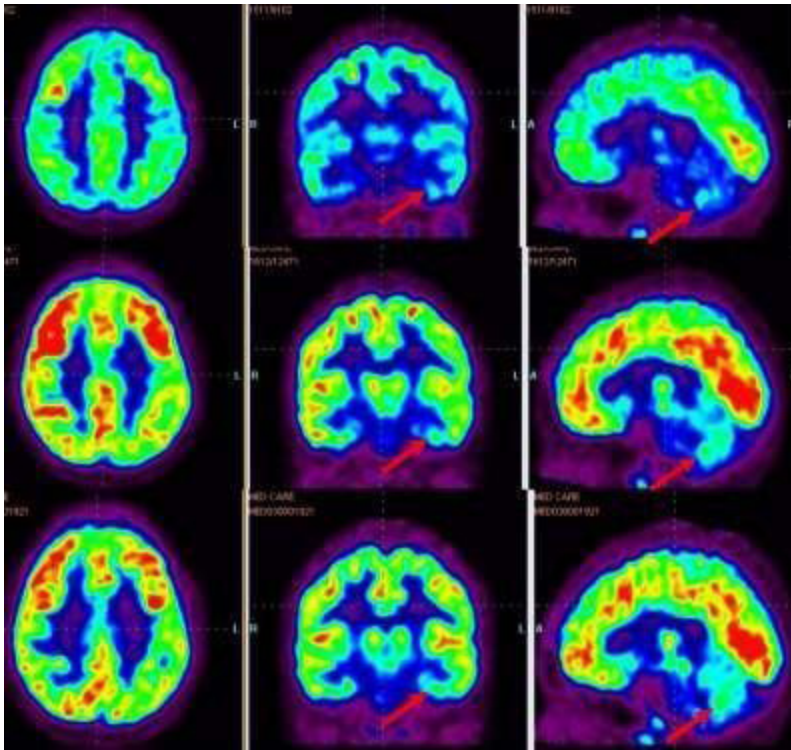


Figure 14: PET-CT scan taken before and after the stem cell therapy showing significant changes in the brain metabolism.

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

Adverse Events:

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

Conclusion

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

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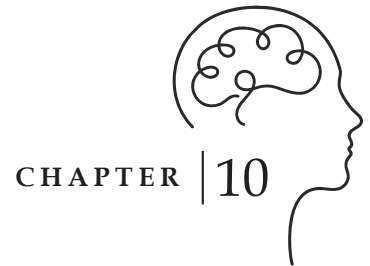
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Security is mostly a superstition. It does not exist in nature nor do children of men as a whole experience it. Avoiding danger is no safer in the long run than outright exposure. Life is either a daring adventure or nothing."

- Hellen Keller



Role of Cell / Stem Cell Transplantation for Cerebral Palsy

*Neurodevelopment in cerebral palsy can be accelerated by trophic,
paracrine and neuroregenerative properties of stem cells.*

Cerebral palsy is defined as “a disorder of movement and posture due to a defect or lesion of the immature brain”. (1) It is known to affect 2/1000 live-born children. CP may result from a variety of prenatal, perinatal or postnatal 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, behavioural issues and seizures. Risk factors for cerebral palsy include prenatal anemia, improper nutrition, infections, premature delivery, etc.

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

Unmet medical needs

The incidence of CP is increasing at an alarming rate throughout the world. Recent medical advances have improved medical care, but still many challenges remain in the management of these disorders. CP remains an incurable disorder. Hence, establishing a standard therapeutic approach is the focus of researchers and clinicians all over the world. Although the available treatment options are helpful in managing the symptoms to some extent, none of them repair the underlying damaged brain. There are no definitive treatment options to accelerate the development of cerebral palsy patients.

Role of stem cell therapy in Cerebral palsy

Hypoxic ischemia is the most common risk factor of CP prenatally and during delivery. Periventricular leukomalacia (PVL), a diffuse 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 leads to disruption in the production of mature OLs, which further leads to disturbance in myelination. (9,10) In CP, microglial activation also instigates the secretion of tumor necrosis factor alpha (TNF- α), interferon gamma (INF- γ), interleukin-1 beta (IL-1 β), superoxide radicals, nitrogen species, glutamates, adenosine which exerts a toxic effect on neurons

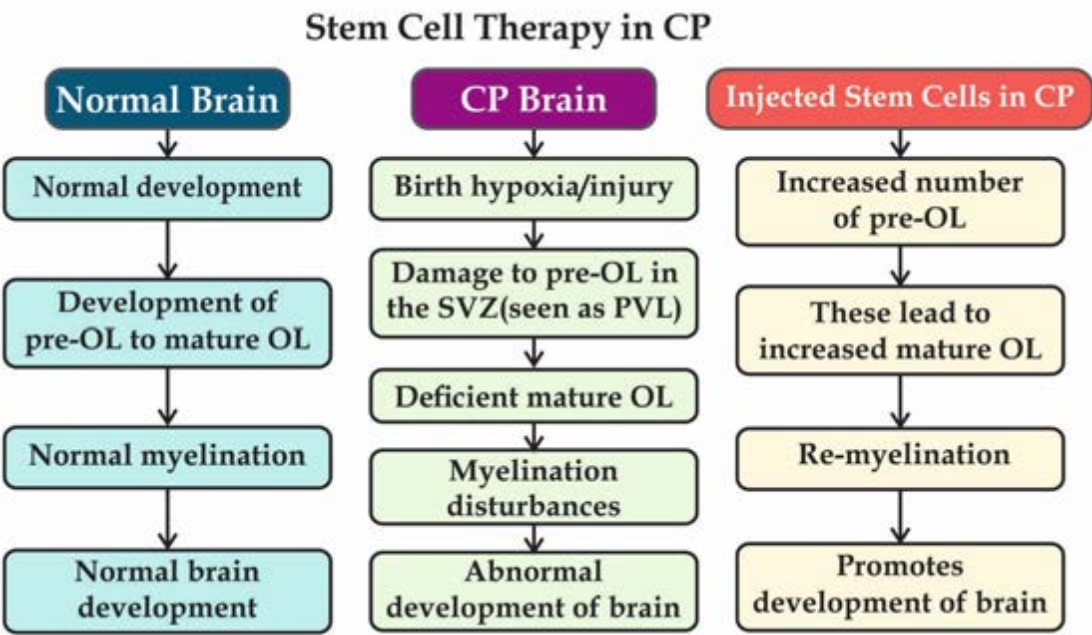


Figure 1: Stem cell therapy in cerebral palsy

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 of $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and 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.

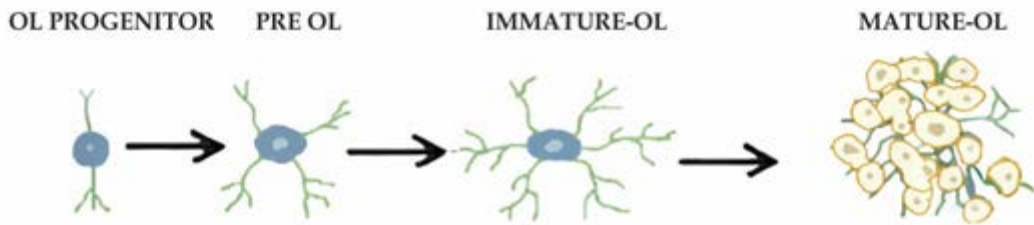


Figure 2: Phases of Oligodendrocyte development

Animal studies

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

Human studies

49 studies, which include 32 clinical studies and 17 case reports, have been published to demonstrate the effects of various types of stem cells in cerebral palsy. These studies used different cells, such as bone marrow-derived stem cells, umbilical cord blood cells, Olfactory Ensheathing cells, embryonic cells and neural cells.

Many studies used bone marrow-derived stem cells such as bone marrow mononuclear cells, mesenchymal cells, enriched bone marrow progenitor cells and total nucleated cells. (27-36) These cells were administered either intravenously or intrathecally. All the authors reported these cells to be safe and efficacious. Neurological and functional improvements were observed in all these studies. Umbilical cord blood cells have also shown a positive outcome after intravenous and intra-

thecal routes of transplantation. (37-49) No major adverse events were recorded in any study. Shroff et al. administered human embryonic stem cells intravenously in 91 patients. 63 patients showed improvement; however, seizures were recorded as a side effect of the intervention. (50) Seledtsov et al. carried out a controlled study injecting a cell suspension from immature nervous and haematopoietic tissues. Their findings suggested that cell therapy was an effective, safe and immunologically justified method of therapy for patients with cerebral palsy. (51) Chen et al. published the results of a randomized controlled study of intracranial administration of olfactory ensheathing cells. (52) They reported these cells to be safe and efficacious. Similarly, Luan et al. and Chen et al. administered neural stem cells and neural stem cell-like cells intracranially and intrathecally in a large number of cases in China and reported it to be an effective treatment for CP. (53-55) Whereas Rah et al. demonstrated the neuroregenerative potential of intravenous G-CSF and autologous peripheral blood stem cells in CP through a randomized, double-blind study. (56)

A study by Boyali et al. includes four CP sufferers who require assistance to stand or walk. Using umbilical cord-derived MSCs (UC-MSC), all of these patients got allogeneic MSCT six times as $1 \times 10^6/\text{kg}$ intrathecally, intravenously, and intramuscularly. The Wee Functional Independence Measure (WeeFIM), Gross Motor Function Classification System (GMFCS), and Manual Ability Classification Scale (MACS) were used to evaluate the patients both before and after treatment. We measured spasticity using the Modified Ashworth Scale (MAS). Results showed that both sides' MAS scores significantly improved following the intervention. According to the WeeFIM data, there was no discernible difference between motor and cognitive functions. Twelve months following the intervention, there was a substantial difference between the GMFCS and MACS scores. Results show that UC-MSC therapy improves motor skills to some extent and has a good impact on spasticity.

In a study by Lv, Z. et al. A total of 25 CP patients were assigned to the treatment, which received an intranasal infusion of Neural stem cells NSCs loaded with nasal patches and rehabilitation therapy, or the control group, which received rehabilitation therapy. Safety and modifications to the Gross Motor Function Measure-88 (GMFM-88), Activities of Daily Living (ADL) scale, Sleep Disturbance Scale for Children (SDSC), and a few modified scales were the main outcomes. The FBN and VBM analyses were the secondary endpoints. Twenty-four months following therapy, patients in the treatment group appeared to have improved GMFM-88 and ADL, Fine Motor Function, Sociability, Life Adaptability, and Expressive Ability in comparison to the control group. Following NSC therapy, the FBN analysis revealed a significant drop in brain network energy compared to baseline, and the VBM analysis revealed a significant increase in gray matter volume in the treatment group. According to findings, children with cerebral palsy may benefit

from the well-tolerated intranasal administration of NSCs.

Our results

Published data

In 2014 Sharma et al. published a study on 40 cases that was carried out to evaluate the benefits of stem cell therapy in cerebral palsy. (27) These cases were administered autologous bone marrow mononuclear cells intrathecally. On follow-up after 6 months, overall 95% of patients showed improvements. The total population was further divided into diplegic, quadriplegic and miscellaneous groups of cerebral palsy. On follow-up, patients showed improvements in various symptoms. (Figure 3-5) In statistical analysis, a significant association was established between the symptomatic improvements and stem cell therapy in diplegic and quadriplegic cerebral palsy. PET-CT done in 6 patients showed metabolic improvements in areas of the brain correlating to clinical improvements.

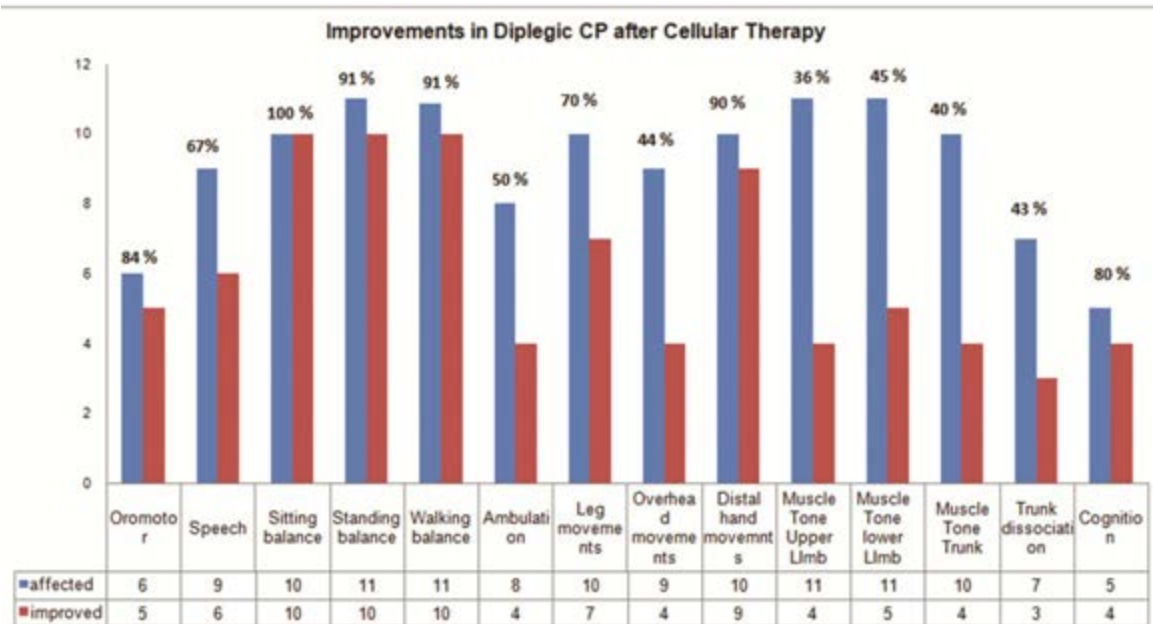


Figure 3: Graph demonstrating improvements in Diplegic CP after stem cell therapy

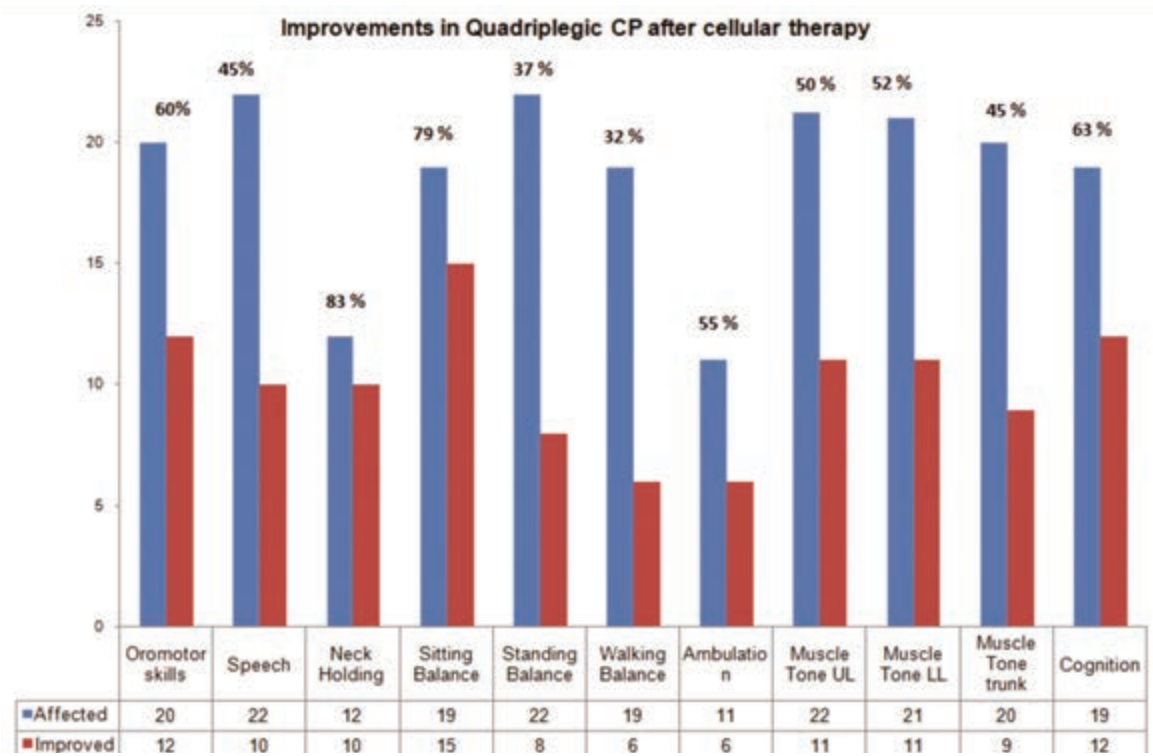


Figure 4: Graph demonstrating improvements in Quadriplegic CP after stem cell therapy

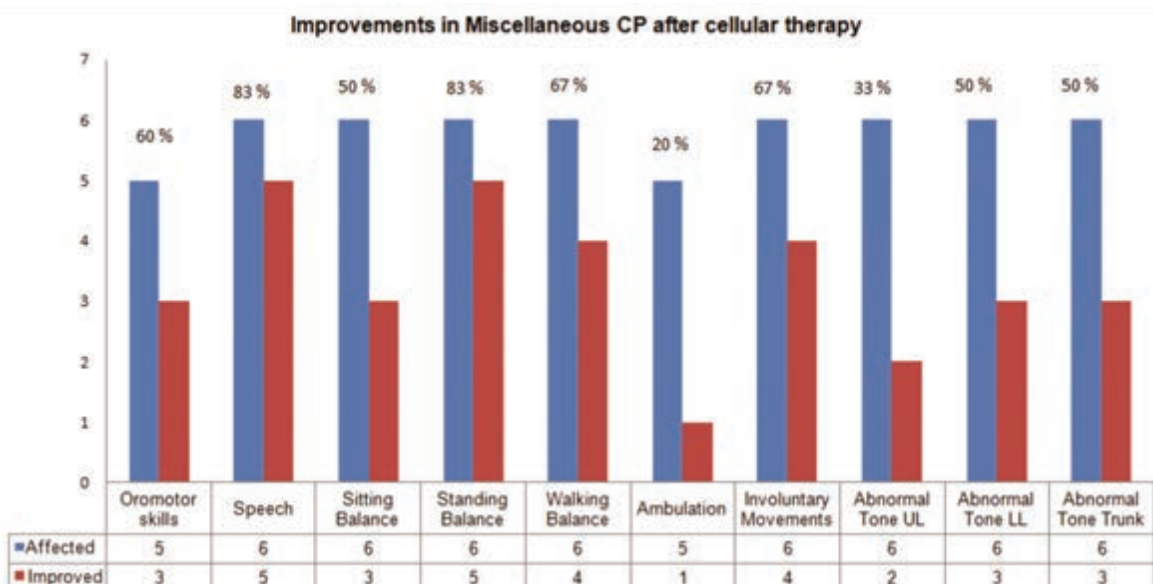


Figure 5: Graph demonstrating improvements in miscellaneous types of CP after stem cell therapy

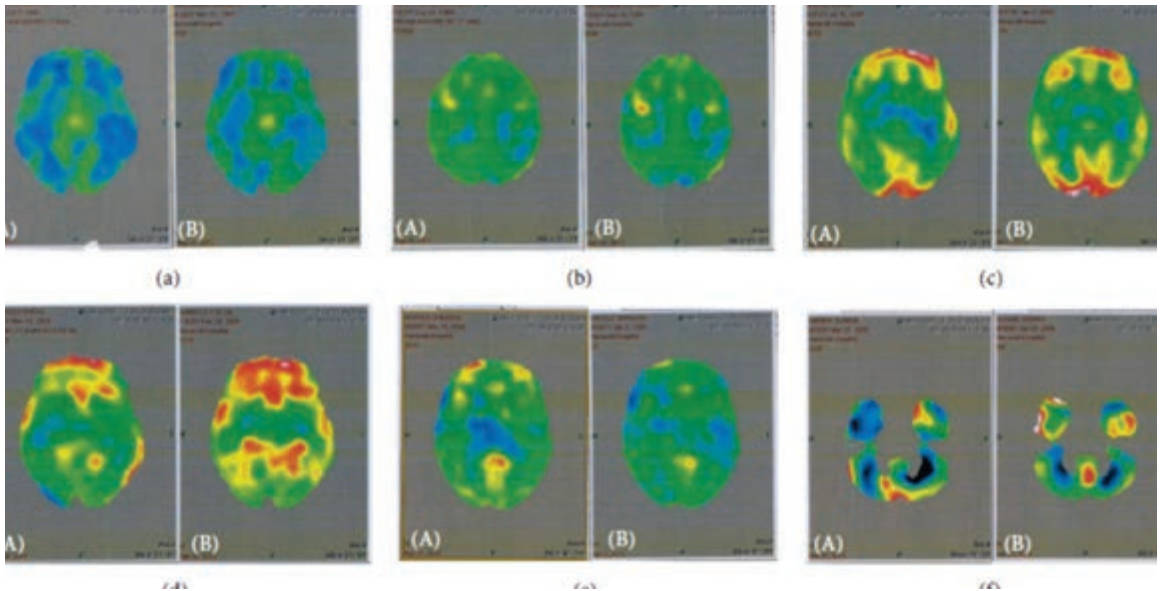


Figure 6: *Improvements in PET-CT scan images of (A) pre- and (B) post-intervention showing increased metabolic activity in various areas. Blue areas indicate hypometabolism, green areas indicate normal metabolism, yellow areas indicate slightly high metabolism and red areas indicate high metabolism.*

Case reports

In the years 2020 and 2019, we published 2 case reports across journals: one on a 4-year-old male child with spastic diplegic CP and the other on a 27-year-old female diagnosed as spastic triplegic cerebral palsy who underwent two intra-thecal transplantations with autologous BMMNC along with neurorehabilitation program. Patients were followed up at regular intervals and outcome measures such as Berg Balance Scale (BBS), Modified Ashworth scale (MAS) and Functional Independence Measure (FIM) were used to quantify outcome. A comparative positron emission tomography-computed tomography (PET CT) brain scan was performed before and after intervention, which revealed improvement in the metabolism of the anterior cingulate lobe, parietal cortex, medial temporal cortex, thalamus, basal ganglia, bilateral paracentral lobule and cerebellum. No major adverse effects were noted after both the interventions.

During follow-up, significant improvements were observed in both cases w.r.t. motor control, coordination, balance, sitting tolerance, memory, sitting posture, gait pattern, trunk, and pelvic alignment. On an objective scale, patients' BBS, FIM scores showed improvements, and qualitative changes were recorded. The results suggest that BMMNC transplantation is a potentially safe and effective therapy for adults with CP. Hence, cellular transplantation in adult CP may help address the complications and improve their quality of life.

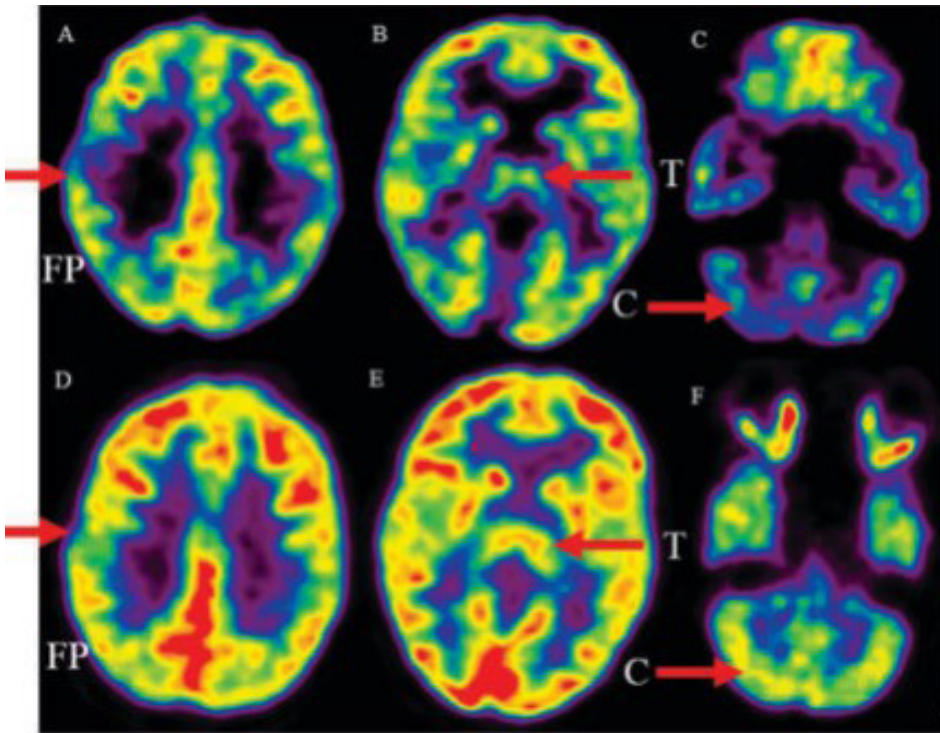


Figure 7: (A-C) Pre-cell transplantation PET CT scan images showing hypometabolism in bilateral fronto-parietal cortex (FP), thalamus (T), and cerebellum (C) regions, respectively. (D-F) Post-cell transplantation PET CT scan images demonstrating improved metabolism in these areas

2. From 2012 to 2018, we have published 8 case reports in various national and international peer-reviewed journals. (57-64) These cases were unique with respect to their age, severity of CP, type of CP, comorbidities such as autism and ID and their outcomes. Outcome measures such as GMFCS, GMFM, and FIM were used to quantify the outcome. A PET-CT scan of the brain was also used to monitor the metabolic changes occurring in the brain after intervention. In all these cases, common symptomatic improvements such as improved trunk strength, limb control, hand functions, walking stability, balance, posture, spasticity and coordination were observed. Transfers such as getting in and out of bed, sitting and getting up from the floor had become easier. Their FIM scores improved, indicating improvement in the ability to perform day-to-day tasks. The case with comorbid ID showed improved IQ scores suggestive of improved cognitive functions. These cases improved neurologically as well as functionally. Their PET CT scans showed improved metabolism of brain areas which were affected before the intervention. The FDG uptake of previously hypometabolic areas had increased after stem cell therapy.

Unpublished data

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

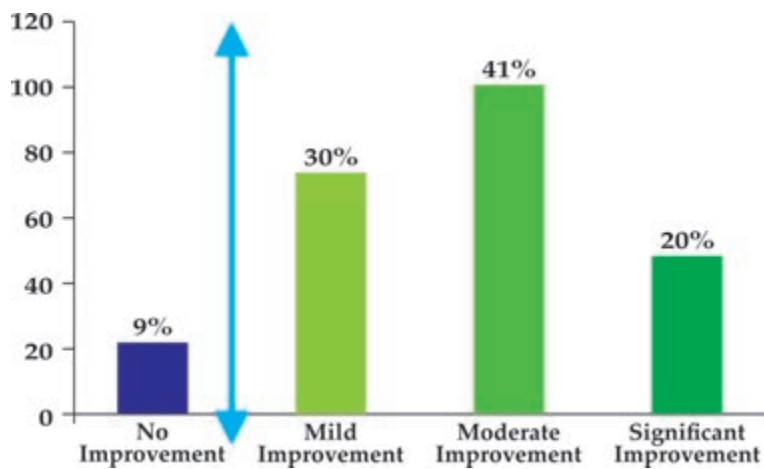


Figure 8: Graph demonstrating improvements after stem cell therapy in cerebral palsy.

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

PET CT Brain in CP

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

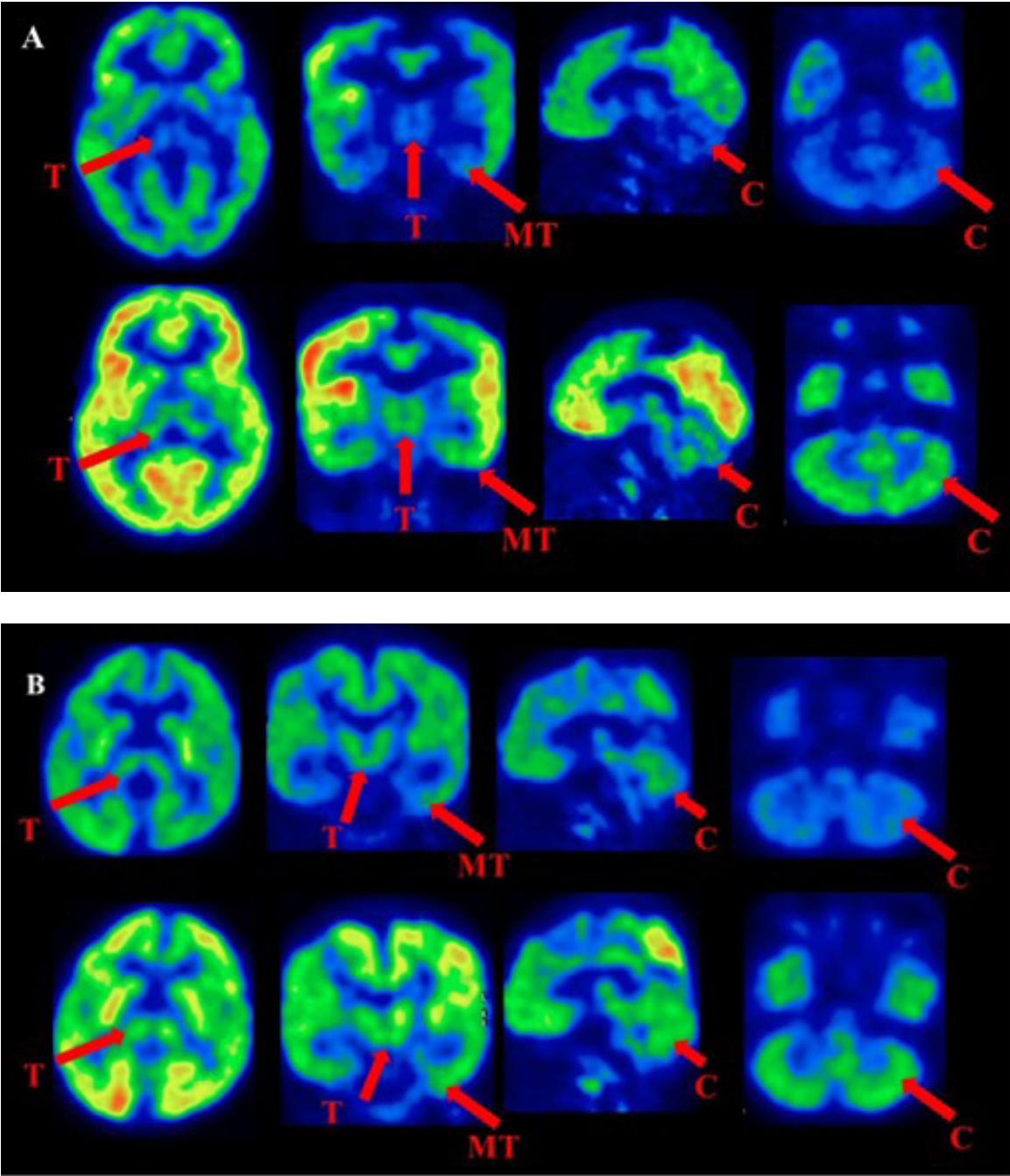


Figure 9 (A & B): Comparative PET CT scans of CP patients performed before and after cell therapy, showing improved metabolism in bilateral hemispheres of the cerebellum (C), thalamus (T), and medial temporal cortex (MT).

Case report

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

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

- Higher mental functions were improved, specifically cognition and orientation. He could understand emotions better and sometimes responded to name-calling as well.
- Sitting and standing posture had improved. He sat and stood more erectly.
- Weightbearing on both upper and lower extremities had improved. He had started doing quadruped and crawling on his own. He could also perform sit-to-stand with support on his own and perform weight shifts on his legs.
- Reflexive development had matured as he had developed equilibrium and anticipatory postural reactions.
- Voluntary control had improved in both upper and lower extremities. Earlier, he could not perform crawling and quadruped. Now, he could crawl and even perform sit-to-stand and kneel standing.
- Trunk-pelvis, pelvis-femoral, and interlimb dissociations had improved from poor to fair.
- Reach-outs in all postures except quadruped had improved. He tried to grasp toys while sitting, kneeling, and standing.
- Balance was better. Earlier, he would not crawl and stand. Now, he started to stand with support. He could stand for 10-15 minutes at a stretch. Also, he started walking with a tripod cycle.

- Transitions were improved. He could perform supine to sit, sit to stand, crawl and walk with support.
- Left hamstring and TA tightness had reduced and right hamstring and TA tightness was not there.
- Speech had improved as vocalization had started.
- Awareness and understanding of relationships were better, as he could now recognize his father.
- Social interaction and play behaviour had improved. He also developed social smile
- He had developed visual tracking too.
- ADLs were age-appropriate;
- The FIM score had improved from 58 to 61.
- GMFM Score had improved from 6.54 to 32.28.
- GMFCS had improved from level V to level IV.

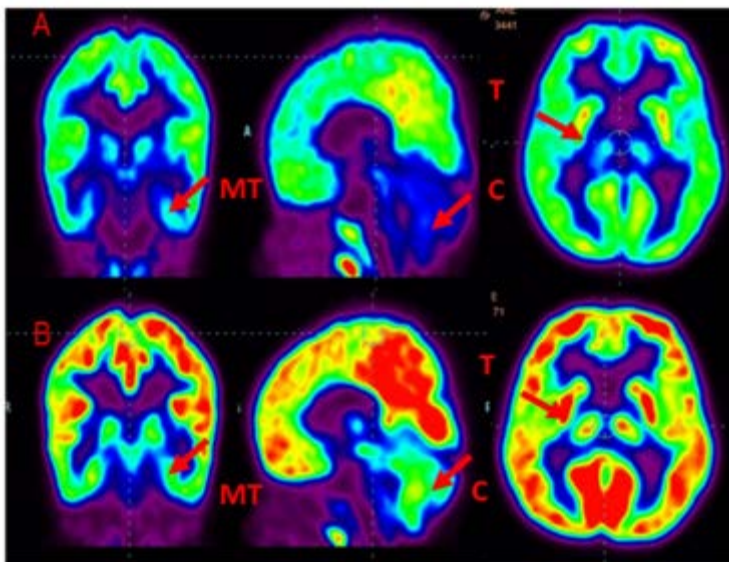


Figure 10: Comparative PET-CT scan showing improved brain metabolism. Before stem cell therapy, a PET-CT scan showed severely reduced metabolism in the bilateral thalamus, medial temporal cortex and cerebellum. Post stem cell therapy, it showed improved metabolism in the bilateral thalamus, medial temporal cortex and cerebellum

Conclusion-

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

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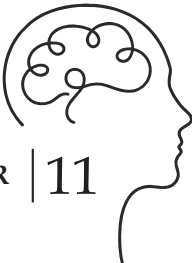
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"What is at stake, in the present moment, is not the future. What is at stake now is the stand you and I take for the future – whether our day-to-day lives could be lived in the context of a reality which we cannot now even imagine. Our work has never 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



CHAPTER | 11

Role of Cell / Stem Cells in Muscular Dystrophy

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

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

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

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

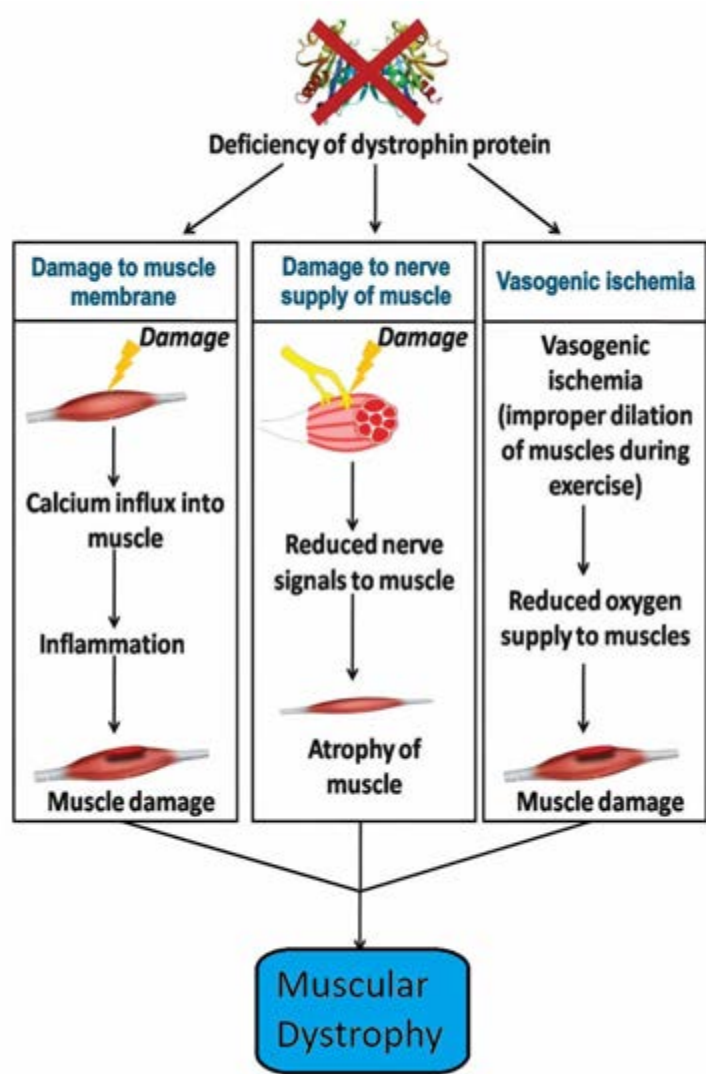


Figure 1: Pathophysiology of the most common muscular dystrophy type, Duchenne muscular dystrophy (DMD).

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

Unmet Medical Needs

The treatments available currently for muscular dystrophy alleviate few symptoms of the disorder but do not act at the cellular level. They fail to carry out the repair and regeneration of the damaged muscles. Also, no treatment repairs the underlying mutation of the genes which cause MD. Gene therapy is being explored and is, though appealing, a treatment option in muscular dystrophy. But due to immune responses and safety issues, it has not been established as a treatment option. No standard therapeutic modality has been successful in halting the progression of the disease or increasing survival. Botulinum toxin type A may be indicated for improvement in joint range and minimizing contractures; however, these effects are temporary. Exon skipping, which makes use of synthetic antisense oligonucleotide sequences, results in the restoration of the reading frame and partial production of functional dystrophin (12). Recently,

Eteplirsen, an antisense oligonucleotide, has received approval but is limited to treatment of patients who have a mutation in the dystrophin gene that is amenable to exon 51 skipping (13). Also, in the study, 6MWT was used as an outcome measure. While the study demonstrated improvement in the 6 MWT distance and a slower rate of loss of ambulation, two of twelve patients still lost ambulation. The drug thus does not provide a cure.

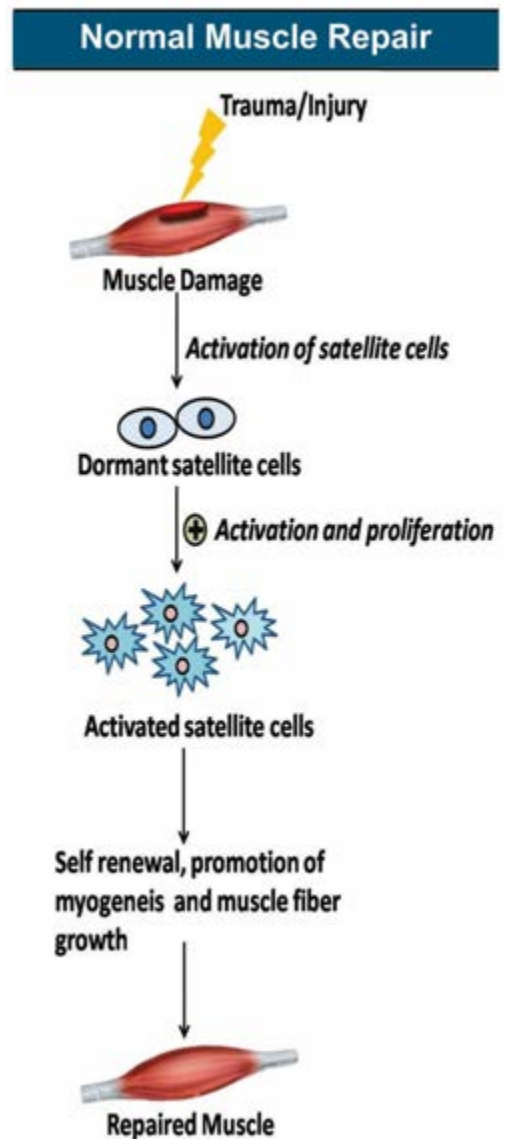


Figure 2: Normal process of muscle repair.

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 (14). Continuous cycles of degeneration and regeneration of muscle fibers exhaust the muscle stem cell pool, leading to muscle being replaced by adipose and fibrotic tissue. Stem cell therapy holds promise as a treatment for muscular dystrophy by providing cells that can both deliver functional muscle proteins and replenish the stem cell pool (15).

The mechanisms by which stem cells may function and reverse the effects of cell death include differentiation, cell fusion, and secretion of cytokines or paracrine effects (16-18). These cells have the capacity to mobilize and exert their reparative effects at the site of injury. They are known to enhance angiogenesis and contribute to neovascularization by producing signaling molecules such as vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF2) (19). Along with an increase in angiogenesis, they also promote tissue remodeling, prevent apoptosis, decrease inflammation, release growth factors and activate the satellite cells (20). In animal studies, these cells have been shown to produce the deficient proteins and make new muscle cells which fuse with the host fibers. Satellite cells,

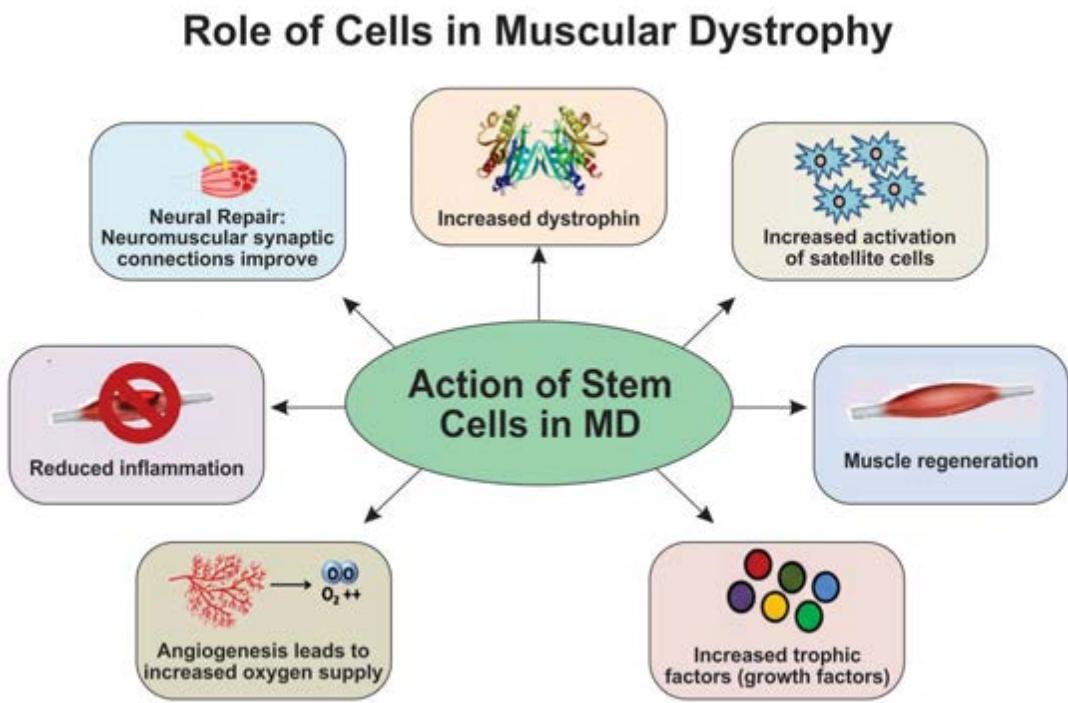


Figure 3: Role of stem cells seen in DMD (in other forms of muscular dystrophy, there is an increase in the deficient protein based on the type).

the adult skeletal muscle progenitor cells, are commonly considered to be the main cell type involved in skeletal muscle regeneration. Further, stem cell-derived exosomes, which are small membrane vesicles and are responsible for intercellular communication, promote muscle regeneration by enhancing myogenesis and angiogenesis.

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

Precautionary measures before stem cell therapy in MD

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

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

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

decrease in FA values is a positive outcome of a treatment measure.

Preclinical studies

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

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

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

Clinical studies

Around 32 studies, which include 16 clinical studies and 16 case reports, are published demonstrating the effect of stem cell therapy in different types of muscular dystrophies. Various types of stem cells are being explored, such as autologous bone marrow mononuclear cells, allogenic umbilical cord stem cells, bone marrow mesenchymal cells, muscle precursor cells, muscle-derived 133+ cells, and myogenic cells.

Torrente et al. [42] tested the safety of autologous transplantation of muscle-derived CD133+ cells in eight boys with Duchenne muscular dystrophy in a 7-month, double-blind phase I clinical trial. No local or systemic side effects were observed in all treated DMD patients. Treated patients had an increased ratio of capillaries per muscle fibre with a switch from slow to fast myosin-positive myofibres. On the other hand, donor myoblasts injected into muscles of 12 patients with DMD

did not show any significant difference in muscle strength between arms injected with myoblasts and sham-injected arms [43]. 4, however, had low levels of donor dystrophin. In another study, 3 DMD patients received injections of myogenic cells [44]. Dystrophin-positive myofibres were observed 4 weeks later in all the patients. In another single case report, donor-derived dystrophin was found in the muscles of a patient who was injected with allogenic muscle precursor cells [45].

Double transplantations of autologous bone marrow mesenchymal stem cells and umbilical cord mesenchymal stem cells were tested for safety and efficacy in progressive muscular dystrophy [46]. Treatment efficacy was observed in 68 of 82 patients (82.9% efficacy) and was found to be safe. In another single-blind study, 11 DMD patients received umbilical cord mesenchymal stem cells [47]. Stabilisation or improvement in muscle strength was found in all the treated patients, while the control group showed a decline in muscle strength at the 1-year follow-up. An 11-year-old DMD boy underwent umbilical cord blood stem cell transplantation [48]. Serum creatine kinase levels declined from 6000 U/L to 2200 U/L post intervention together with improvements in walking, turning the body over, and standing up, 6 weeks post intervention. Umbilical cord-derived hematopoietic stem cell transplantation, on the other hand, was not found to be efficacious in DMD [49].

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

Phase 1 clinical trial by Lee et al. explored the safety and tolerability of EN001 developed by ENCell, allogeneic early-passage Wharton's jelly-derived mesenchymal stem cells that originate at the umbilical cord as a potential treatment option for patients with DMD. Two equal-sized subgroups of six paediatric DMD participants were created: one for low-dose EN001 (5.0×10^5 cells/kg) and another for high-dose EN001 (2.5×10^6 cells/kg). To evaluate the safety of EN001, all subjects were observed for 12 weeks following administration. DLT, or dose-limiting toxicity, was assessed two weeks after delivery. Serum creatine kinase levels were measured to assess exploratory efficacy, and at week 12, functional assessments such as spirometry, myometry, the North Star Ambulatory Assessment, and the 6-minute walk test were carried out and compared with the baseline values. The findings showed that EN001 did not result in any significant side effects and was safe and well tolerated by DMD patients. Larger-scale future research using a randomized controlled trial design and repeated doses could validate EN001's effectiveness.

Side Effects

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

Our results

In 2013 Sharma et al. published a study in the “Cell Transplantation” journal carried out on 150 patients diagnosed with muscular dystrophy. On a mean follow-up period of 12 months± 1 month, 86.67% of cases showed symptomatic and functional improvements, with 6 patients showing muscle regeneration and a decrease in fatty infiltration on Musculoskeletal Magnetic Resonance Imaging (MSK MRI) and 9 showing improved muscle electrical activity on Electromyography (EMG). 53% cases showed increase in trunk muscle strength, 48% an increase in upper limb strength, 59 % an increase in lower limb strength and about 10% showed an improved gait pattern.

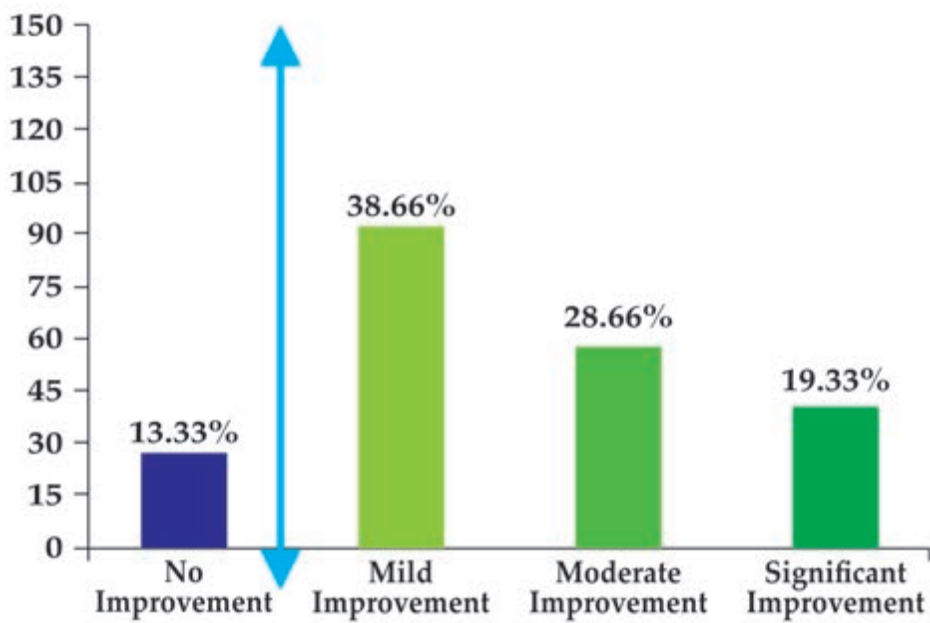


Figure 4: Graph showing improvements in muscular dystrophy patients after stem cell therapy. Y-axis = number of patients (n=150).

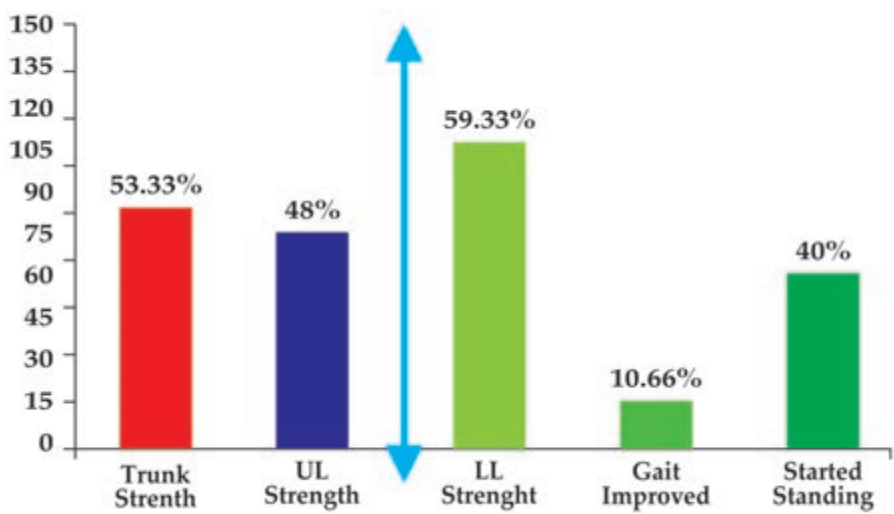


Figure 5: Graph showing symptomatic improvements in muscular dystrophy patients after stem cell therapy. The number of patients showing improvements in trunk strength, upper limb (UL) strength, lower limb (LL) strength, gait pattern, and standing function are shown. y-axis = number of patients (n = 150).

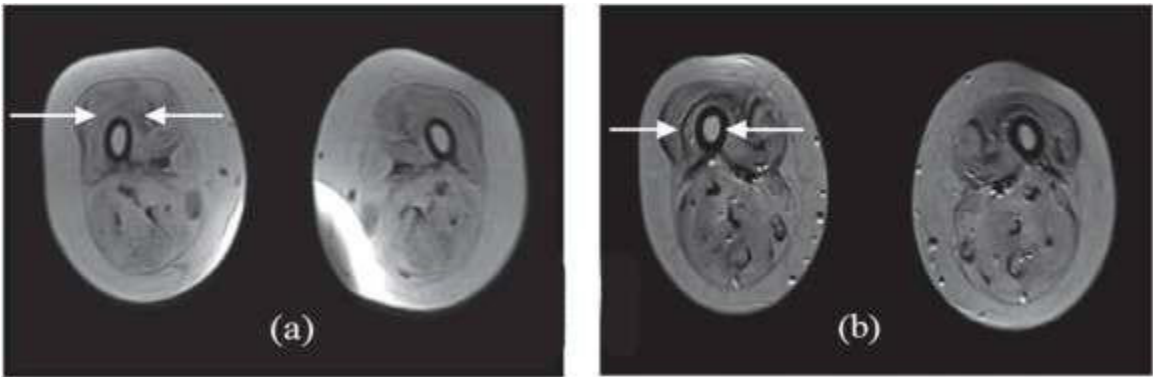


Figure 6: Figures a & b show the musculoskeletal MRI images before and after stem cell therapy, respectively. Figure b shows regeneration of muscle in vastus medialis and vastus lateralis.

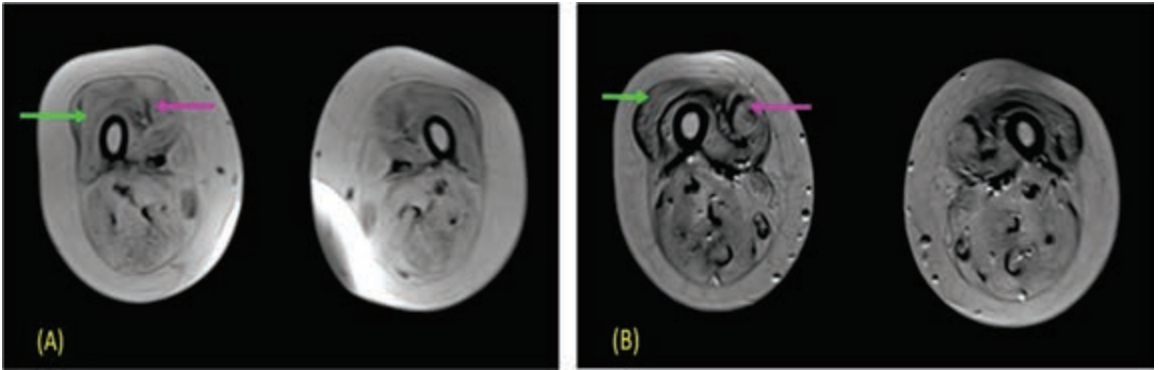


Figure 7: A & B show MRI musculoskeletal images before and after stem cell therapy. Regeneration of muscles is seen in Fig B.

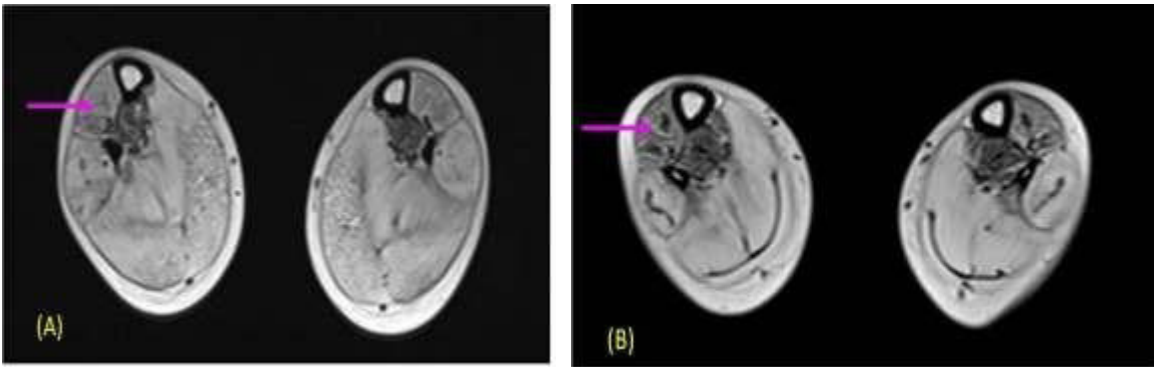


Figure 8: The figures A & B show MRI musculoskeletal images before and after stem cell therapy, respectively. Regeneration of muscles is seen in Figure B.

2. In 2015 Sharma et al. published an individual study comprising LGMD patients. This study included 65 patients diagnosed as LGMD that underwent autologous bone marrow mononuclear cells intrathecally and intramuscularly. While 59 cases completed the study, 6 were lost to follow up. These cases were divided into 3 groups depending on the number of transplantations administered. Group 1 included the cases who underwent one stem cell therapy. (N=31). Group 2 included cases who underwent two transplantations (N=24). Group 3 included cases who underwent 3 transplantations (N=4). In group 1, 97% of the patients showed improved function. The total percentage of strength improvement ranged from 84% to 100% in all the muscles. In group 2, 96% of the patients showed an improved function. The total percentage of strength improvement ranged from 90% to 100% in all the muscles. In group 3, of the four patients, one patient deteriorated in his FIM score, whereas two patients improved, and one maintained functional status. Most of the patients had maintained muscle strength.

Unpublished data

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

Duchenne Muscular Dystrophy

A total of 139 boys detected with DMD underwent autologous bone marrow mononuclear cell intrathecal and intramuscular transplantation. The 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 and 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 them showed distal rod (45-55) exon deletions, 7 showed proximal rod (3-21) exon deletions, 2 showed both proximal and distal rods, 4 showed deletion of exons in other regions and 13 patients showed no deletions but mutations.

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

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

Table 1 : Matched pair Wilcoxon Sign Rank test analysis of outcome measures pre- and post-therapy

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 2 : Matched pair Wilcoxon sign rank test analysis of modified manual muscle testing scale.

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

	Comparison Group	Intervention Group	Test Statistics
Total Number of Patients	35	42	-
Percentage of patients currently non-ambulatory	65%	23%	-

Predicted time till loss of Ambulation	142 months	204 months	0.004

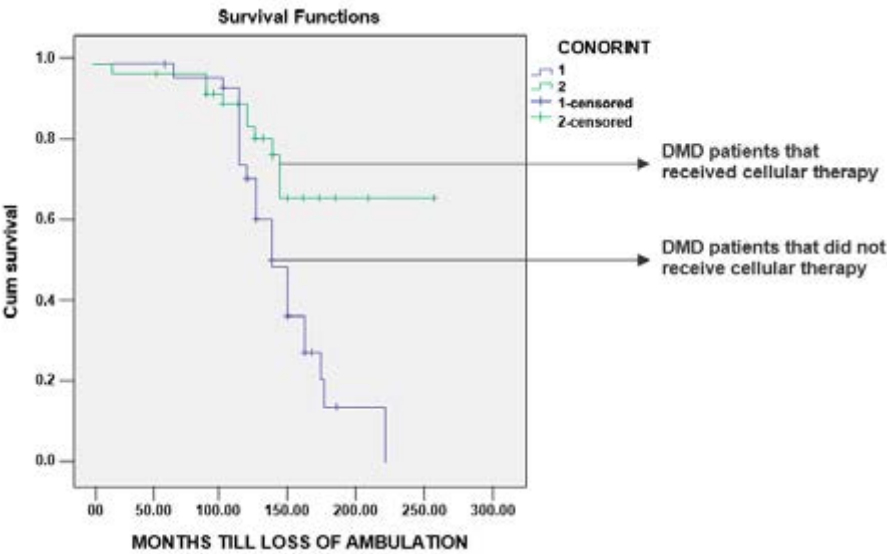


Figure 9: Kaplan-Meier curve analysis of time till loss of ambulation in patients with and without stem cell therapy

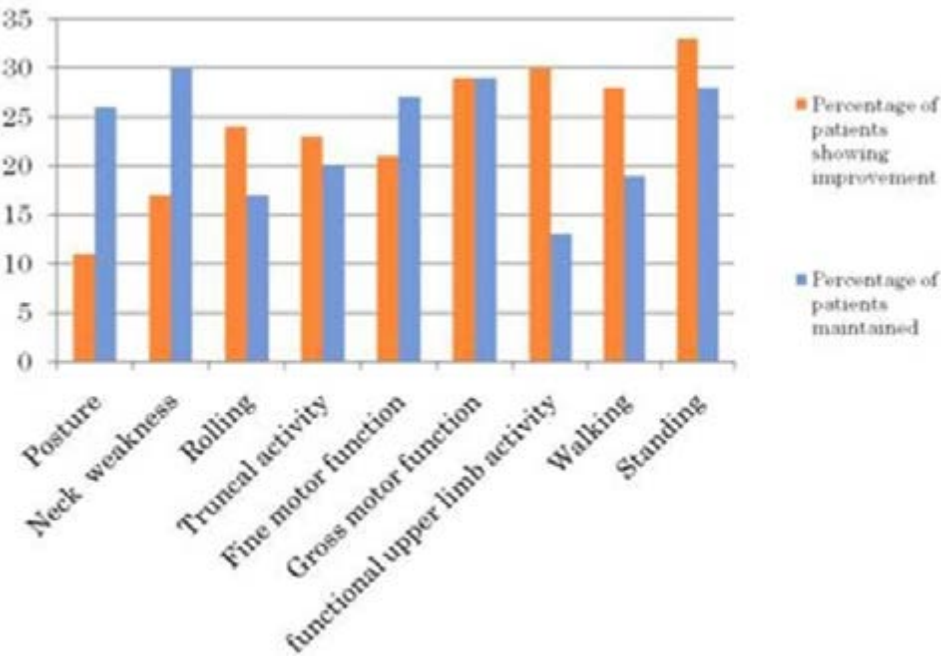


Figure 10: Percentage analysis of symptomatic improvements in the patients with stem cell therapy.

Table 4: Percentage analysis of modified manual muscle testing scale

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	Flexors	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 Extensor	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

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

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

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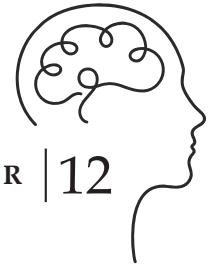
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"Neurosurgeons would be happy if they could make the spinal cord regenerate thus helping thousands of paraplegics all over the world. Sustained efforts in this direction are the Immediate need of the future."



Dr. B. Ramamurthi

Founding father of Neurosurgery in India



CHAPTER | 12

Role of Cell / Stem Cells In Spinal Cord Injury

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

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A spinal cord injury (SCI) is damage to the spinal cord caused by trauma such as road traffic accidents (RTAs), falls from heights or non-traumatic events such as infection, loss of blood supply, compression by cancer or 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). Severe cervical spinal damage results in quadriplegia, whereas an injury to the thoracic or lumbar spine leads to paraplegia.

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

The current treatment for SCI includes surgical interventions, medicines and re-

habilitation. Their main goal is to stabilize the spine and prevent any secondary complications.

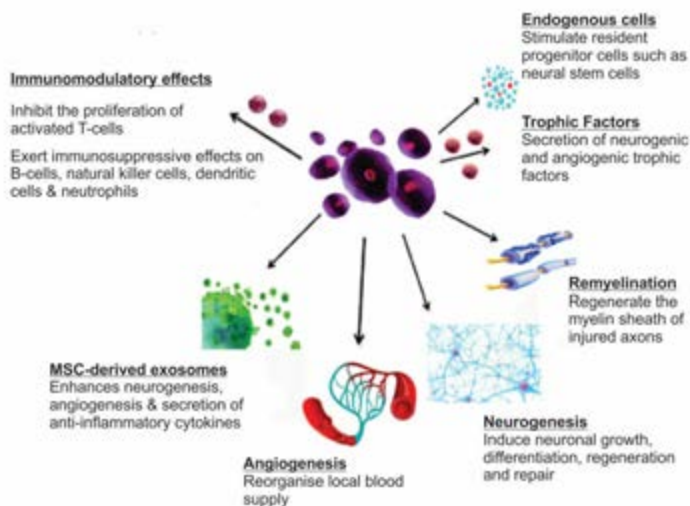
Unmet Medical Needs

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

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

Stem cell therapy in spinal cord injury

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



ROLE OF STEM CELLS IN SPINAL CORD INJURY

Extensive research has been carried out in the past few decades in order to develop stem cell transplantation as a therapeutic intervention for SCI. It mainly focuses on replacing the lost or damaged

cells and promoting axonal growth and remyelination of axons. The cells migrate to the site of injury and initiate the repair process. They release trophic factors to stop neuronal degeneration and stimulate angiogenesis. These factors also activate the quiescent cells and recruit them to the injured site. Experimental models have demonstrated the formation of functional neuronal circuits promoting functional recovery. (4-6)

Pre-clinical studies

Various animal studies have been conducted in the past to establish the 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 behaviour, and the differentiated fates of experimental cells have been studied extensively in vitro. Moreover, the survival, proliferation, differentiation, and effects on promoting functional recovery of transplanted cells have also been examined in different animal SCI models. (46-54) These pre-clinical studies have helped translate the use of stem cells in humans, initiating an array of human clinical studies.

Clinical Studies

So far, 72 studies, of which 60 are clinical studies and 12 are case reports, have been conducted demonstrating a beneficial effect of stem cell therapy in more than 1000 patients. (55-108) Stem cells from different lineages and sub-types have been used in these studies, which include autologous bone marrow-derived mononuclear stem cells, bone marrow-derived mesenchymal stem cells, bone marrow-derived stromal cells, adipose-derived mesenchymal stem cells, hematopoietic progenitor cells, peripheral hematopoietic cells, activated Schwann cells, olfactory ensheathing cells and umbilical cord mesenchymal stem cells. Improvements have been observed clinically in the form of improved functions and objectively in the form of improved ASIA scores and MRI changes. Post-therapeutic adverse events were observed in a few patients, such as fever, headache, nausea and vomiting, tingling sensations, spasms, and neuropathic sensory symptoms including burning and pain sensations.

A study by McKenna et al. evaluated the safety of oligodendrocyte progenitor cells (LCTOPC1) derived from human pluripotent stem cells administered between 7 and 14 days postinjury to 5 patients with T3 to T11 neurologically complete spinal cord injury (SCI). The frequency and severity of adverse events associated with the LCTOPC1 injection served as the primary goal for safety. Neurological func-

tion as determined by sensory and lower-extremity motor scores was the secondary goal. The findings showed that, with a 98% follow-up of participants over the first ten years of the clinical trial, no adverse events associated with LCTOPC1 had been observed. No syrinx formation, increasing tumors, more spinal cord injury, or neurological impairment was observed. According to MRI findings, T2 signal alterations in 80% of patients were consistent with the development of a tissue matrix at the site of injury. Although future adverse events cannot be completely ruled out, the experiment shows that patients can tolerate this cell well for up to ten years.

Our Results

Published data:

1. Sharma et al. in 2020 published an open-label study in “International Journal of Health Sciences” including 180 sub-acute and chronic SCI patients who received intrathecal autologous BMMNCs along with neurorehabilitation were taken. Patients were followed up after an average of 9 ± 7 months. They were assessed for functional symptomatic changes and the outcome measures used were the functional independence measure (FIM) and the walking index for SCI (WISCI). The non-parametric Wilcoxon signed-rank test was used to test the statistical significance of the difference in the scales WISCI, FIM, and before and after transplantation. Patients showed symptomatic improvement in sitting/standing balance, bed mobility, trunk stability, upper limb function, mobility, sensation, bowel/bladder functions, and activities of daily living with no serious adverse events. Scores on FIM and WISCI showed statistically significant improvement. It was found that 69.44% of patients showed improvement in FIM scores and 37.22% of patients showed improvement in WISCI scores. In subgroup analysis, it was found that early intervention and more than one dose of BMMNCs demonstrate a better functional outcome. Younger patients demonstrated better improvements in functional independence. Both cervical and dorsolumbar levels of injury show significant improvements in motor and sensory deficits.

Table 1: Percentage improvement in various symptoms of spinal cord injury

Symptoms	Affected	Improved	Percentage
Sitting	163	118	72.39
Bed Mobility	172	113	65.69
Standing	177	113	63.84
Postural Hypotension	41	25	60.97
UL Function	68	38	55.88

Mobility	180	95	52.77
Activities of daily Livings	149	75	50.33
Walking	179	90	50.27
Trunk Stability	177	78	44.06
Trunk Muscle Strength	177	70	39.54
Sensation	177	64	36.15
Bladder	176	46	26.13
Bowel	173	41	23.69
Spasticity	168	22	13.09

2. In 2019, Sharma et al. published a detailed analysis of chronic thoracolumbar SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells (BMMNCs) followed by neurorehabilitation. The study sample included 110 thoracolumbar SCI patients. The outcome was recorded at a mean follow-up of 2 years $\pm$ 1 month. The outcome measures were the Functional Independence Measure (FIM) score, the American Spinal Injury Association scale (ASIA) and detailed neurological assessment. Data was statistically analyzed using McNemar’s test to establish significance between the change in symptoms and the intervention.

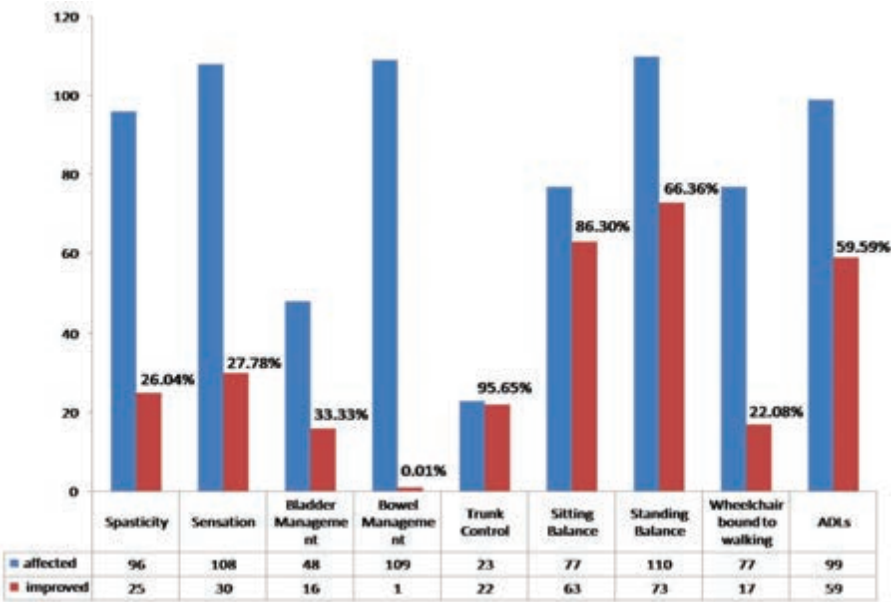


Figure 1: Symptomatic improvements in patients with spinal cord injury after stem cell therapy. The x-axis denotes symptoms presented in the patient population, and the y-axis denotes the number of patients. (ADLs – activities of daily living).

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

Table 2: Statistical significance for each symptomatic/functional change using McNemar’s test

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

Table 3: Objective improvements evident on electromyography (A) and functional magnetic resonance imaging (B) after stem cell therapy in selected patients

	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 - Ankle	7.2	14.8
	L Tibial (knee) - Gastrocnemius - Ankle	10.2	11.7
Patient 2	L Comm Peroneal - EDB - Ankle	0.8	3.0
	R Comm Peroneal - Tib Ant - Fib Head	1.6	3.4
	L Comm Peroneal - Tib Ant - Fib Head	1.8	4.3
	R Tibial (knee)-AH-Ankle	7.0	8.0
	L Tibial (knee) - AH-Ankle	7.9	8.3
	R Tibial (knee)-Gastrocnemius-Ankle	6.2	18.7
	L Tibial (knee)-Gastrocnemius-Ankle	2.5	17.2

3. In 2013, Sharma et al. published a detailed analysis of chronic cervical SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells followed by neurorehabilitation. (99) This study includes 56 patients with chronic cervical SCI. The outcome was recorded at a mean follow-up of 2 years $\pm$ 1 month. The outcome measures were Functional Independence Measure (FIM) score, American Spinal Injury Association scale (ASIA) and detailed neurological assessment. Data was statistically analyzed using McNemar’s test to establish significance between the change in symptoms and the intervention. 37 out of 50 (74%) showed improvements. A statistical analysis using McNemar’s test established a significant association of these symptoms with the intervention. No major side effects were noted in the duration of 2 years in both the studies. A better outcome was observed in thoracolumbar injury as compared to cervical injury, suggesting that the level of SCI greatly influences the recovery of the patient. Both studies demonstrated statistically significant clinical and functional outcomes.

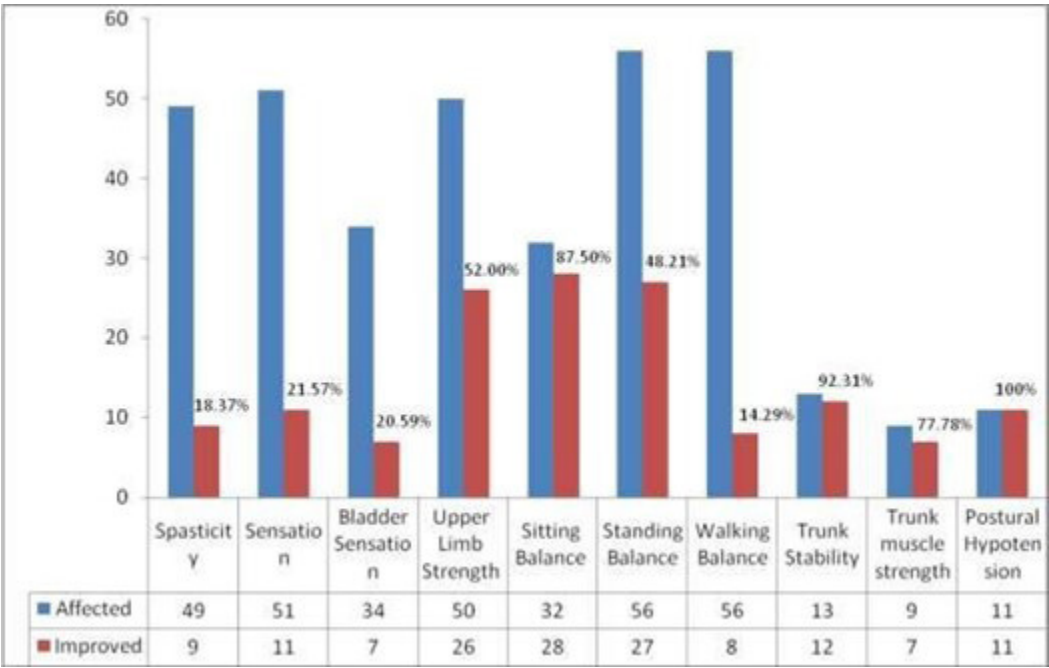


Figure 2: Graph demonstrating symptomatic improvements in chronic cervical SCI patients after cell therapy

Table 4: McNemar’s test: Table demonstrating the statistical analysis for each symptomatic improvement in cervical SCI using McNemar’s test.

Symptom	No.of patients affected	No.of patients improved	Mc Nemar's test value	P value
Spasticity	49	9	7.1111	*0.00766
Sensation	51	11	9.0909	*0.002257
Bladder Sensation	34	7	5.1428	*0.02334
Upper Limb Strength	50	26	24.03846	*<0.000001
Standing Balance	32	28	26.03571	*<0.000001
Sitting Balance	56	27	25.03704	*0.01333
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

Table 5: Percentage analysis of improvements: Table demonstrating a detailed analysis of various factors and the improvements.

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

Table 6: Comparison between the outcomes of cell transplantation in cervical SCI and thoracolumbar SCI.

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%
Standing Balance	87.50%	81.81%
Sitting Balance	48.21%	66.36%
Trunk Stability	92.31%	95.65%
Postural Hypotension	100.00%	100.00%

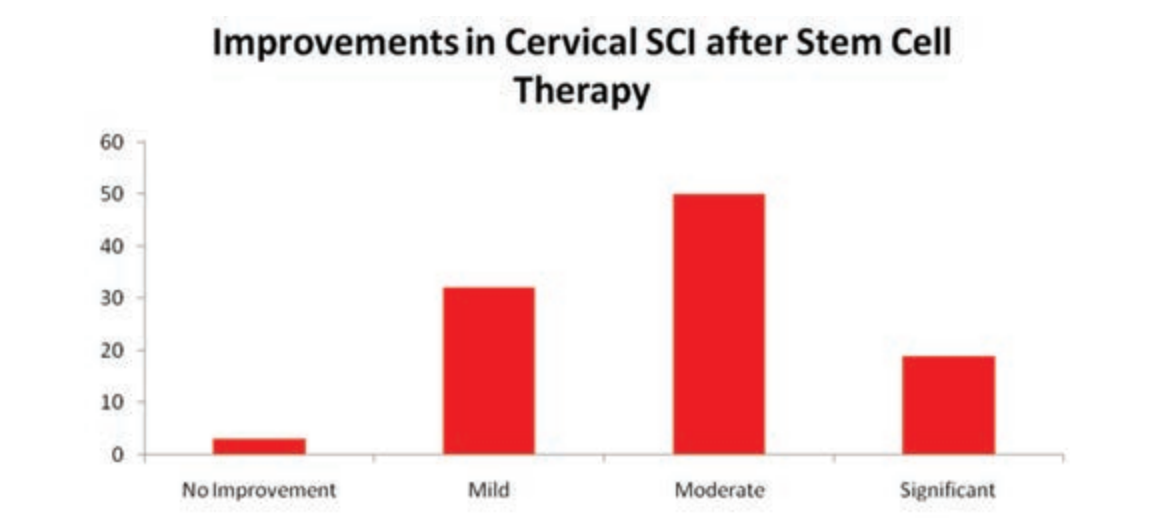


Figure 3: Improvements seen in cervical SCI after intrathecal administration of autologous BMMNCs.

Conclusion

It has long been considered that spinal cord injury is an irreversible condition causing permanent debilitation. However, recent advancements in the field of regenerative medicine have disproved that assumption. While stem cell therapy is not a cure for SCI, the neuro-regenerative and reparative effect of stem cells enhances the outcome of the currently available rehabilitative and medical treatments. The safety and efficacy of stem cell therapy has been established in the clinical studies done so far. Factors that can influence the outcome of stem cell therapy in SCI are (A) level of injury (patients with thoraco-lumbar level of injury have a

better prognosis than those with cervical level of injury), (B) incomplete SCI have a better prognosis than complete injury, and (C) the earlier the treatment is initiated, the better the prognosis (D). Rehabilitation is essential for maximizing functional recovery in patients. Thus, the benefits of stem cell therapy are clear to see in the clinical studies conducted so far and the results seen have been very encouraging and offer much hope for the future.

Future Directions

In SCI, rapid loss of the oligodendrocytes is recorded. The quiescent endogenous ependymal cells which are activated after the injury are unable to differentiate into the required cells of oligodendrocyte lineage, failing to limit the damage. Also, the microenvironment of the injured spinal cord prevents neuronal differentiation of the transplanted cells due to the pro-gliogenic signals. Hence, future research should focus on manipulating the cells before transplantation or infusing neurotrophic growth factors in order to stimulate the endogenous cells and modulate them towards producing more oligodendrocytes. (114) The future of regenerative medicine in SCI lies in combining the use of stem cells with nanodrug delivery systems. (115) Recently, the stem cells are being co-transplanted with nanospheres, improving the cell survival and neurological functions in the animal models. However, their long-term safety needs to be assessed. Cells of varied origin, such as dental pulp, adipose tissue and other induced pluripotent cells, are being studied extensively to test their potency, safety, feasibility and efficacy in SCI. (116-118) Nanofiber scaffold formulations have been used successfully for targeted cell delivery into the body organs. (119) Neuralstem has developed NSI-566 neural stem cells which it seeks to deliver directly into the spinal cord gray matter in SCI patients. These cells are expected to integrate with the patient's neural tissue and potentially form new neural circuits to connect and bridge axons above the site of injury with neuron segments below. (120)

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

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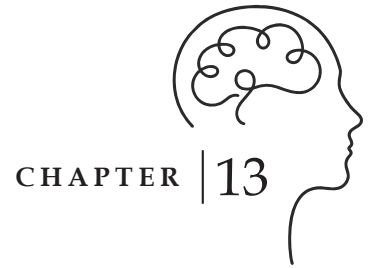
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Our natural power is sapped by the parasites of the centuries: fear, superstition, a view of reality that reduces life's wonders to creaking machinery. If we starve these parasitic beliefs they will die. But we rationalize our fatigue, our inertia;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

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

– Marilyn Ferguson



Role of Cell / Stem Cell Transplantation In Stroke

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

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

Acute medical management is based on the type of stroke where, ischemic stroke is treated by thrombolysis or anticoagulation medications. For hemorrhagic stroke, management is focused on the underlying cause of the bleed, which is reduce

blood pressure or treatment of aneurysm etc. Medical and surgical strategies aim at prevention of recurrence of stroke. Stroke rehabilitation remains the cornerstone for patients with stroke and should be initiated as early as possible. Most return of function is seen in the first few months, and then improvement falls off with the “window” considered officially by U.S. state rehabilitation units and others to be closed after six months, with little chance of further improvement.

Unmet medical needs

With the current treatment approaches, medical, surgical or rehabilitative, the pathophysiological processes and the resultant damage occurring at the micro-cellular 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 an acute stroke

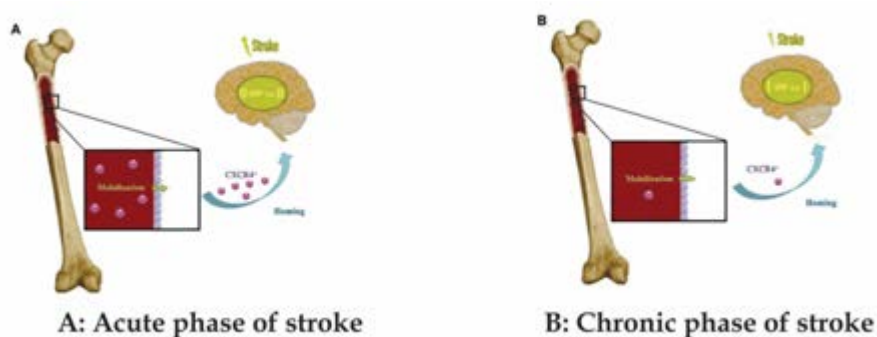


Figure 1: Natural process of mobilization of endogenous bone marrow stem cells after stroke.

starts decreasing as the chronic stage approaches. Therefore, as time passes by, the rate of recovery also reduces in the chronic stage. This forms the rationale that if a greater number of stem cells are supplied to the injured area in the chronic stage, it may hasten and increase the chances of recovery.

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

Stem cell therapy in Stroke

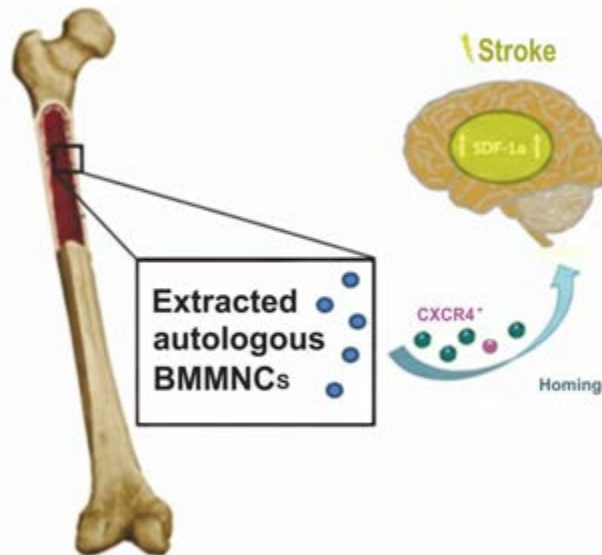


Figure 2: *The transplanted autologous BMMNCs provide a large number of stem cells in the chronic phase, which mimic the natural pathway (depicted in figure 1) to repair the damaged brain areas.*

Pre-clinical studies:

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

A systematic review and meta-analysis of the preclinical studies using MSCs for stroke was conducted by Quynh et al. (2016). They identified 46 relevant studies that analyzed the effect of MSC transplantation for Ischemic stroke. Out of these, 44 studies showed a significant improvement in the neurological severity score and other motor function tests. There was also a significant reduction in infarct

size of the treated rats. Greater improvements were noted in rats treated with intracerebral routes followed by intra-arterial and intra-venous routes. The study did not find any significant change in the outcomes when the cells were delivered within 24 hours of the stroke or after 24 hours of the stroke. The mechanism of action in the acute stage is presumed to be the reduction of ongoing injury to neural tissue, whereas in the subacute stage, stem cells promote neural repair (15).

Clinical studies:

A total of 59 studies that included 50 clinical studies and 9 case reports were published demonstrating the benefits of stem cell therapy in stroke. Different cell types studied in these articles were autologous bone marrow mononuclear cells (BMMNCs), autologous and allogenic mesenchymal cells (MSCs), Fetal porcine cells, neural stem cells (NSCs), immature neurones and hematopoietic cells, and olfactory ensheathing cells (OECs). Different routes of administration studied in the articles were Intracranial (IC), Intravenous (IV), Intraarterial (IA), Intrathecal (IT), Subarachnoid.

All these studies unanimously suggested that the transplantation of various types of stem cells was safe using various routes of administration. Although some adverse events were noted in these studies, none of them were related to cell transplantation. Many studies showed that there was a statistically significant difference in the pre- and post-measures of the Barthel Index, Modified Rankin Scale, National Institute of Health Stroke Score, Functional Independence Measure and Frugal Myer Scale after stem cell therapy. Along with the functional improvements, there were improvements in the metabolism as measured on PET-CT scan, and some studies showed a reduction in the lesion size on MRI. (16-43)

Our results

Published data

In 2014, Sharma et al. published a study to demonstrate the effect of intrathecal administration of autologous bone marrow mononuclear cells (BMMNCs) on the recovery process of patients with chronic stroke. (43) 24 patients diagnosed with chronic stroke were administered cell therapy, followed by multidisciplinary neurorehabilitation. They were assessed on functional independence measure (FIM) objectively, along with assessment of standing and walking balance, ambulation, and hand functions. Out of 24 patients, 12 improved in ambulation, 10 in hand functions, 6 in standing balance, and 9 in walking balance. Patients with age less than 60 years showed a higher percentage of improvement in the areas of ambulation, hand functions, and sitting and standing balance, as compared to the patients with age more than 60 years. They also showed improvement in the FIM scores. Time since the stroke episode also seemed to have an effect on the recovery of

patients. The percentage of improvement was higher in patients whose episode of stroke was less than 2 years old, as compared to those patients whose stroke was older than 2 years. Patients with ischemic type of stroke had better outcomes in all the mentioned areas, as compared to those with hemorrhagic stroke. Also, patients with right brain involvement showed a higher percentage of improvement in the areas 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.

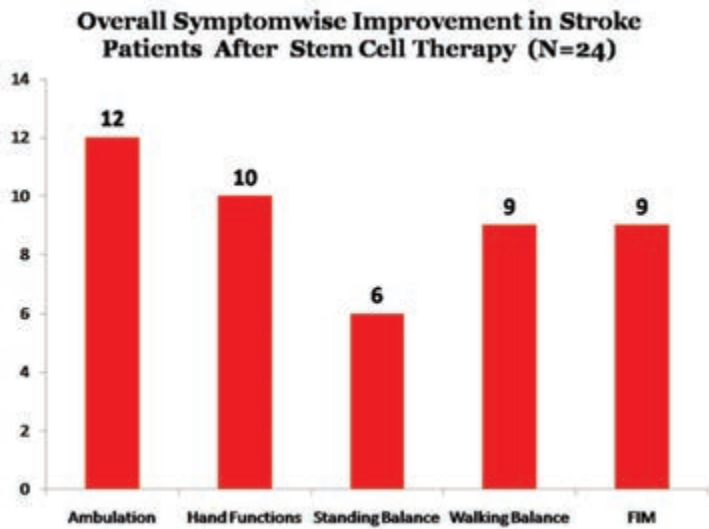


Figure 3: Symptom-wise improvements seen in patients of stroke after intrathecal administration of autologous BMMNCs.

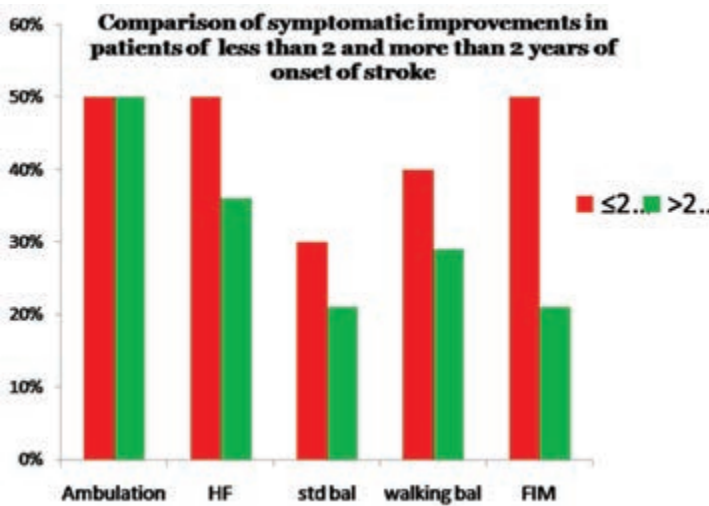


Figure 4: Comparison of symptomatic improvements in patients according to duration since onset of stroke.

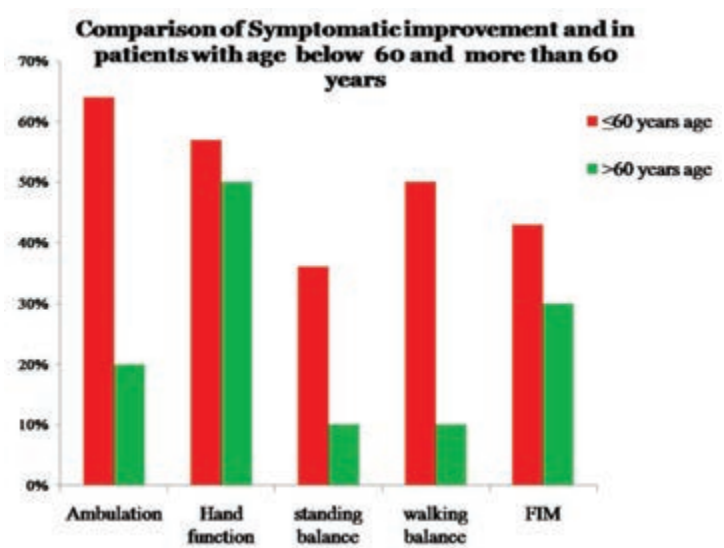


Figure 5: Age-wise comparison of symptomatic improvements in patients of stroke.

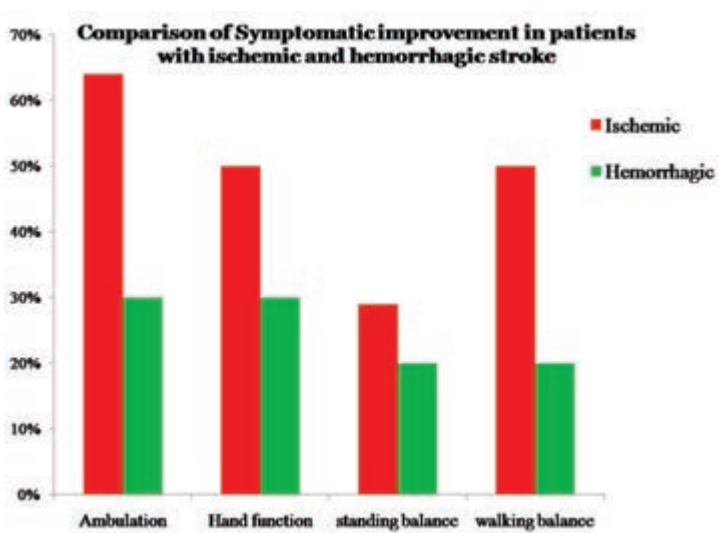


Figure 6: Comparison of symptomatic improvements according to the type of stroke.

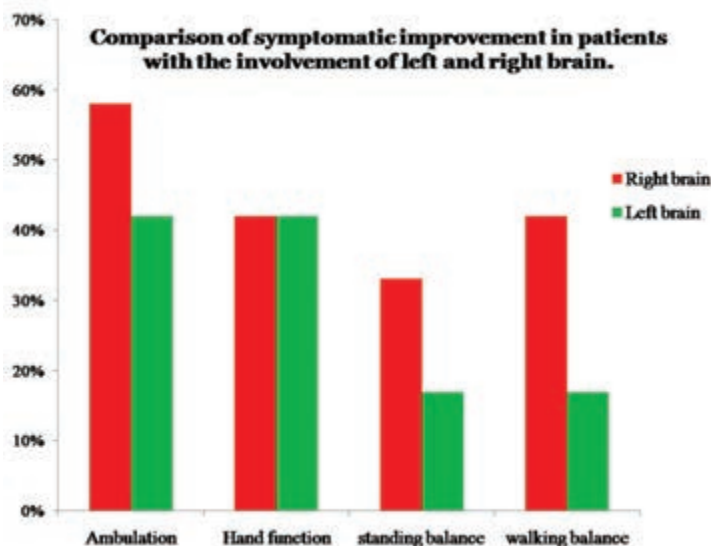


Figure 7: Comparison of symptomatic improvements according to side of the brain involved.

Case Report

1. In 2019 Sharma et al. published a case report in "International Journal of Science and Healthcare Research". The case report included a 50 year old male suffering from ischemic stroke due to middle cerebral artery infarct for the last 4 years who underwent intrathecal administration of autologous bone marrow mononuclear cells followed by neurorehabilitation. He presented with right hemiparesis, dysarthria and memory deficits. He underwent cellular therapy twice at an interval of 6 months. FIM, BBS, Beck Depression Inventory, reach test Forward/Backward/Right/Left (F/B/R/L), and MRS were the outcome measures that were used. Brain glucose metabolism was measured using a high-resolution PET/CT scan. After intervention, the voluntary control, memory, ambulation and speech improved. The recovery was also marked by an improvement on the Berg Balance scale (50 to 52), the Beck Depression Inventory scale (23 to 9) and the Reach score (4/3/2/2 to 10/4/5/6). FIM and MRS were also maintained. A comparative Positron Emission Tomography-Computer Tomography (PET CT) scan of the brain 6 months after cellular therapy showed improvements in the bilateral frontal cortex, parietal cortex, thalamus, cerebellum, medial temporal cortex, right basal ganglia, right temporal cortex, and cingulate cortex, which correlated with clinical improvements. No new complaints or neurological worsening were reported in the patient. Cellular therapy along with neurorehabilita-

tion was safe and beneficial. Cellular therapy assisted the impaired areas of the brain in recovery, as demonstrated on PET CT scan even at a chronic stage.

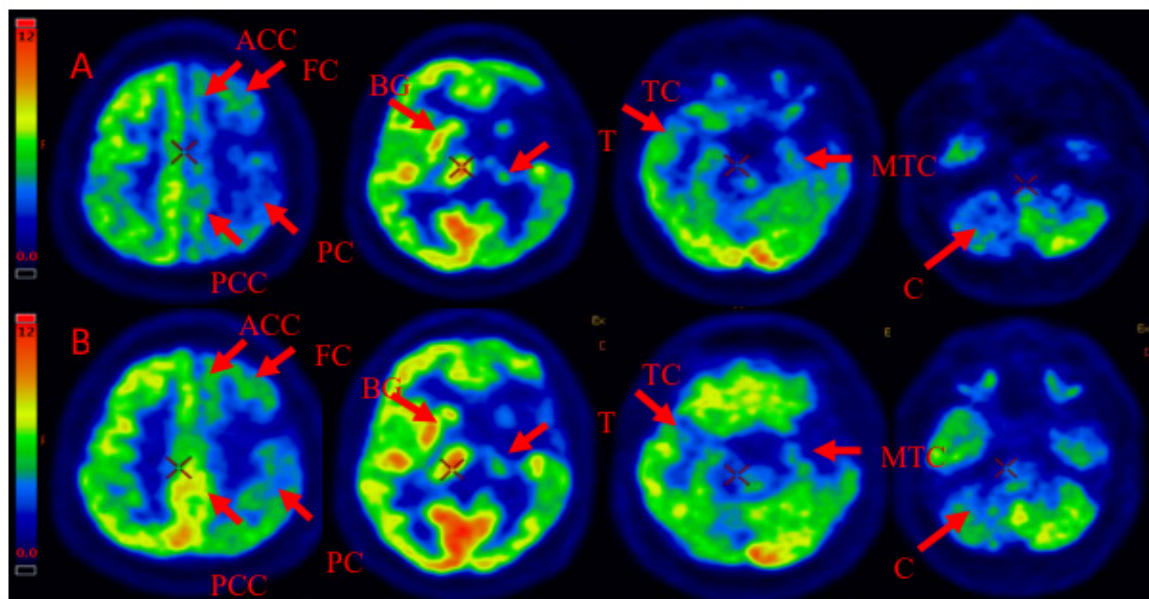


Figure 8: A] The PET CT scan before cellular therapy showed hypometabolism in the left frontal cortex (FC), parietal cortex (PC), cingulate cortex (ACC – anterior cingulate cortex, PCC – posterior cingulate cortex), temporal cortex (TC), basal ganglia (BG), thalamus (T), and right cerebellum (C). B] The post-PET scan following cellular therapy showed significant improvement in the bilateral frontal cortex (FC), parietal cortex (PC), right BG, right TC, cingulate cortex (ACC – anterior cingulate cortex, PCC – posterior cingulate cortex), and mild improvement in the bilateral thalamus, medial temporal cortex and bilateral cerebellum (C).

2. From 2012 to 2016 we published 4 case reports of patients with different types of stroke in various peer-reviewed journals. These cases underwent intrathecal autologous bone marrow-derived mononuclear cell transplantation. All these patients showed functional and neurological improvements on follow-up. Improvements were seen in symptoms such as walking and standing balance, hand grip, voluntary control, spasticity, speech, mobility, etc. These patients also showed improved brain metabolism on a PET-CT scan of the brain. (44-47)

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

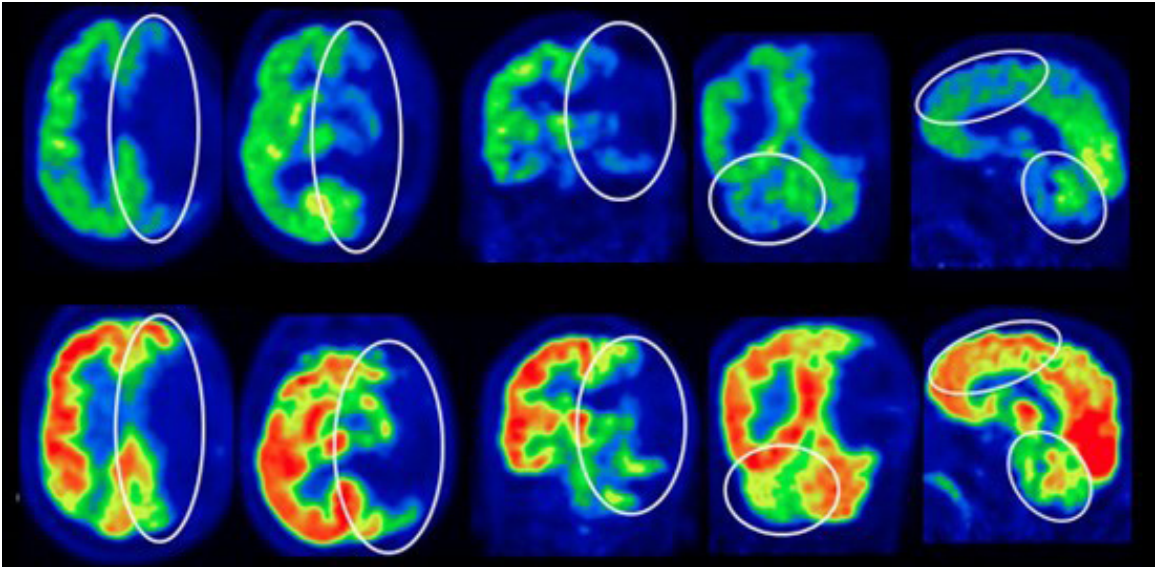


Figure 9: Comparative PET CT scans of a stroke patient, exhibiting left MCA infarct (dark penumbra can be seen on the left side, which extends into the right cerebellar hemisphere). The scan shows improvement in regions with infarct after cell therapy. Signals can be seen extending into affected regions, including cortical regions, right cerebellar hemisphere, and bilateral thalamus.

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 the 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 take into account acute and chronic strokes. This is crucial to analyze the effect of cell therapy at various stages. Appropriate dosage remains unclear. A dose-response correlation is an important aspect of cell therapy. Routes of administration are an important area which decides the intensity of effect of cell therapy. Objective imaging needs to be introduced into clinical trials to get an insight into the physiological processes occurring at the cellular level after cell therapy to strengthen the results obtained.

Conclusion:

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

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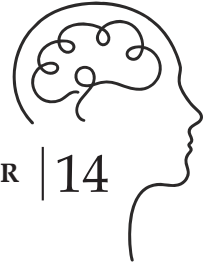
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"Do not fear to defend new ideas even the most revolutionary, your own faith is what counts most. But have the courage also to admit an error as soon as you have proved it to yourself, that your idea is wrong. Science is the graveyard of ideas. But 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



CHAPTER | 14

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

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

.....

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

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

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

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

Pathophysiology of ALS

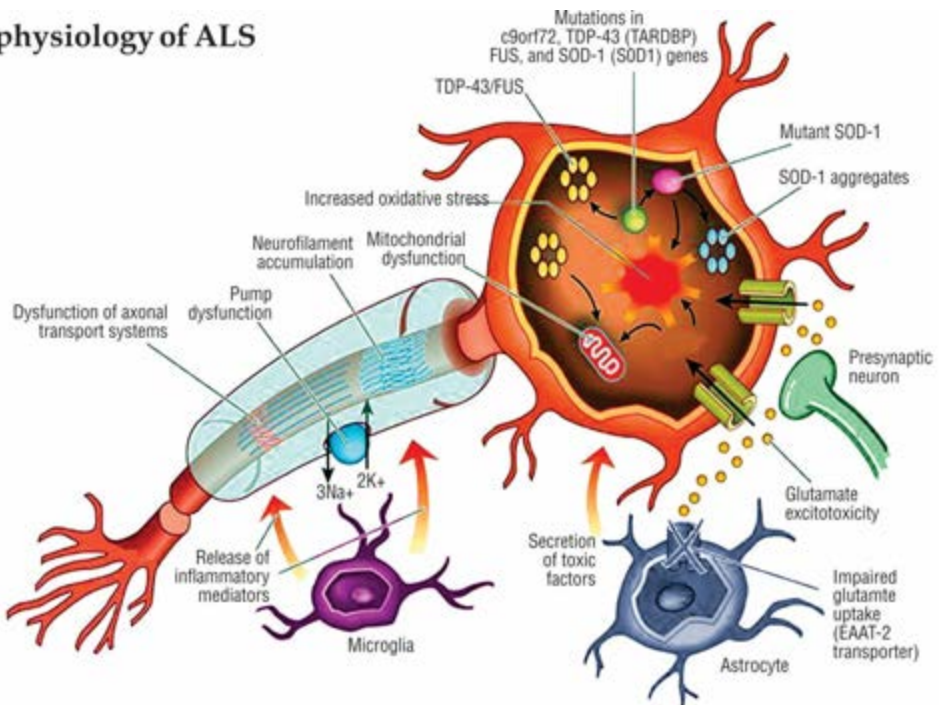


Figure 1: A diagram depicting various pathophysiological mechanisms causing ALS/MND – genetic mutations, glutamate toxicity, inflammation and neurotoxicity due to glial cell response, axonal dysfunction, increased oxidative stress and mitochondrial dysfunction.

Unmet medical needs

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

Stem cell therapy for ALS

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

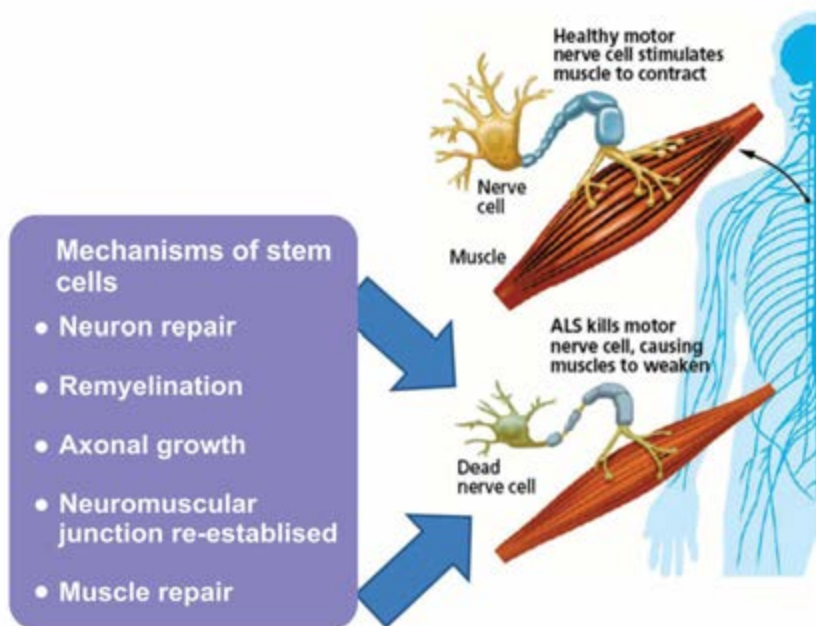


Figure 2: Mechanism of action of stem cells in MND/ALS.

Replacement of degenerated motor neurons is the ultimate goal of transplantation therapy but various factors influence the outcome of the transplanted cells. Survival of the cells in the host environment, their neurogenic potential, actual neurogenesis at the target site and formation of neuronal connections over long distances are some of the factors [32, 33]. As the transplantation science evolves, these factors could be monitored to gain appropriate outcomes, but currently the aim of transplantation is to protect the existing motor neurons and attempt to bring about 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 in on the site of injury [34]. They further demonstrate immunomodulatory, anti-inflammatory and cytoprotective properties [35]. The factors secreted by these cells bring about neo-angiogenesis [36]. These paracrine effects lead to neuroprotection and subsequent alteration in the disease course and progression [37].

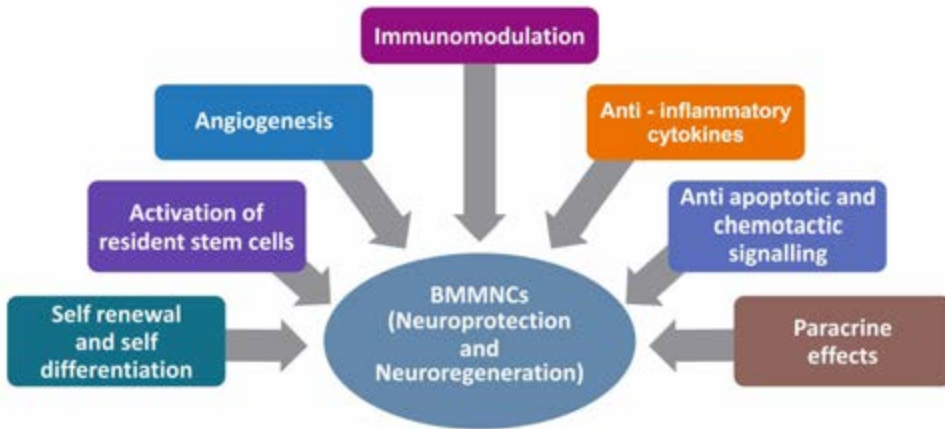


Figure 3: Mechanism of Action of cellular therapy in ALS

Animal Studies

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

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

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

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

neurotrophic factors and the reduction of microgliosis and macrogliosis.

Human studies

45 studies, which include 34 clinical studies and 11 case reports, have been published to demonstrate the effects of various types of stem cells in motor neurone disease/amyotrophic lateral sclerosis.

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

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

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

Olfactory ensheathing cells extracted from human fetal olfactory bulbs were injected into the bilateral corona radiata in 15 ALS patients who were compared to 20 untreated controls [22]. Over a 4-month follow-up period, positive and beneficial results were observed as a five-point difference in the ALSFRS-R. In another

study, 327 patients received injections of olfactory ensheathing cells into the spinal cord, the bilateral corona radiata, or both [23]. They reported improved ALS functioning rating scale and normalized electromyographical findings 4 weeks after transplantation, with no differences between the three groups. Studies have demonstrated the safety of intraspinal infusion of autologous bone marrow mononuclear cells [24-26]. Deda et al. demonstrated reinnervation in 7 of 13 patients on electro neuro myography following intraspinal infusion of autologous bone marrow mononuclear cells, 1 year post treatment [26]. Clinical improvements were seen in 10 patients. Intrathecal transplantation of autologous bone marrow mononuclear cells was found to be safe and effective in slowing down disease progression in 5 studies [27-31].

A study by Nam, J. Y. et al. investigated the long-term survival benefits of treatment with intrathecal lenzumestrocel (autologous bone marrow-derived mesenchymal stem cell (BM-MSC) product). The investigation comprised 157 individuals who got lenzumestrocel and whose symptoms lasted for less than two years. The BM-MSC group had a substantially higher chance of surviving than the external control group. The BM-MSC group had a substantially decreased hazard ratio for death, according to multivariate Cox proportional hazard analysis, which also suggested that numerous injections were more successful. Furthermore, a year following the initial treatment, no significant adverse medication reactions were discovered during the safety evaluation. The current study's findings demonstrated that lenzumestrocel medication improved long-term survival in actual ALS patients.

Our results

Published results

1. In 2021, Sharma et al. published a study including 216 individuals with confirmed ALS in the "Journal of Neurorestoratology". This was a retrospective analysis, which lasted for ten years and five months. Of these, 150 were in the intervention group, which received autologous BMMNCs and conventional treatment, and 66 were in the control group, which received only standard care. Using Kaplan-Meier analysis, the expected survival durations of the control and intervention groups were calculated and compared. Within the intervention group, the survival length of patients with various onset types and hormonal statuses was compared. According to Kaplan-Meier analysis, the intervention group's survival length was 91.7 months, whereas the control group was 49.7 months ($p = 0.008$). No significant side effects associated with the operation or cell therapy were noted by any of the patients. Patients with limb onset (102.3 months) compared to bulbar onset (49.9 months), premenopausal women (93.1 months) versus postmenopausal women (57.6 months) ($p = 0.002$), and pre-andropaus-

al men (153.7 months) versus post-andropausal males (56.5 months) ($p = 0.006$) had significantly higher estimated survivals ($p = 0.013$) within the intervention group. With the potential for long-term positive effects and improved survival, cell therapy employing autologous BMMNCs in conjunction with conventional treatment is a viable and secure option for ALS. Premenopausal women, men under 40, and patients with limb onset showed superior therapy success.

Table 1. Effect of prognostic factors on survival duration within the intervention group.

Prognostic factors	Estimated survival duration (months) as calculated by Kaplan–Meier survival analysis	Statistical significance
Onset type Bulbar onset Limb onset	49.9 102.3	0.013*
Hormonal status (Women) Premenopausal women Postmenopausal Women	93.1 57.6	0.002*
Hormonal status (Males) Preandropausal men Postandropausal men	153.7 56.5	0.006*

***Indicates a statistically significant difference between the groups.**

2. In 2015 Sharma et al. published a retrospective controlled cohort study of 57 patients [27]. Out of these, 37 patients underwent autologous bone marrow mononuclear cell transplantation in addition to standard rehabilitation and Riluzole. The survival duration since the onset of the disease of this group was compared with a control group that consisted of 20 patients who did not receive cell transplantation. Survival duration was computed using a Kaplan-Meier survival analysis and compared using a log-rank test. The effect of age at onset, type of onset and lithium on survival duration in the intervention group was analyzed. The 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 6) (Table 1). Survival duration was significantly ($p=0.039$) higher in people with the onset of the disease below 50 years of age (Figure 5). Limb onset and lithium also showed a positive influence on the survival duration (Figure 7 & 8). Mean survival duration of the intervention group was also higher than the survival duration of ALS patients in previous epidemiological studies (Table 3).

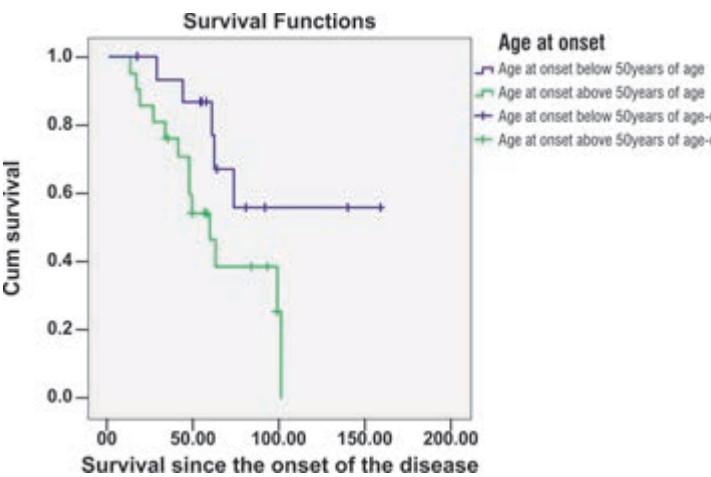


Figure 5: Kaplan-Meier survival comparison for the patients in the intervention group below and above 50 years of age at the onset.

Table 2: Survival analysis

Survival Analysis	Intervention Group	Control Group
Total Mortality	48.64%	50.00%
Range of survival Duration (months)	13 - 158	26 - 84
Mean Survival duration (months)	87.76 (10.45)	57.38 (5.31)

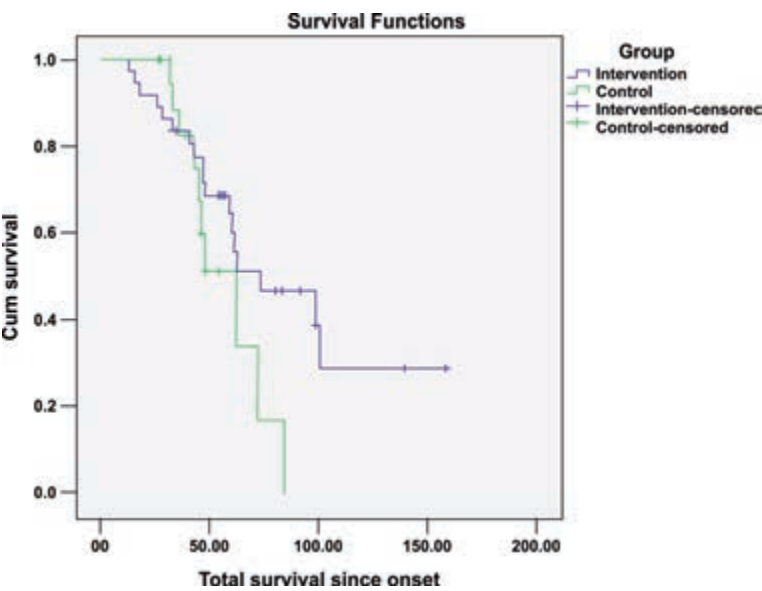


Figure 6: Kaplan-Meier survival analysis comparing the mean survival duration of the intervention and control groups.

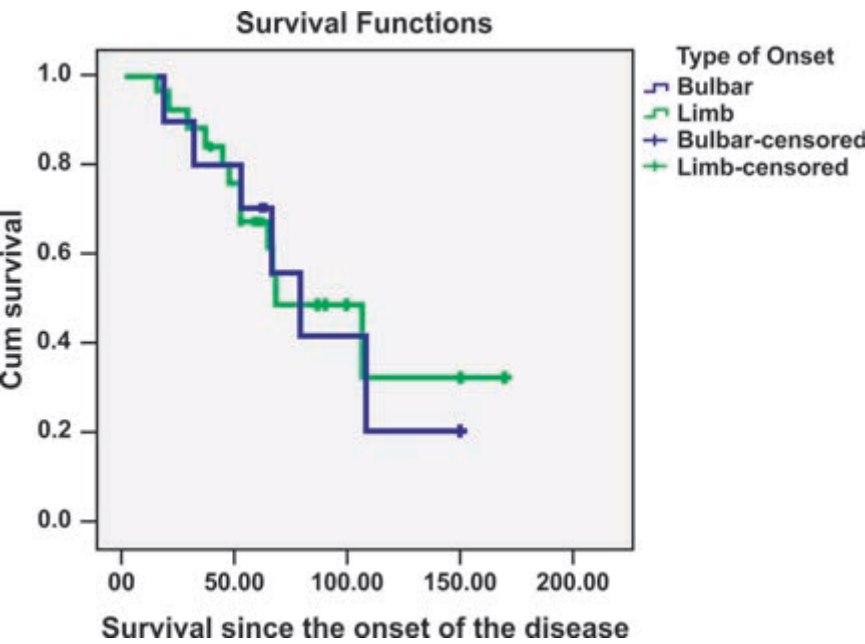


Figure 7: Kaplan-Meier survival comparison for the patients in the intervention group with bulbar onset and limb onset.

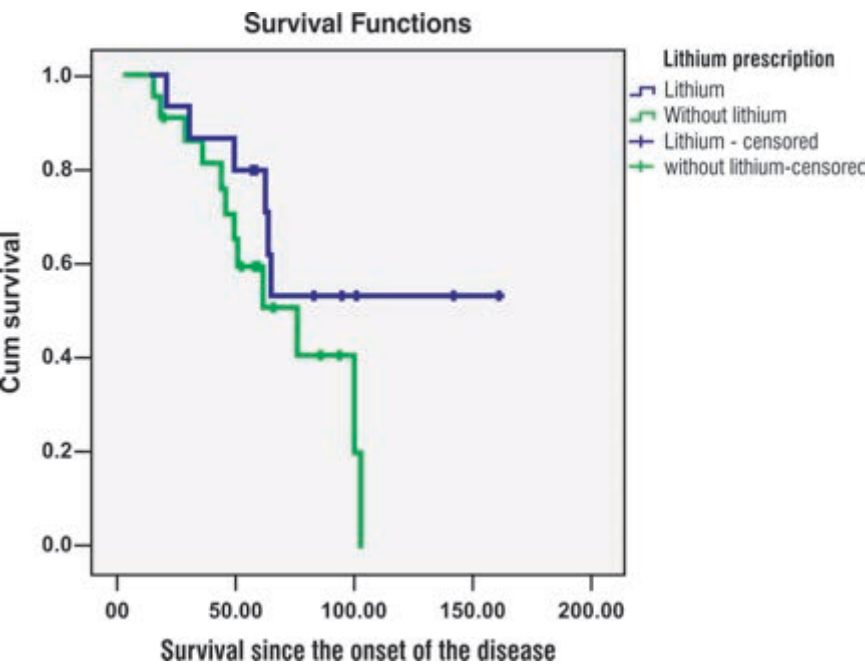


Figure 8: Kaplan-Meier survival comparison for the patients in the intervention group with and without Lithium prescription.

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

Author	Year	Country	Sample Size	Mean age of the population in years (SD)	Mean or median Survival since onset	Comparison with the results if our study
Haverkamp et al. [38]	1995	USA	1200	55.7	37.5 Months	88.86 Months
Forbes et al. [39]	2004	Scotland	1226	Men – 65.2 Women	25 Months, 5-year survival probability – 11%	88.86 Months 5-year survival probability – 62.3%
Milu et al. [40]	2005	Italy	79	64.4	39.2 Months	88.86 Months
Osuntukun et al. [41]	1974	Nigeria	92	39.2 (1.6)	Dead – 73 Months Alive – 79 Months	
Radhakrishnan k [42]	1986	Libya	23	51	42 Months	88.86 Months
Norris et al. [43]	1993	USA	708	Range - 25 - 74	24-44 years - 71.5 Months 45 - 54 years - 35 Months 55 - 74 years - 32.5 Months	
Alcaz S [44]	1997	Serbia	58	Men - 56.2 Women - 56.6	2-year survival probability – 62% 5-year survival probability – 27% 7-year survival probability – 27%	

Eisen et al. [45]	1993	USA	246	Not available	< 40 years - 98.4 Months 61 - 70 - 31.2 Months	
Traynor et al. [46]	2000	Ireland	388	Men - 63.3 Women - 64.4	Definite ALS - 27 Months Probable - 30 Months	Definite ALS - 88.86 months
Sorenson et al. [47]	2002	USA	77	63	23 Months	88.86 Months
Tysnes et al. [48]	1991	Norway		60.9 Range - 34 to 82	28 Months	88.86 Months
Turner et al. [49]	2003	England	769	Long Survivors - 43, Short survivors - 57, Others - 62	43 Months	88.86 Months

Conclusion of the study:

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

Case report

In 2020 we published 2 case reports across journals: one about a 47-year-old male suffering from ALS for 2 years and another about a 40-year-old male ALS patient, suffering for the past four years, whose treatment involved multiple doses of intrathecal transplantation of autologous bone marrow-derived mononuclear cells and long-term lithium, followed by multidisciplinary neurorehabilitation and standard Riluzole treatment. Patients were followed up at a regular intervals and outcome measures such as the Berg Balance Scale (BBS), Functional Independence Measure (FIM), Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRSr), and Six Minute Walk Test (6MWT) were used to quantify the outcome. No major adverse effects were noted after both the interventions. After follow-up, symptomatic improvements were seen in speech, swallowing, stamina, walking and muscle strength; fasciculations and cramps reduced drastically for the 47-year-old patient, and significant improvements were observed in ambulation, reach, stamina, inspiratory and expiratory flow, and leg movements for the

40-year-old patient. The highlight was the maintenance of the patient’s condition in view of a degenerative prognosis. On an objective scale, patients’ ALSFRSr, BBS, 6MWT, and FIM scores showed improvements, and qualitative changes were recorded; these improved outcomes were indicative of the slowing down of disease progression. Multiple doses of intrathecal autologous cell therapy along with rehabilitation and lithium, in addition to standard riluzole treatment, is a novel approach for decelerating disease progression and qualitatively improving living conditions for ALS patients and their caregivers.

Neuroprotective effects of female reproductive hormones in combination with cellular therapy

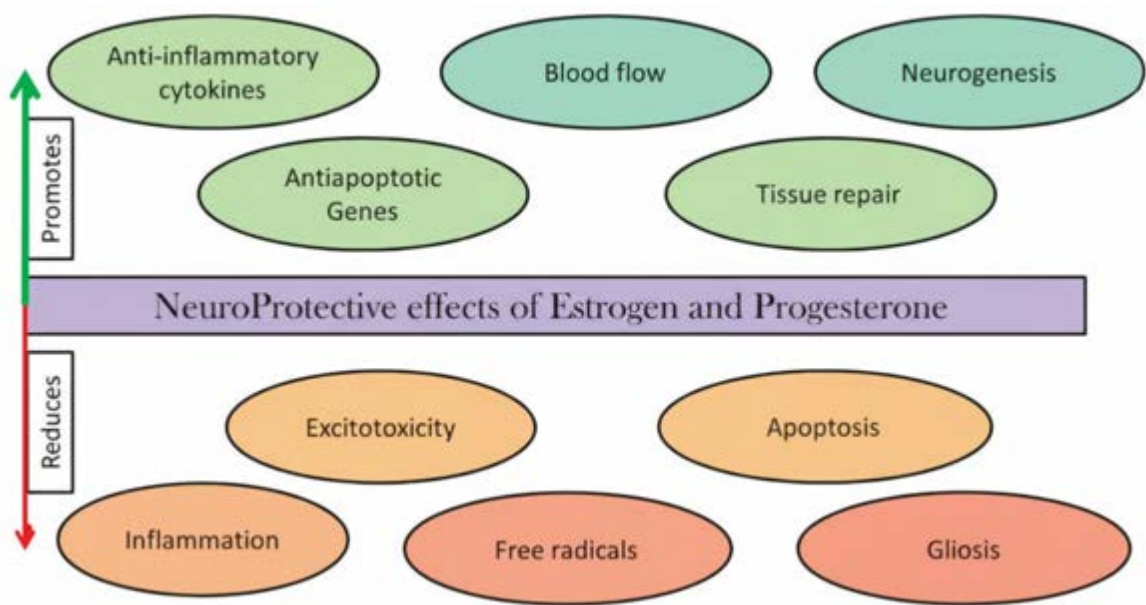


Figure 9: Neuroprotective effects of Estrogen and Progesterone

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

Upon comparing the disease progression and survival percentages, we found that pre-menopausal women have the slowest disease progression and no mortality in the 1.5 years of follow-up period. This was followed by men below the age of 50 years, women and men above the age of 50 years. The disease progression of the 4 groups is given in Figure 10, Table 4.

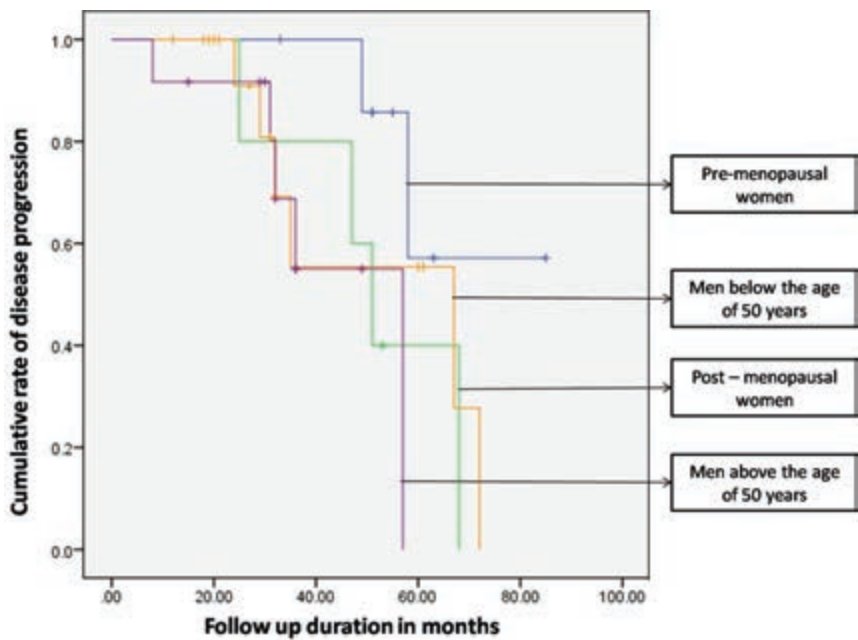


Figure 10: Disease progression of the 4 subgroups of ALS patients treated with cell therapy

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

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

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

Future directions

Gene therapy

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

Nur-Own cells transplantation

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

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

Combination of gene therapy and stem cell therapy

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

Induced pluripotent stem cells (iPSCs)

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

Effect of Reproductive hormones

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

Conclusion

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

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

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

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

1. Younger patients below the age of 50 years
2. Women, especially premenopausal women
3. Patients in the early stage of the disease
4. Patients who have still not developed swallowing, breathing and speech problems

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

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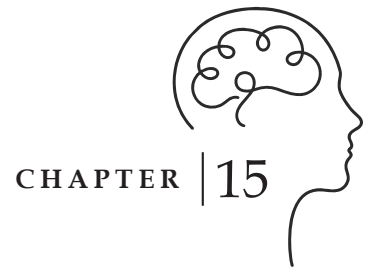
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To believe that, what has not occurred in history will not occur at all, is to argue disbelief in the dignity of man.

- Mahatma Gandhi



Role of Cell / Stem Cells in Traumatic Brain Injury

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

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

Unmet medical needs

The past 2 decades have seen great advancements in understanding the molecu-

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

Stem cell therapy in TBI

Due to the brain’s limited capacity to regenerate the damaged neurons, the intervention should aim at halting the degeneration and replacing the lost and damaged neurons. (4) In the past few years, cell therapy has gained attention as a prospective therapeutic option 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, includ-

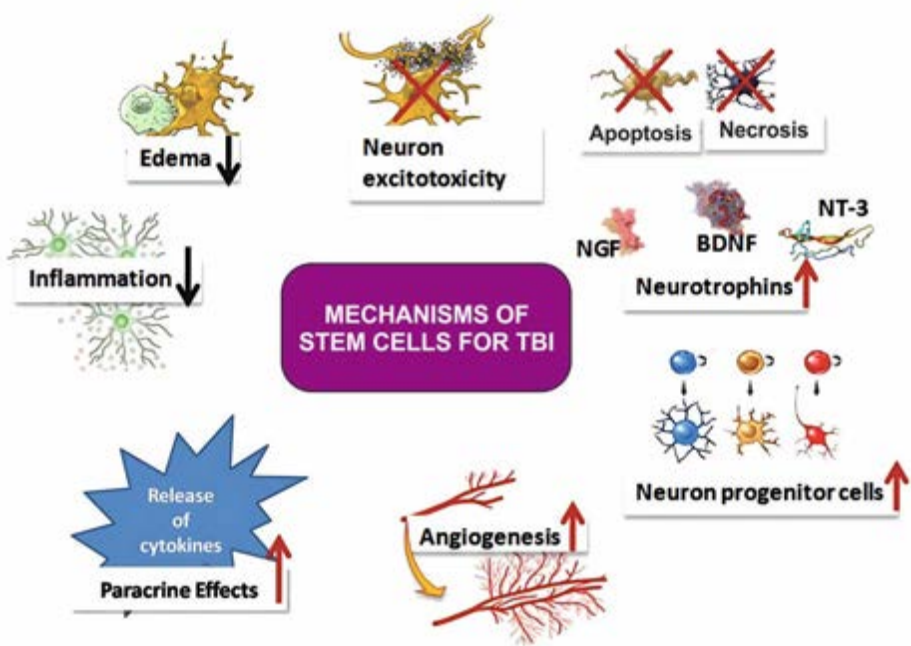


Figure 1: Mechanisms of stem cells for TBI

ing 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), and basic fibroblast growth factor (bFGF), which stimulate the endogenous neuroprotection and repair. (7,9)

Animal studies

Various experiments on animal models have been carried out to test the safety and feasibility of different types of cells. Reiss et al. transplanted embryonic cells in experimental rats and recorded dramatic improvements but the safety of administration could not be established. (9) A 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 mechanisms. (10-15) Bone marrow stem cells were also found to be efficacious in modulating the inflammation-associated immune cells and cytokines in TBI-induced cerebral inflammatory responses. (16, 17) Studies also showed that bone marrow-derived mesenchymal cells (BMMSCs) migrate to the injured areas and increase expression of neurotrophic factors such as vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF). BMMSCs also upregulate the protein expression levels of synaptophysin, a synapse protein which is downregulated after TBI, resulting in neuromotor functional recovery. (18) In an experimental study, rats injected with BDNF gene-modified umbilical cord mesenchymal stem cells (UCMSCs) led to improvements in behaviour and other neurological functions.

(19) In 2016, a study conducted on Wistar rats to investigate the capacity and sensitivity of diffusion-derived magnetic resonance imaging suggested that cell intervention executed at 6 hours accelerates the brain remodeling process and results in an earlier functional recovery. (20)

Few studies were conducted recently to demonstrate the homing and migration of transplanted cells in rat models of TBI using fluorescence labelling. Dong et al. tracked the distribution of human umbilical cord mesenchymal stem cells (MSCs) in large blood vessels of traumatic brain injury rats. They found that intravascular migration and homing of MSCs in rats which received MSCs transplantation and new angiogenesis in MSCs-transplanted blood vessels. (21) Guo et al. demonstrated the integration of intravenously injected EPCs into the injured brain tissue. These EPCs enhanced recovery by contributing to neurogenesis. (22.) Lin et al. showed that transplanted human neural precursor cells integrate into the host neural circuit, which was detected by fluorometric Ca^{2+} imaging and nerve tracing, and ameliorate neurological deficits in a mouse model of traumatic brain injury. (23.)

Clinical Studies

14 studies, which include 11 clinical studies and 3 case reports, have been published to demonstrate the effects and safety of various types of stem cells in traumatic brain injury.

One of the first studies was published in 2008 by Zhang et al. They assessed the safety and feasibility of a combined procedure to deliver autologous mesenchymal stromal cells in 7 patients with traumatic brain injury. (24) They found that neurological functions improved significantly at 6 months after cell therapy. In the IA series, Cox et al. performed a phase 1 clinical trial to demonstrate the safety and feasibility of allogeneic BMMNCs in children with severe traumatic brain injury. 10 children were included in the study. Glasgow Outcome Scores at 6 months showed 70% with good outcomes and 30% with moderate to severe disability. (25) Followed by this, they published the results of their phase 2 clinical trial in 2017, which included 25 patients: 15 in the intervention group and 10 in the control group. They recorded positive functional and neurological outcomes and also downregulated inflammatory biomarkers. (26) Similar studies were published by Sharma et al. and Liao et al. in 2015 demonstrating the positive effect of BMMNCs. Wang et al., in 2013, conducted a study on patients with sequelae of TBI. They administered 40 patients with umbilical cord mesenchymal stem cells and observed improved neurological functions and self-care in these patients as compared to the controls. (27) Vaquero et al. in 2017 showed improvement and progressive increase in brain glucose metabolism after intrathecal administration of autologous mesenchymal stromal cells using 18 FDG PET-CT in 3 patients. (28) In 2017, Wang Z et al demonstrated the safety and feasibility of neural stem cell transplantation in patients with severe TBI. They observed that the majority of patients experienced improved neurological function on follow-up along with increased serum levels of nerve growth factor and brain-derived neurotrophic factor. (29)

Our Published Results

1. In 2020 Sharma et al. published a study in "Cell Regeneration" to investigate the effects of cell transplantation; we studied 50 individuals with persistent TBI. Neurorehabilitation and intrathecal autologous bone marrow mononuclear cell transplantation were part of the strategy. The average length of follow-up was 22 months. Six months following their initial intervention, fifteen patients received a second round of cell. As an outcome measure, the functional independence measure (FIM) was employed to assess the patients' functional changes. To examine the patients' symptomatic improvements, a percentage analysis was conducted. Overall, 92% of patients exhibited improvements in neurological status, cognition, attention and concentration, muscle tone, coordination, activities of daily living, voluntary control, memory, oromotor skills, lower limb activities, ambulation,

trunk & upper limb activities, speech, posture, communication, and balance while sitting and standing. The pre-intervention and post-intervention ratings were subjected to statistical tests in order to assess their relevance. Following the intervention, there was a statistically significant (at $p < 0.05$ with p -value 0) increase in the FIM scores on the Wilcoxon signed rank test. Patients with mild TBI who were less than 18 years old and had less than five years since their injury experienced better results from the intervention. Ten patients had comparative Positron Emission Tomography-computed tomography (PET CT) scans to track how the operation affected brain function. To ascertain their impact on the result of cell transplantation, variables including age, the number of doses taken, the amount of time since the injury, and the severity of the injury were also examined. In regions of the brain that were linked to the clinical alterations, ten individuals displayed better metabolism.

Table 1: Result of statistical analysis of FIM scores

Scale	Mean score before cellular therapy	Mean score after cellular therapy	Z Value	Significance
FIM	55.84	62.2	-4.7821	Significant

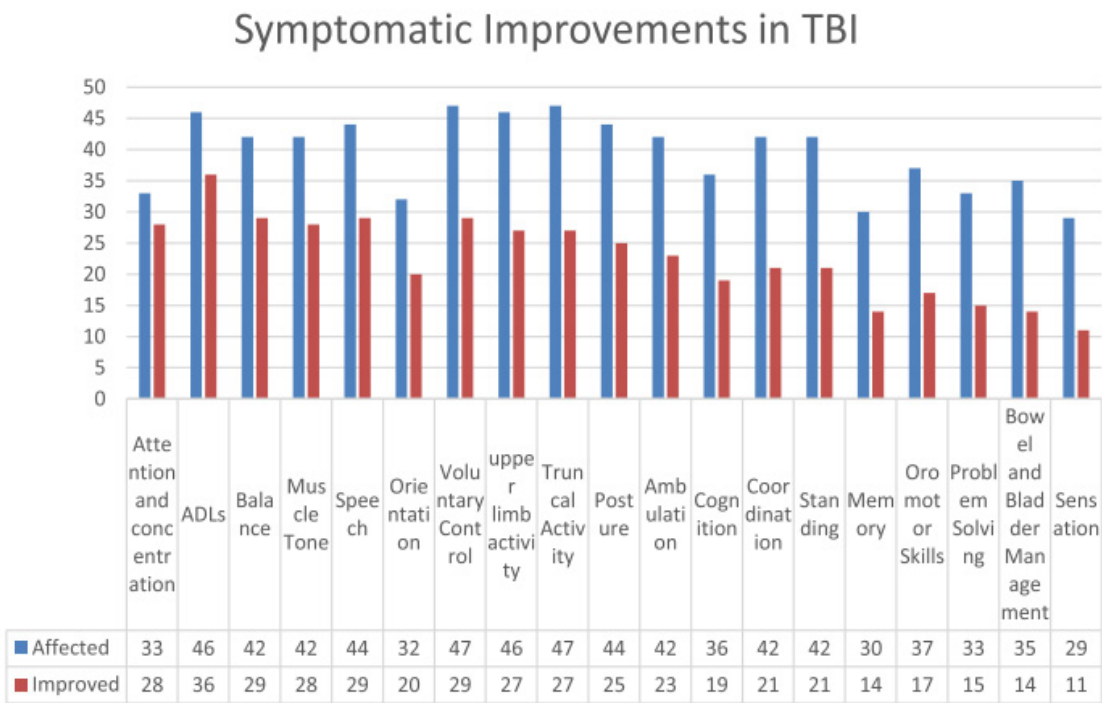


Figure 2: Graph demonstrating symptomatic improvements in patients with chronic TBI after cell transplantation

2. In 2015 Sharma et al. conducted a study on 14 cases who were administered autologous bone marrow mononuclear cells intrathecally. (30) The follow-up was done at 1 week, 3 months and 6 months after the intervention. The Functional Independence Measure scale, the SF-8 Health Survey Scoring and the disability rating scale were used as outcome measures. At the end of six months, a percentage analysis was carried out for improvement in every symptom, as discussed in Figure 3. 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 are core measures of the clinical and functional improvements demonstrated by these patients.

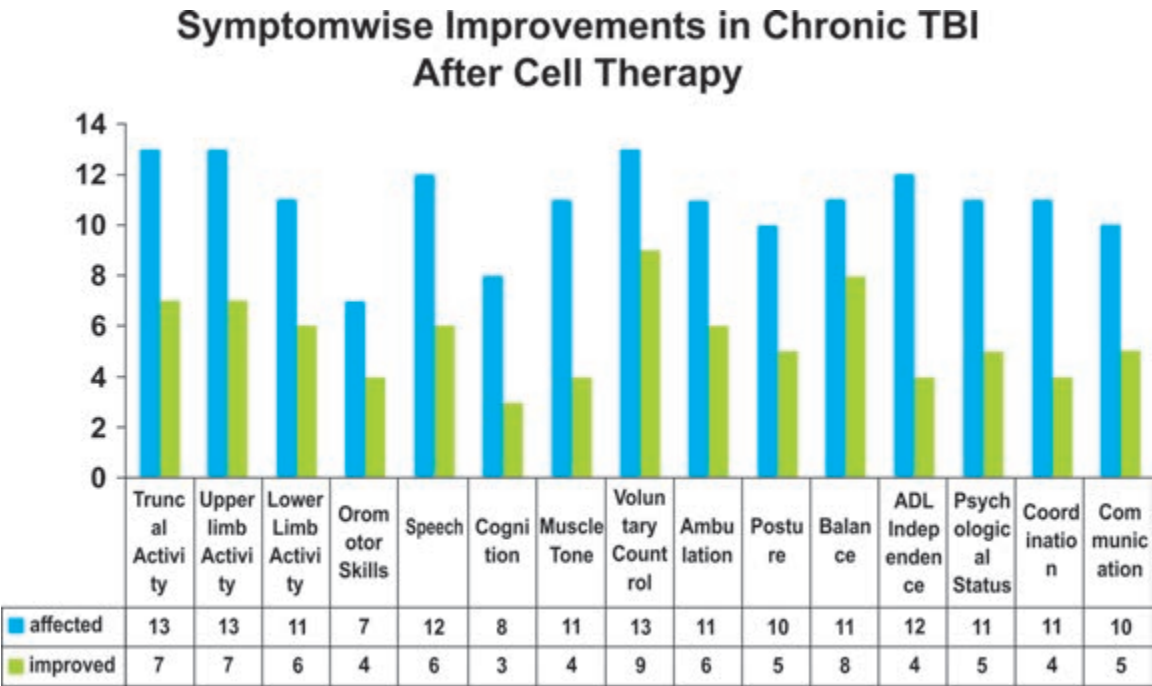


Figure 3: Symptom-wise improvements in chronic TBI patients seen after intrathecal administration of autologous BMMNCs

Table 2: SF8 scores before and after intervention suggesting improved quality of life.

	SF8 (Pre)		SF8 (Post)	
Patient No.	PCS 8	MCS 8	PCS 8	MCS 8
1	28.4	32.5	39.2	40.8
2	44.4	52.1	51.4	59.8

3	47.1	35.4	48.6	43.2
4	33.4	51.5	43	55.9
5	39	64.1	43.1	62.7
6	43.7	58.8	50.1	58.3
7	37.8	44.7	41.5	47.2

Table 3: Areas of the brain showing improved metabolism in PET CT scan and their correlation to the clinical function improvement

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

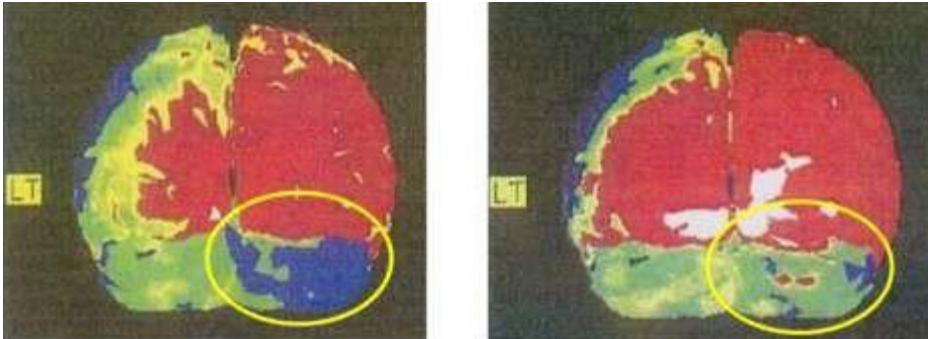


Figure 4: PET CT Scan showing improved metabolic activity, which is indicated by decrease in blue areas after stem cell therapy

PET CT scan as a monitoring tool

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

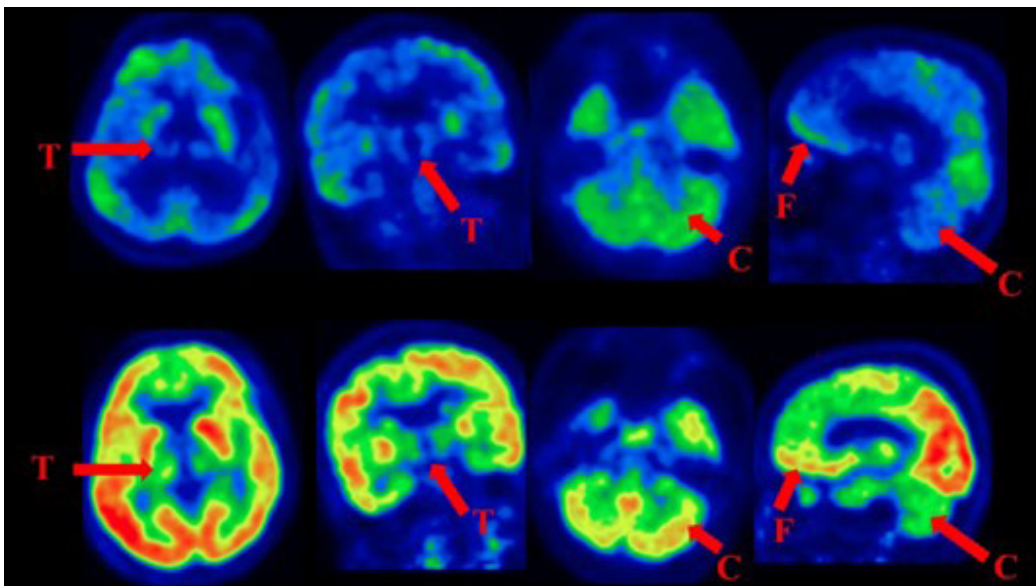


Figure 5: Comparison image of pre and post cell therapy in TBI patient. The areas - Cerebellum (C), Thalamus (T), Frontal (F) marked with arrows depict the improved brain regions after cell therapy.

Conclusion

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

Future directions

Studies focusing on combining the efficacy of HBOT and stem cell therapy to target the neurological damage should be established. Future studies should identify the ideal cells for therapeutic use, along with the ideal route of administration. The optimum quantity of cells, frequency of doses and the time interval between consecutive doses should be established to optimize this intervention. The ideal time of injection of stem cells should also be determined as in the acute phase, inflammation and pathological metabolic changes make the endogenous environment inhospitable for the injected cells. In chronic phase the gliotic changes may affect the efficacy of cell therapy. It is also important to track the changes occurring in the brain after intervention paving way for more research to be conducted on the monitoring tools.

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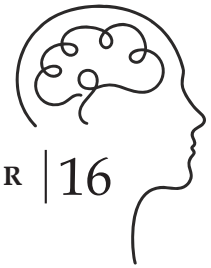
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“Strength does not come from physical capacity. It comes from an indomitable will.”

– Mahatma Gandhi



CHAPTER | 16

Role of Cell / Stem Cell Therapy for Intellectual Disability

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

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

Pathophysiology

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

Unmet Medical Needs

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

Stem Cell Therapy for Intellectual Disability

In the case of intellectual disability, any damage to the brain is permanent and irreversible, 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 the hippocampus and cerebral cortex areas of the brain, which further lead to faulty information processing and consecutively affect cognition and adaptive behaviour. [7] To reverse the damage caused to the central nervous system, only a neurorestorative therapy like stem cell therapy would be beneficial.

Stem cells have a unique property of homing and targeting specific damaged areas on administration that require the right combination of signaling molecules from the injured tissue and the corresponding receptors on stem cells. They survive, migrate, proliferate and differentiate into the required cell types. (8) They not only replace the dead cells but also stimulate the endogenous cells and prevent further damage. Their paracrine activities, such as secretion of growth factors, angiogenesis, neurogenesis, immunomodulation, decreasing inflammation, etc., also help in the repair process. (9) This could help repair the disrupted neuronal networks in ID and hence improve the information processing.

Animal studies have shown that administration of stem cells may support the ability for structural brain repair as well as cognitive improvement. Published studies involving direct stem cell interventions in intellectual disability (ID) are limited. To date, only four studies comprising one clinical study and three case reports have investigated the effects of stem cell therapy in individuals with ID.

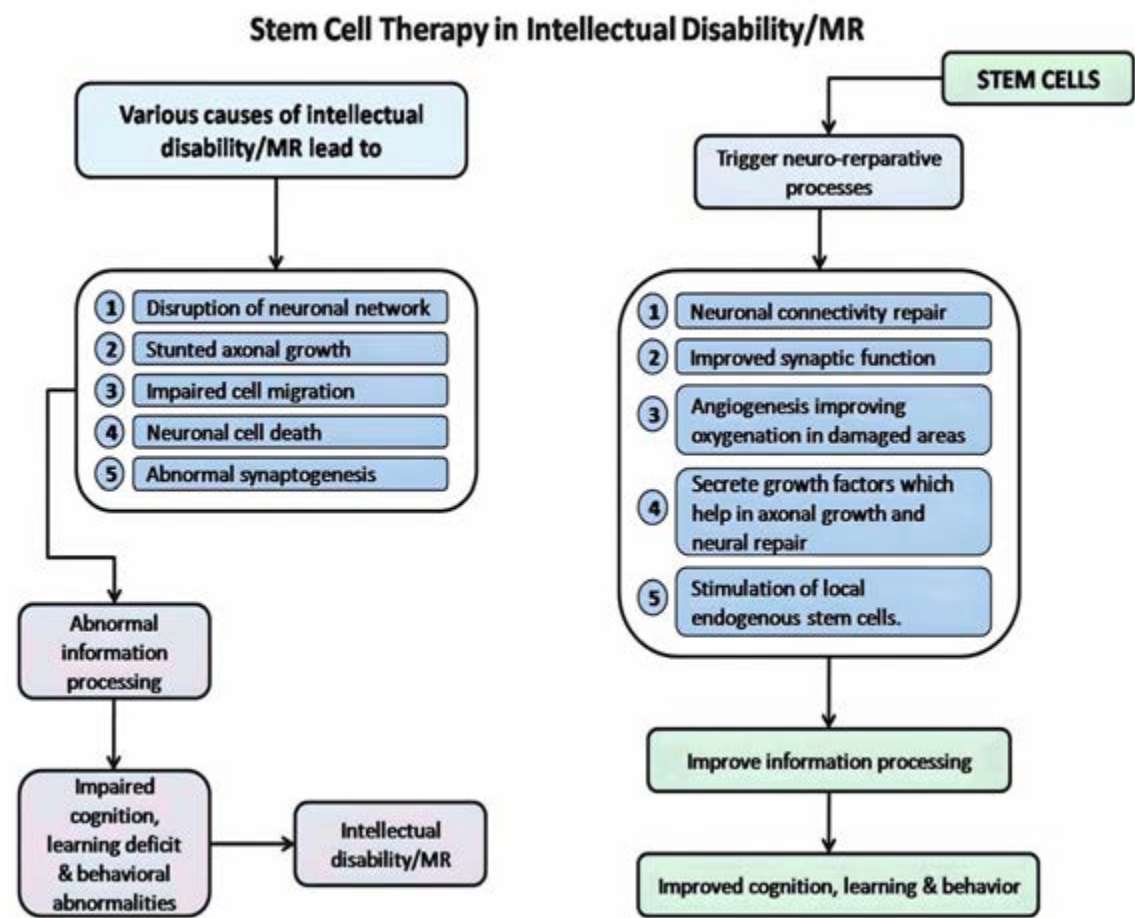


Figure 1: Stem Cell Therapy in intellectual disability

Our Results:

Published data

In 2018 Sharma et al. published the first study in “Stem Cell Research & Therapy” in the world demonstrating the efficacy of intrathecal autologous bone marrow mononuclear cell transplantation in individuals with ID. (10) It was an open-label proof-of-concept study which included 58 patients. These patients were divided into two groups: the intervention group (n = 29) and the rehabilitation group (n = 29). The intervention group underwent stem cell therapy and standard neurore-

habilitation, while the rehabilitation group underwent only standard neurorehabilitation.

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

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

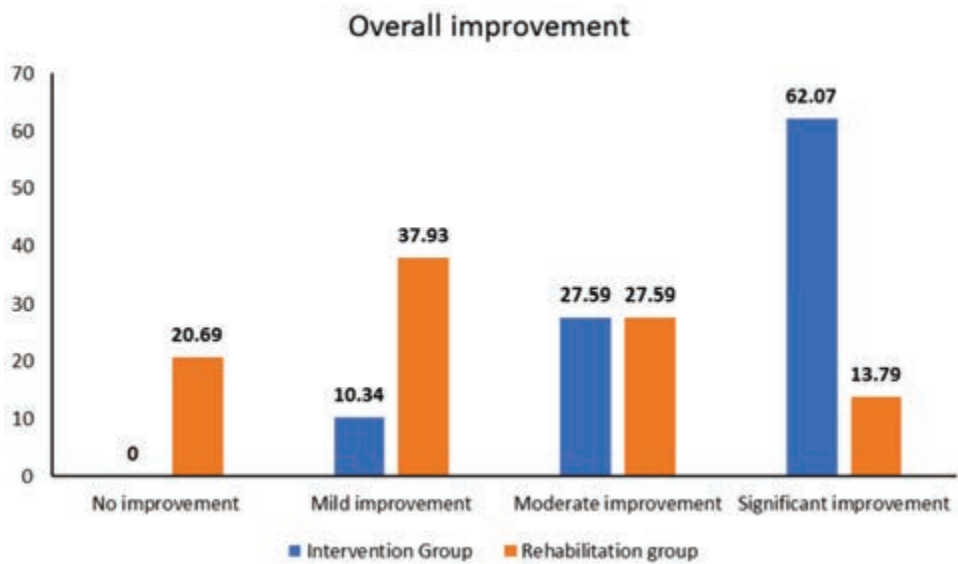


Figure 2: Graph demonstrating a comparison of overall percentage improvements in ID between the intervention group and rehabilitation group.

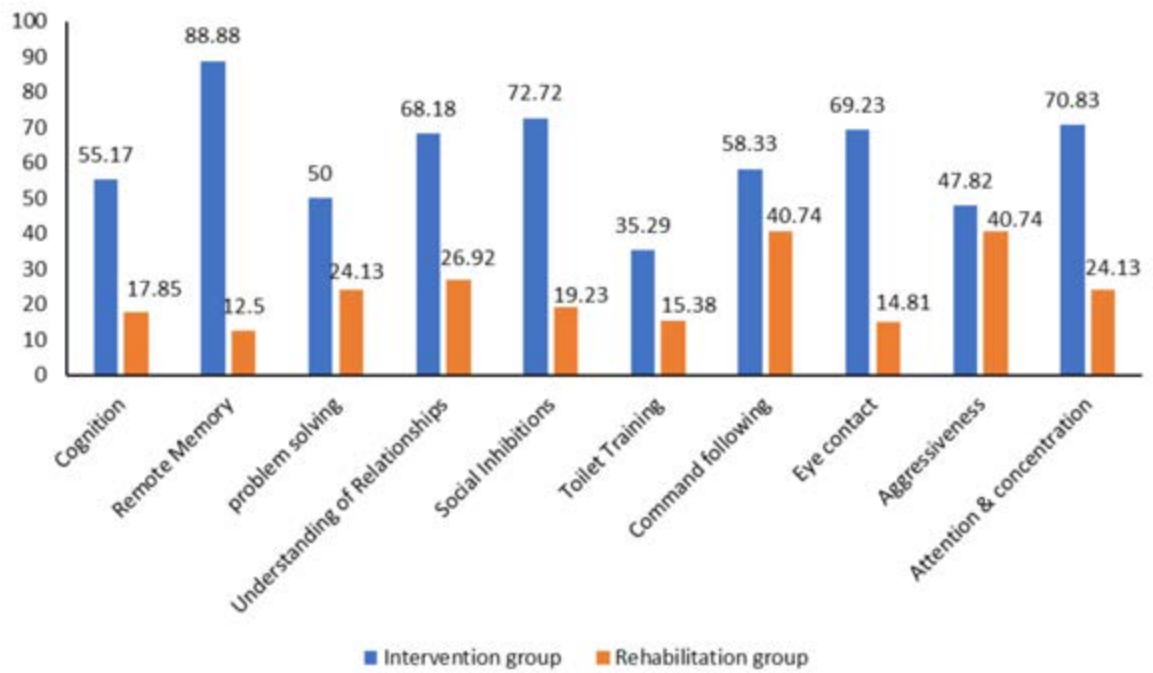


Figure 3: Graph demonstrating a comparison of overall percentage improvements in the symptoms ID between the intervention group and rehabilitation group.

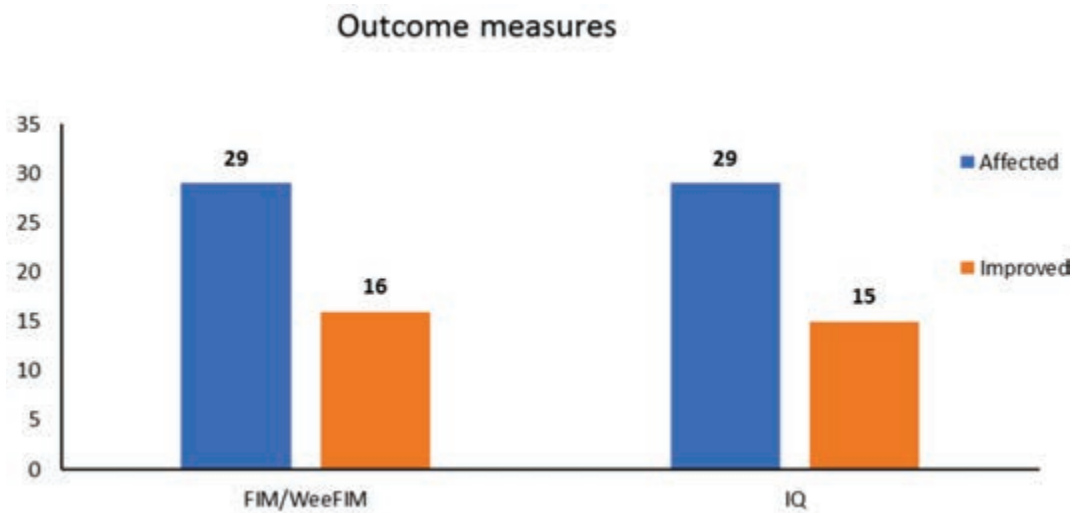


Figure 4: Graph demonstrating improvements in the outcome measures (FIM/WeeFIM and IQ) in patients with ID in the intervention group, six months after cellular therapy

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

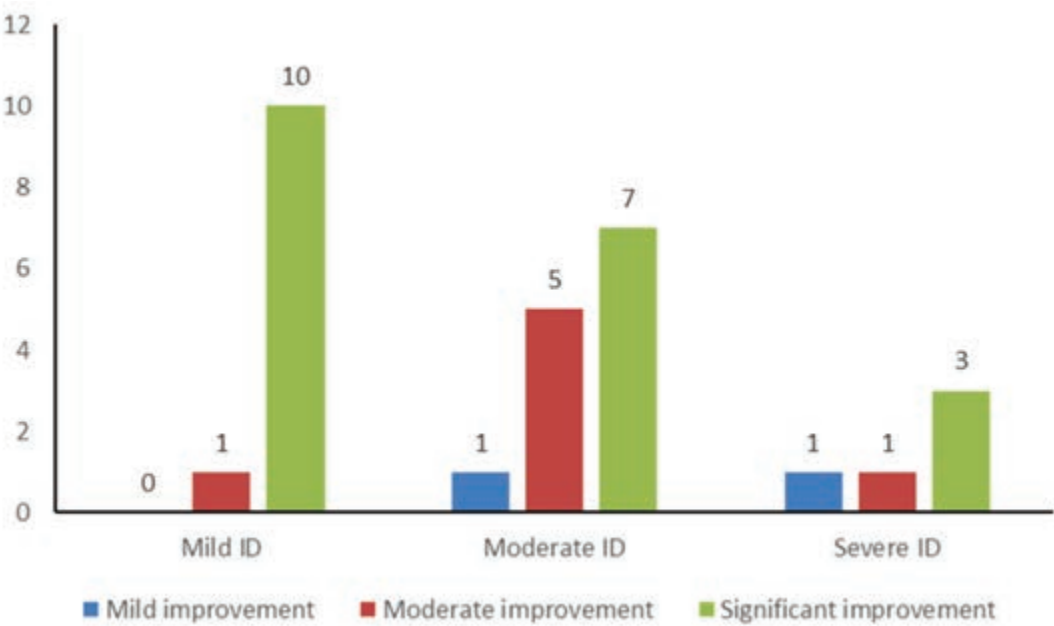


Figure 5: Graph showing the comparison of improvement in patients in the intervention group with severity of ID.

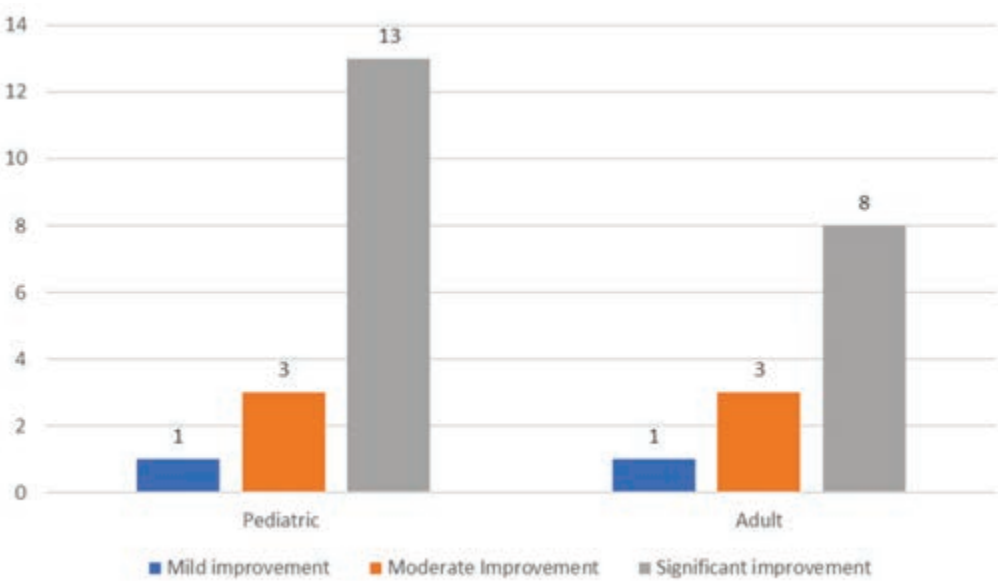


Figure 6: Graph showing the comparison of improvement in patients in the paediatric and adult populations of the intervention group.

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

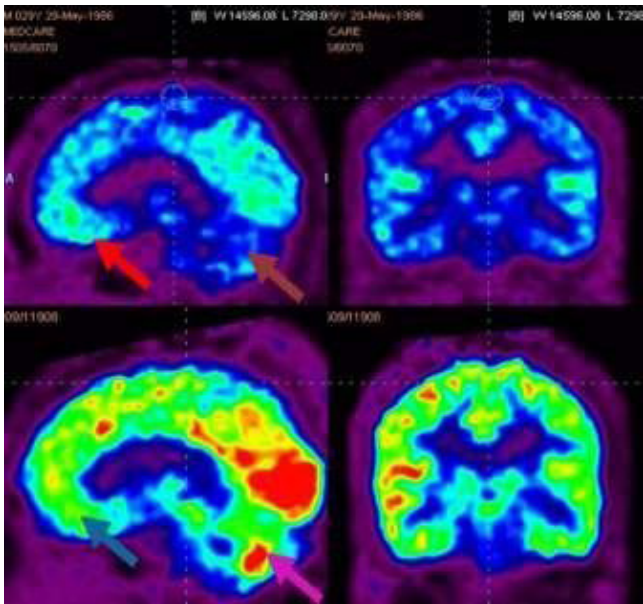


Figure 7: The top row indicates the PET CT Scan image before stem cell therapy showing reduced metabolism in the prefrontal, frontal (red arrows), and cerebellum (brown arrow); row indicates improved 18F-FDG metabolism after intervention in the prefrontal, frontal (blue arrows), and cerebellum (pink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

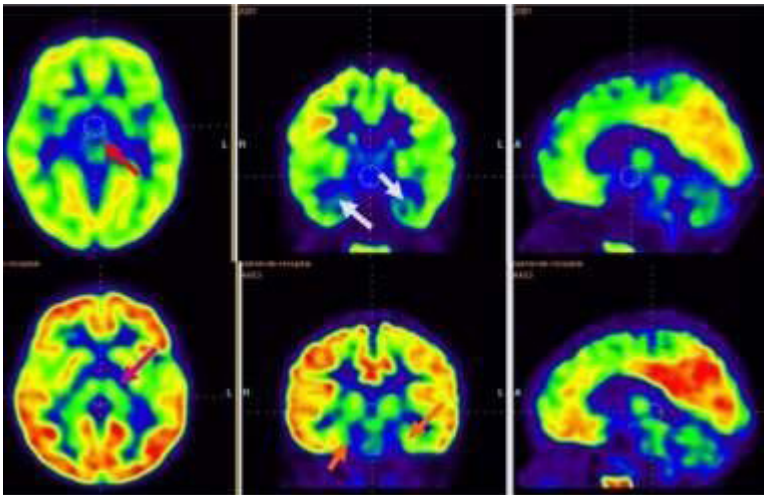


Figure 8: The top row indicates the PET CT scan brain image before stem cell therapy, showing reduced metabolism in the thalamus (red arrow) and mesial temporal structures (white arrow). The Below row indicates improved 18F-FDG metabolism after therapy in the thalamus (pink arrow) and mesial temporal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

Case report

1. In 2019 Sharma et al. administered autologous bone marrow mononuclear cells intrathecally in a 20-year-old male patient diagnosed as ID with ASD. The patient was followed up at regular time intervals and adverse events were monitored. IQ, the Binet Kamath Test of Intelligence and ISAA were outcome measures used to quantify the outcome. A comparative positron emission tomography-computed tomography (PET CT) brain scan was performed after intervention. Over 3 years of follow-up, there were no adverse events and Symptomatic improvements such as attention and concentration, interest in studies , sitting tolerance, Motor planning, social interaction & Eye contact improved, and he was now able to fold clothes and operate a computer. Hyperactivity, aggressive behavior And self-biting, stereotypical behaviour, irritability, Inappropriate laughter and irrelevant speech, and temper tantrums were also reduced. IQ scores improved from 46 to 56 and On the Binet Kamath Test of intelligence, his mental age improved to 108 months (pre-cell therapy: 88 months); scores on ISAA improved from 134 to 70. Comparative positron emission tomography-computed tomography. The brain showed improved metabolism in bilateral frontal and temporal cortices and the cerebellum. Autologous bone marrow mononuclear cell therapy may be effective to improve quality of life in individuals with ID with ASD even in adulthood.

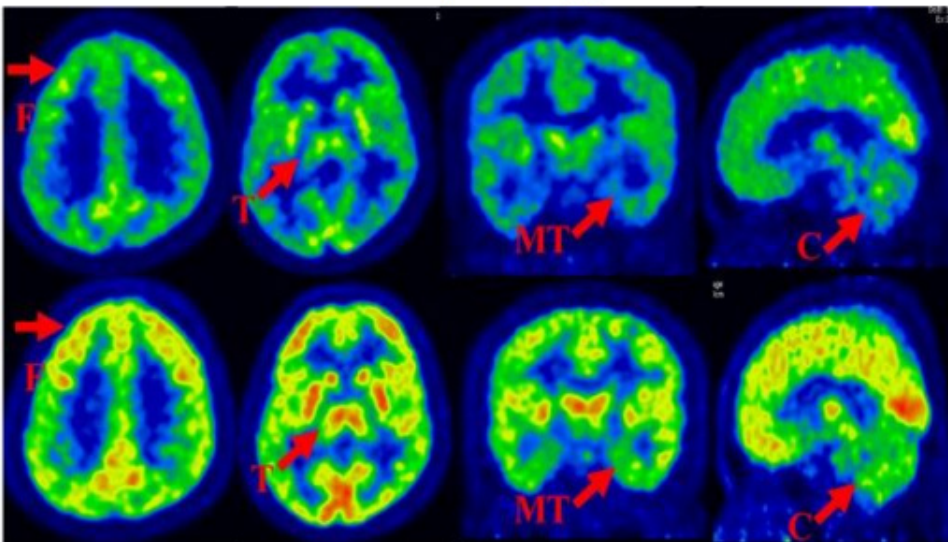


Figure 9: Representative PET CT brain images of the patient with Intellectual Disability and Autism Spectrum Disorder. Top row indicates sections before the intervention of cell therapy. The bottom row indicates PET CT images 2 years post cell therapy. The arrowheads indicate the regions of interest (ROI).

Table 1: Comparison of Binet Kamath Test of Intelligence Pre- and post-cell therapy

	Pre cell therapy	Post cell therapy
Basal age (in months)	84	84
Terminal age (in months)	96	120
Mental age (in months)	88	108
IQ	46	56
Degree of retardation	Moderate ID	Mild ID

2. In 2015 and 2017 We published 2 case reports of individuals diagnosed with moderate ID. They were administered with autologous bone marrow mononuclear cells intrathecally. No major adverse events were recorded in either case. On follow-up, they showed improvements in cognition, attention and concentration, behaviour (hyperactivity, aggression, and temper tantrums had reduced), sitting tolerance, speech, eye contact, etc. These improvements correlated with the improved brain metabolism of the areas responsible. (11,12)

Conclusion-

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

Future Directions

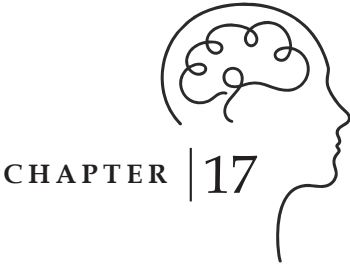
Due to the heterogeneous nature of the disorder, it is a challenge to find a definite cure for intellectual disability. Genetic factors play a major part in intellectual disability (ID) and gene therapy has provided novel insights in treating the genetic aberrations underlying ID. But gene therapy cannot replace the lost neurons and

also poses practical difficulties that have prevented it from being a clinically feasible and viable option for the treatment of ID. Stem cell therapy addresses the core damage occurring in the brain of an individual with ID. The combined approach of gene therapy and stem cell therapy along with standard rehabilitation may help in addressing the gene defect as well as the neuronal dysfunction in the brain. It is also advocated to find the most effective type of cell, the number of cells required and the frequency of the doses to effectively treat the underlying neurological deficit. Future studies should consider the use of PET-CT scans as a monitoring tool and substantiate the effects of cellular therapy in ID. Large-scale, multicentre, and randomized controlled trials are recommended to further establish the safety and efficacy of cellular therapy in ID.

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Role of Cell / Stem Cell Therapy In Cerebellar Ataxia

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

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

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

Unmet medical needs

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

protect the unaffected areas.

Mechanism of action of Stem Cell Therapy:

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

Animal studies

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

Human Studies

A total of 7 studies, comprising 4 clinical investigations and 3 case reports, have been published reporting the effects and safety of diverse stem cells in cerebellar ataxia. Most of the studies used umbilical cord blood cells (13-16). All of them showed that these cells result in neurological and functional improvements. Amariglio et al. transplanted human fetal neural stem cells intracerebellarly and intrathecally in 1 patient with Ataxia Telangiectasia. However, the patient developed a host-derived brain tumor. (17) Shroff et al. transplanted human embryonic stem cells intravenously and intramuscularly in 3 cases of ataxia and reported it to be efficacious. (18) Tsai et al. recently published the results of their Phase I/IIa clinical study conducted on 6 patients with spinocerebellar ataxia type 3. They transplanted allogenic adipose tissue-derived mesenchymal stem cells intravenously. These

cells were safe, as there were no adverse events recorded and also, there was minimal clinical improvement reported. (19)

Published Case report

1. In 2019 Sharma et al. presented a case of an 18-year-old female diagnosed with sensory ataxia who was intrathecally administered autologous bone marrow mononuclear cells (BMMNCs) along with neurorehabilitation. The patient was followed up regularly. Outcome measures such as the Berg Balance Scale (BBS), Functional Independence Measure (FIM), Brief Ataxia Rating Scale, and Modified International Cooperative Ataxia Rating Scale were used to quantify the outcome. Twelve months after intervention, Bed mobility, upper limb fine motor activities and gross motor activities, Sitting and standing balance were Improved Ataxic gait had reduced, and she could walk independently. signs such as ataxia, postural tremors, intention tremors and dysmetria improved. Functional Independence Measure score improved from 106 to 107. The Berg Balance Scale improved from 18 to 34. The Brief Ataxia Rating Scale improved from 7 to 5. The Modified Interna-

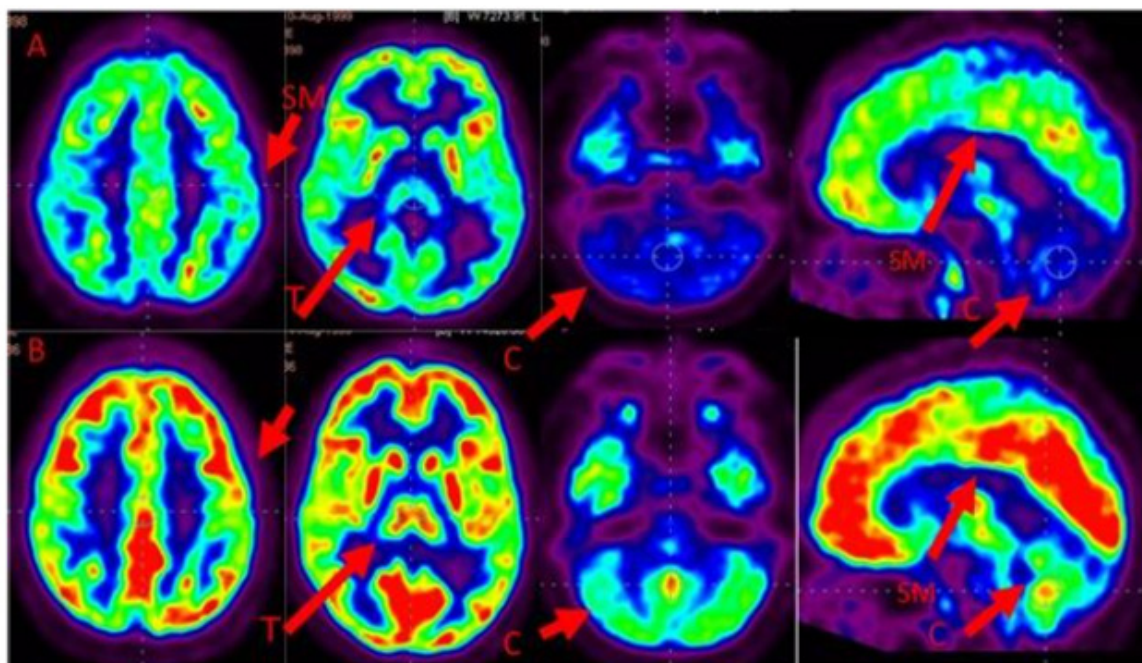


Figure 1: FDG -PET/CT image of a sensory ataxia patient showing reduction of hypometabolism. In the bilateral cerebellum (C). The images were normalized to the SCENIUM normal healthy brain Volumetric template. (A) A PET image before cellular therapy showed reduced metabolism in the bilateral cerebellum (blue – severe damage, yellow-green – normal). (B) A PET image of six months after cellular therapy showed improved metabolism in bilateral cerebellum

tional Cooperative Ataxia Rating Scale improved from 26 to 24. Comparison of the Positron Emission Tomography Computed Tomography (PET CT) image before and 12 months after cellular therapy showed improved metabolism in the bilateral sensory motor cortex, thalamus and cerebellum. These PET findings correlated with symptomatic improvements. The clinical improvements along with PET CT findings suggest that cellular therapy is a beneficial therapeutic modality for sensory ataxia. No major adverse effects were seen.

- 2. In 2014 Sharma et al. presented a case report of a 33-year-old female with Spinocerebellar Ataxia (SCA) who was treated with autologous BMMNCs intrathecal transplantation followed by standard rehabilitation. (20) She had severe impairment of dynamic balance, coordination, speech, gross and fine motor control. Ambulation was dependent, requiring support from two people with an ataxic gait. Functionally, she scored 86 on the Functional independence measure (FIM) and 62 on the 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 the 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 mono-

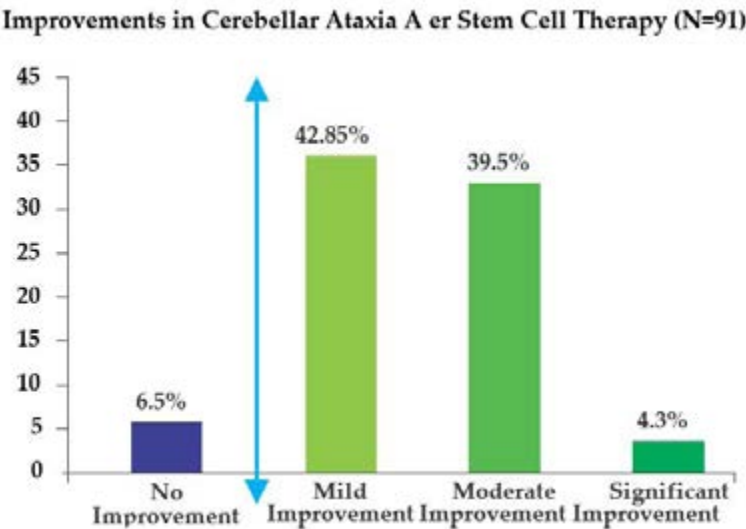


Figure 2: Improvements seen in cerebellar ataxia patients after intrathecal administration of autologous BMMNCs.

nuclear cells in 91 cases of cerebellar ataxia. Symptoms such as co-ordination, ambulation, hand functions, stamina/fatigue, trunk balance and standing were analysed. On follow-up, 93.4% of patients showed improvements, while 6.6% showed no improvement. 49.45% of patients showed mild improvements, 39.5% showed moderate improvements and 4.3% showed significant improvements.

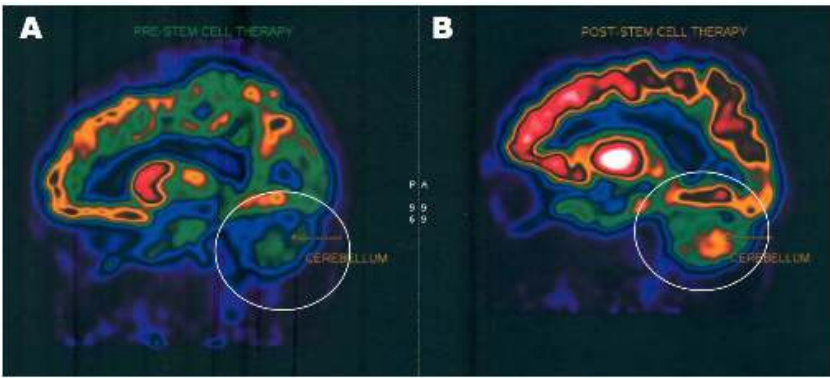


Figure 3: PET CT Scan showing improved metabolic activity, which is indicated by increased orange area in the cerebellum after stem cell therapy, as indicated by the circles.

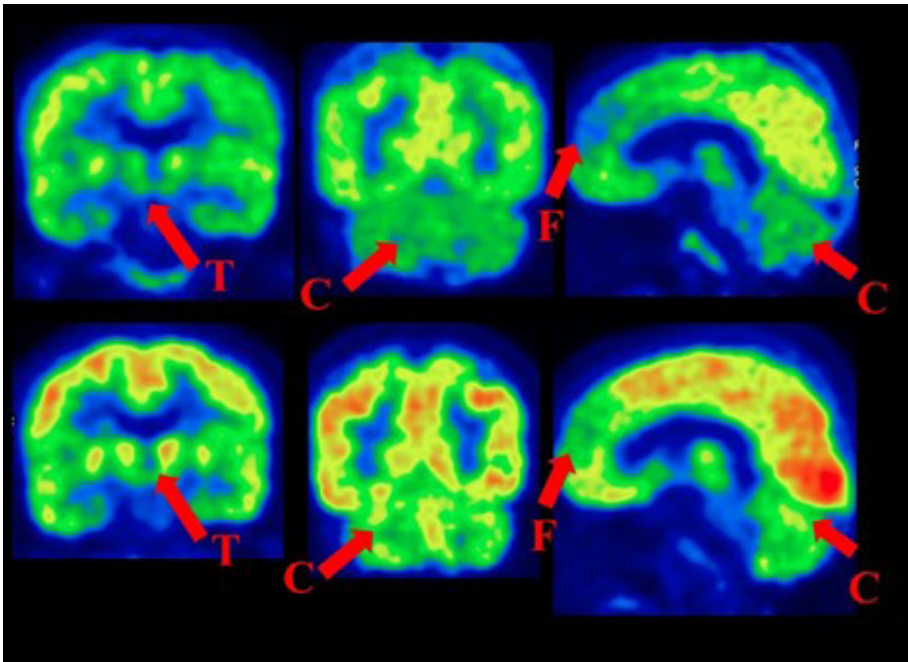


Figure 4: Comparative PET CT scans showing improvements before and after cell therapy in an ataxia patient. A prescan shows reduced metabolism in the bilateral cerebellar region, thalamus, and frontal cortex. Post-scan following cellular therapy shows improvement in Cerebellum (C), Thalamus (T), and Frontal (F) regions of the brain.

Conclusion:

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

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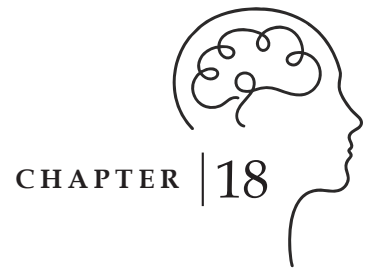
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“If you can dream and not make dreams your master, if you can think but not make thoughts your aim; If you can meet with triumph and disaster and treat those two imposters just the same...if you can fill the unforgiving minute with 60 seconds of distant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

– Rudyard Kipling



Radiological Imaging in Cell / 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 a 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 (fMRI), Diffusion tensor imaging of the muscles (DTI), Positron emission tomography-computed tomography (PET-CT) scan and structural imaging modalities like magnetic resonance imaging (MRI-MSK) and Electromyography.

Functional imaging modalities

The underlying principle of functional neuroimaging using PET-CT scans is that

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 a 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 (fMRI), Diffusion tensor imaging of the muscles (DTI), Positron emission tomography-computed tomography (PET-CT) scan and structural imaging modalities like magnetic resonance imaging (MRI-MSK) and Electromyography.

Functional imaging modalities

The underlying principle of functional neuroimaging using PET-CT scans is that the changes in blood flow and energy consumption in the form of glucose are associated with brain metabolism and tissue function [21, 22]. fMRI 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 [23].

Positron Emission Tomography - Computed Tomography Scan

A PET-CT scan is a functional imaging technique that represents the physiological activity of the brain as three-dimensional images and reflects the functional processes in the body. The physiological activity of the brain is measured using the FDG (fluorodeoxyglucose) uptake by the brain cells. FDG is an analogue of glucose. Glucose transporter proteins transport FDG to the brain cell where it undergoes the metabolic processes. Once it has been converted to glucose 6 phosphate, it cannot be metabolized further and is trapped in the cells, as the cell membrane is impermeable to this molecule [24]. 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 [25]. 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 were distributed evenly in all the areas of the brain. Increased SUV indicates better metabolic activity of the tissue [26]. 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.

MRI Study in Autism:

Large-scale collaborative studies have contributed significantly to our understanding of autism spectrum disease. The EU-AIMS Longitudinal European Autism Project showed that brain development in autism follows an unusual pattern by studying several hundred people throughout childhood and early adulthood. In addition to being measured, differences in cortical thickness and surface area, especially in sensorimotor regions, were also linked to the existence of social and sensory challenges [27, 28]. These findings demonstrate how MRI can correlate clinical characteristics with changes in the developing brain.

MRI in Autism is usually reported to be normal but quantitative MRI analysis has revealed structural connectivity abnormalities in autism. In a large MRI study, Badhe et al. (2024) examined the development of the corpus callosum (CC) – the major fiber tract connecting the cerebral hemispheres – in children with ASD. They found that although callosal length increased moderately with age in ASD (particularly in the anterior midbody segment), the overall size of the corpus callosum was significantly reduced in autism. In particular, autistic children showed a shorter genu and splenium (the anterior and posterior end regions of the CC) compared to age-matched healthy controls. The genu connects the prefrontal cortices and coordinates the functions like decision making, planning, motor control between right and left hemisphere, while the splenium connects the two occipital lobes, allowing to process the visual information and integrating the sensory data between two occipital lobes.

These differences indicate an abnormal developmental trajectory of the corpus callosum in ASD, with the callosal “end-points” (involved in prefrontal and occipital interhemispheric connectivity) lagging in growth. Such callosal underdevelopment aligns with theories of long-range cortical underconnectivity in autism and could underlie the deficits in integration of information between brain hemispheres. Notably, Badhe et al. report that callosal length correlates with age in ASD ($r \approx 0.4$), but callosal volume and height did not change significantly with age, underscoring that the CC in autism not only starts off smaller but also fails to catch up over time. [29].

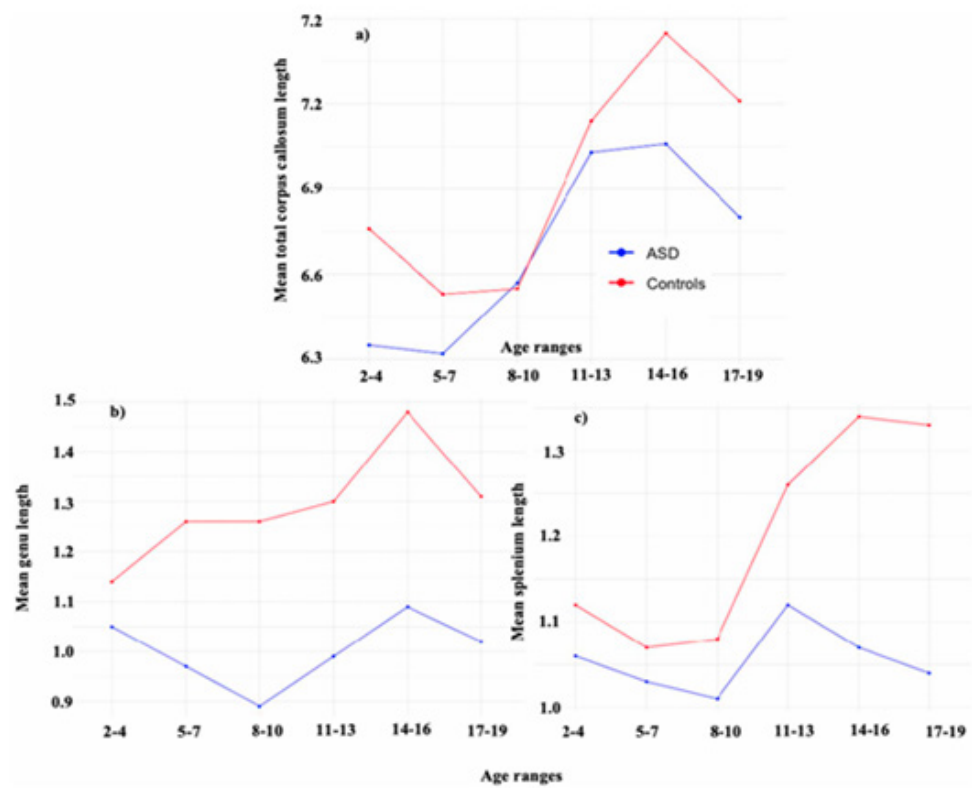
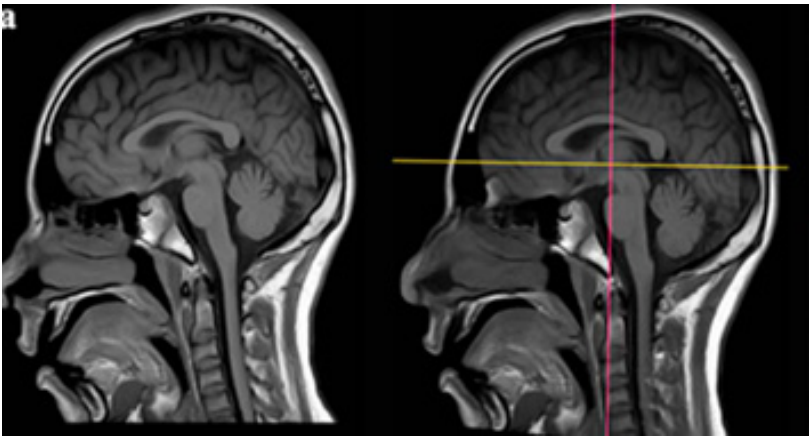
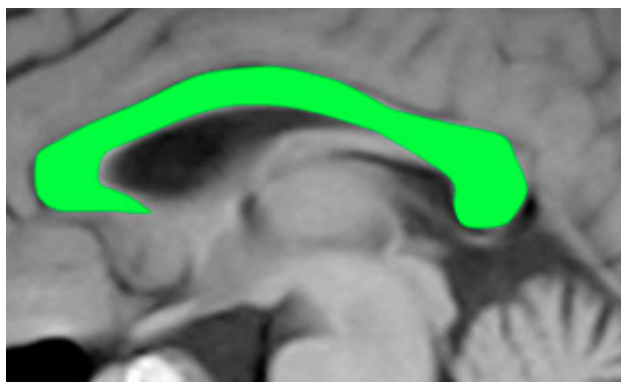


Figure 1: Comparison line plot of mean length of the total (A), genu (B), and splenium (C) of the corpus callosum across groups in ASD and controls.



(a)



(b)

Figure 2: (a) Localization of the corpus callosum as seen on a sagittal slice of a representative anatomical T1-weighted MRI image. (b) Reoriented image zoomed 3-fold, where the corpus callosum is highlighted in green along the mid-sagittal section.

PET-CT study in Autism:

1. A 6-year-old boy diagnosed with 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 the Indian scale for assessment of autism (ISAA). His PET-CT scan showed hypometabolism in the regions of the superior temporal gyrus, amygdala 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 (Figures 3A, B and C). He showed improvement in nonverbal communication, eye contact, social interaction, attention and concentration. Aggressive and repetitive motor behaviour had reduced significantly. His ISAA score also showed improvement; the intensity of all symptoms reduced and therefore the score reduced from 123 to 103. In view of these improvements, he underwent a 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.

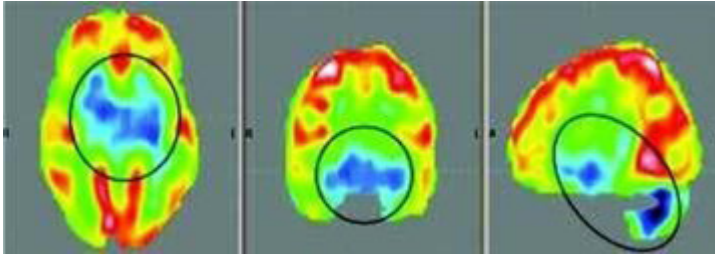


Figure 3A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum

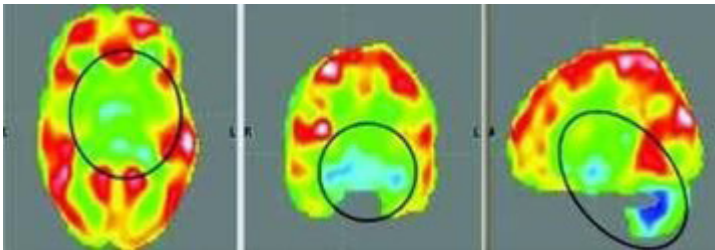


Figure 3B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of the superior temporal gyrus, mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum, indicated by reduction in the blue and black areas

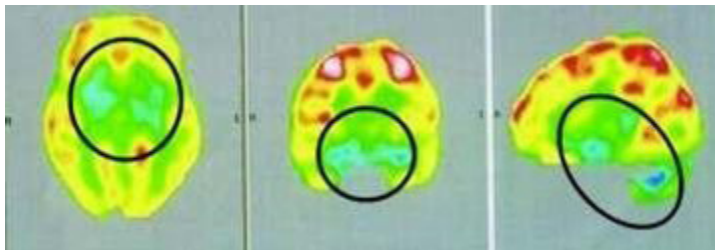


Figure 3C: PET-CT scan 8 months after second cellular transplantation showing maintained increase in the metabolism of the superior temporal gyrus, mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum and reduction in the metabolism cortical regions showing the balancing effect

2. A 13-year-old male and a known case of autism were treated with autologous bone marrow mononuclear cells intrathecal transplantation. He was born full term by normal delivery, with a 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 with 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 the PET-CT scan as increased metabolism in the region of the bilateral occipital lobes and mesial temporal structures.

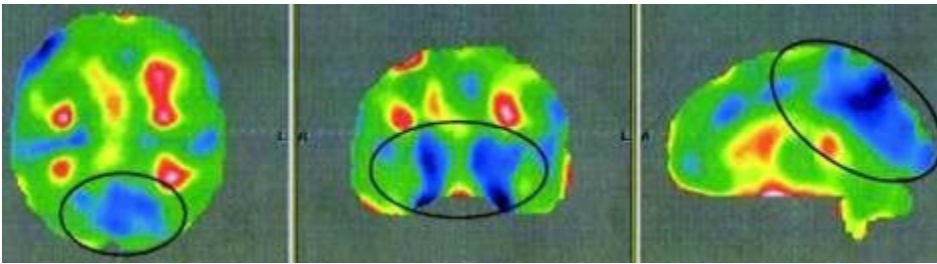


Figure 4A: PET-CT Scan before stem cell transplantation suggestive of reduction in the metabolism of occipital lobes and mesial temporal structures bilaterally

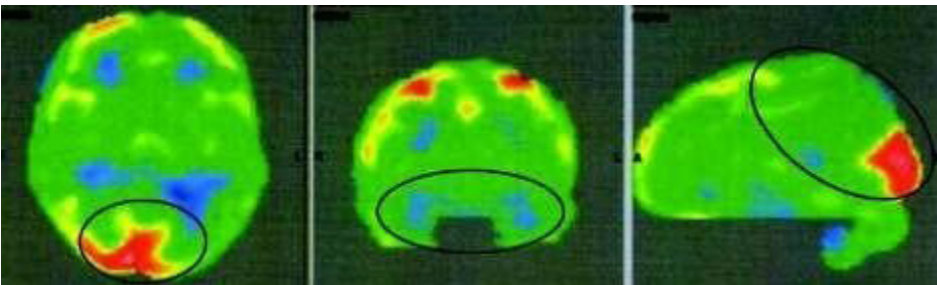


Figure 4B: PET-CT scan after stem cell transplantation showing improved metabolism in bilateral occipital lobes mesial temporal structures

3. A 9-year-old girl with autism underwent autologous BMMNCs intrathecal transplantation. Her main symptoms were poor attention and concentration, social interaction difficulties, difficulty in adapting to a changed environment, the presence of repetitive and strange movements, poor eye contact, irrelevant speech and complete dependence for ADLs. 6 months after the first transplantation, she showed improvements in eye contact, non-verbal communication, and learning; a reduction in laughing without reason; an improvement in command following; an understanding of relationships; reduced hyperactivity; and started picking up well in ADL training. These changes also correlated with the PET-CT scan showing improvement in the metabolism of mesial temporal lobes, amygdala, hippocampus, and cerebellum bilaterally (Figure 5B).

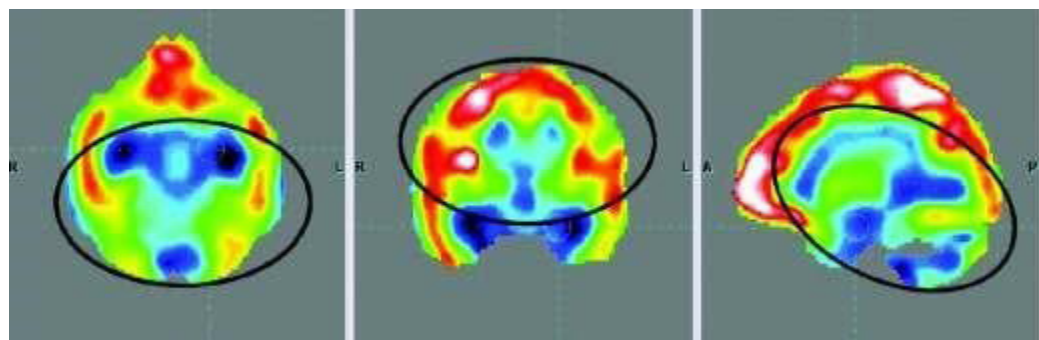


Figure 5A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum

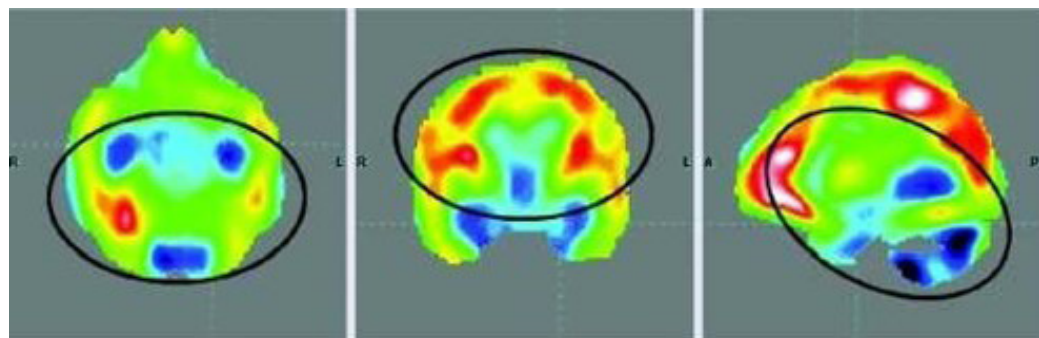


Figure 5B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of mesial temporal lobes, amygdala, hippocampus, and cerebellum bilaterally, indicated by reduction in the blue and black areas

4. An 11-year-old boy with poor attention and concentration, increased hyperactivity, poor social interaction, abnormal presence of stereotypical behaviour, poor eye contact, and monosyllable speech was treated with cellular therapy. The PETCT scan before stem cell therapy was suggestive of reduced metabolism in the temporal lobes, mesial temporal lobes, cingulate and paracingulate regions, cerebellum, amygdala, hippocampus and parahippocampus. Six months after cellular therapy, he showed a reduction in hyperactivity; he could sit in 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 the temporal lobes, mesial temporal lobes, cingulate and paracingulate regions, cerebellum, amygdala, hippocampus and parahippocampus.

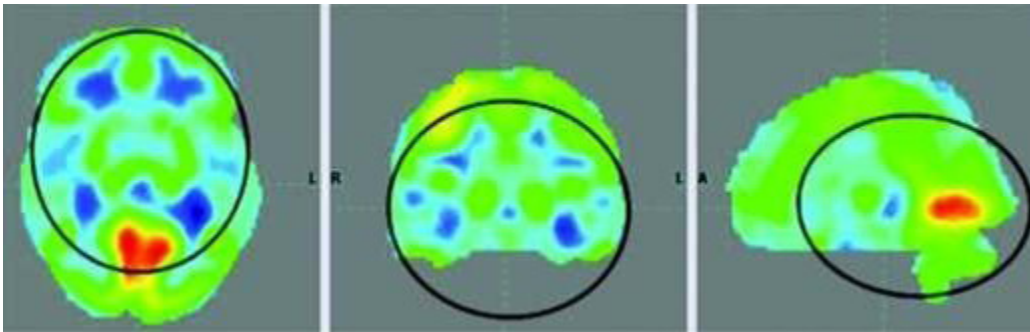


Figure 6A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of mesial temporal lobes, amygdala, hippocampus, cingulate regions and cerebellum

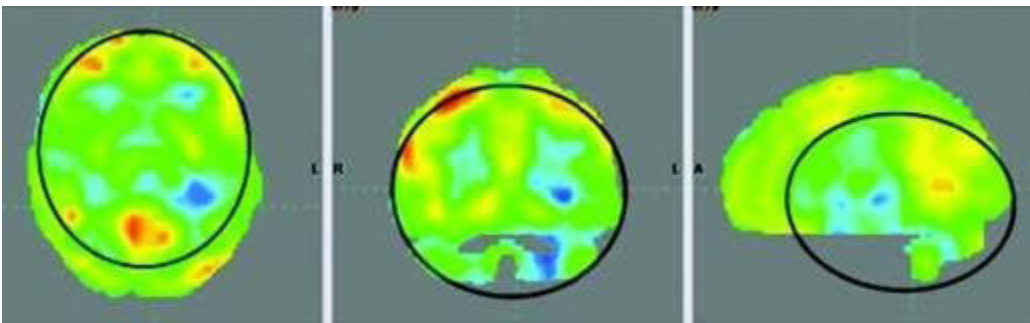


Figure 6B: PET-CT scan after cellular transplantation showing increased metabolism in the regions of mesial temporal lobes, amygdala, hippocampus & cerebellum bilaterally, indicated by reduction in the blue & black areas

5. A 4-year-old 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; and social interaction, attention and concentration improved. These clinical improvements also correlated with the changes observed in PET-CT scan findings.

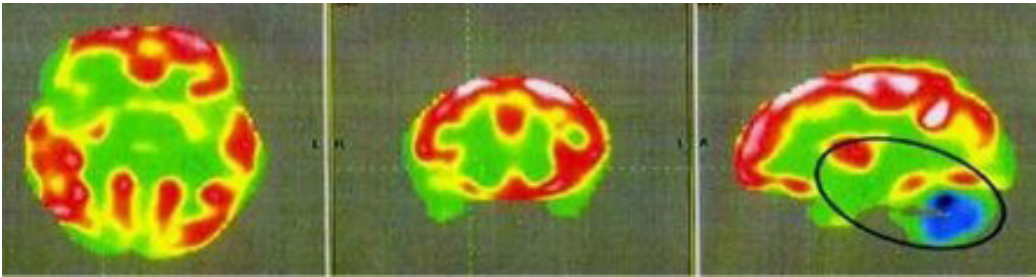


Figure 7A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of bilateral cerebellum

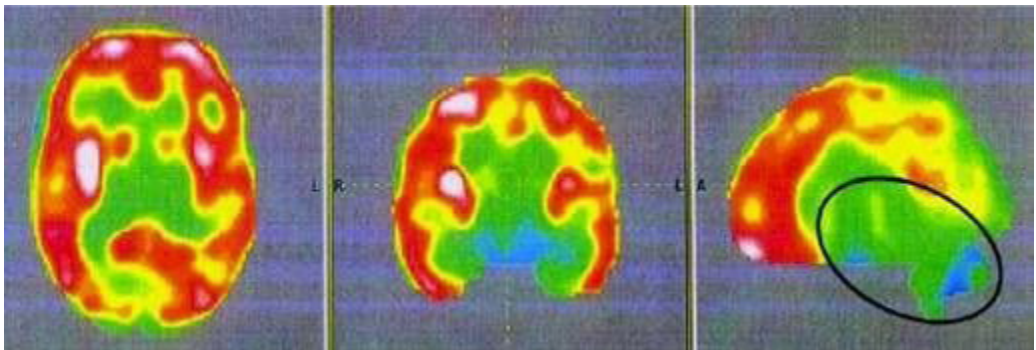


Figure 7B: PET-CT scan after cellular transplantation showing increased metabolism in the regions of cerebellum bilaterally, indicated by reduction in the blue and black areas

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

ity and stereotypical behaviours. A PET-CT scan correspondingly showed improved metabolism in basal ganglia, mesial temporal structures and cerebellar lobes bilaterally.

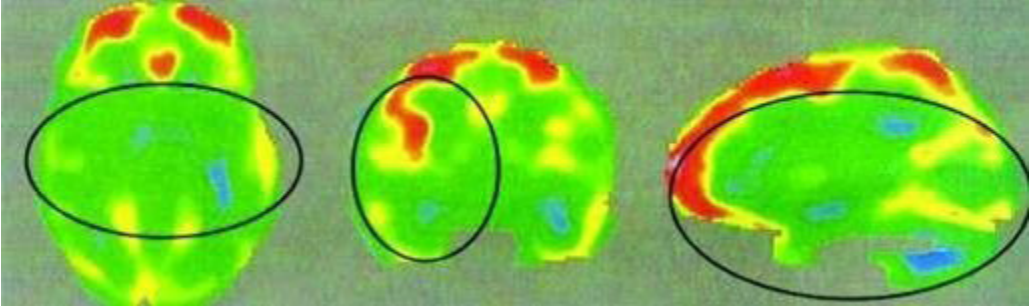


Figure 8A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour and as outlined by the circles in the regions of basal ganglia, mesial temporal structures and cerebellum

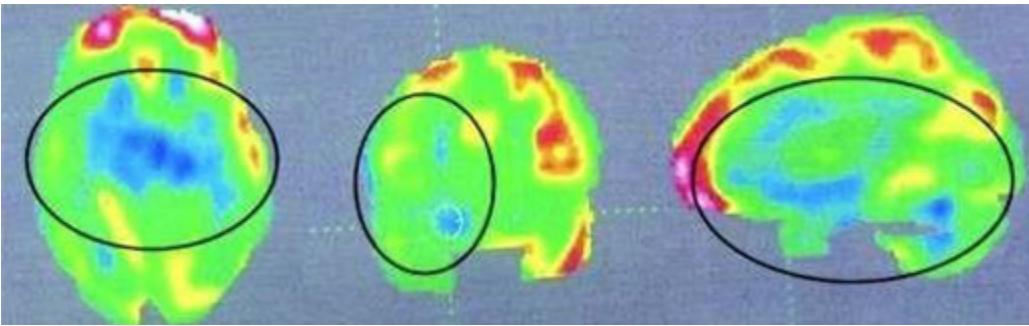


Figure 8B: PET-CT scan after cellular transplantation showing increased metabolism as outlined by the circles in the regions of basal ganglia, mesial temporal structures and cerebellum

7. A 7-year-old boy diagnosed with autism underwent autologous BMMNCs intrathecal transplantation. His main symptoms were poor social interaction, crying spells, hyperactivity, poor eye contact, temper tantrums, poor command following and complete dependence for daily activities. His PET-CT scan (Figure 9A) 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 behaviour. These

improvements correlated with the PETCT scan changes of balancing of the brain metabolism, as shown in Figure 9B.

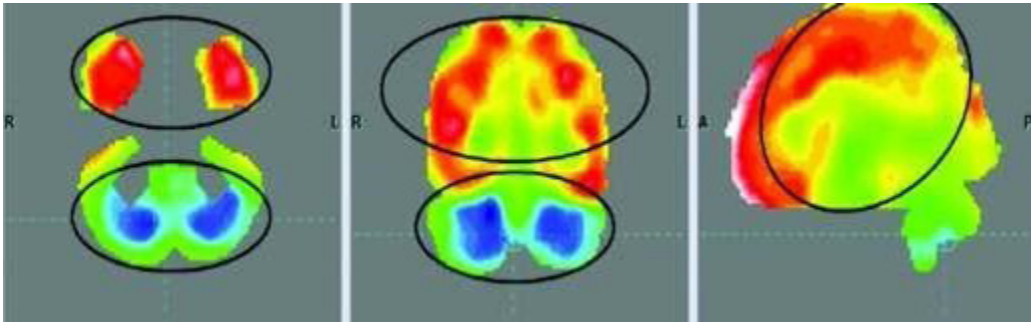


Figure 9 A: PET-CT scan before stem cell therapy showing diffuse increase in the metabolic activity of the cortical lobes and reduced metabolic activity in bilateral cerebellar lobes.

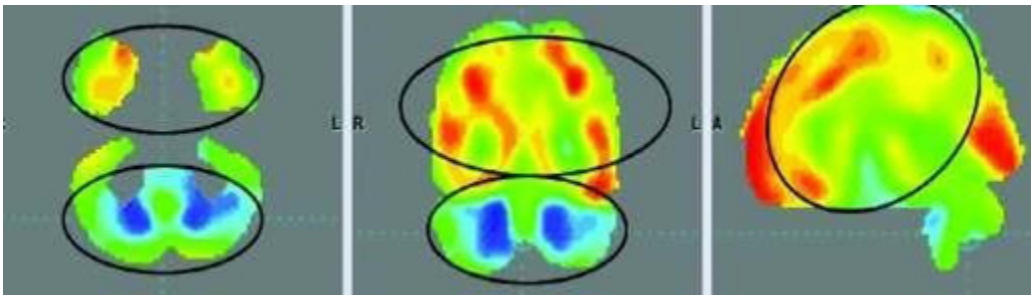


Figure 9B: PET-CT scan after stem cell therapy showing reduction in the metabolic activity of the cortical lobes and increased metabolic activity in bilateral cerebellar lobes, hence highlighting the balancing effect of cellular therapy

Our Publication:

According to our baseline investigation, Sharma et al. (2018) discovered that, in contrast to the pattern of typical development, the global glucose absorption in autistic children falls linearly with age. Children with ASD under 5 years old actually showed higher than normal metabolism in certain regions, whereas older autistic children exhibited pronounced hypometabolism relative to age-matched controls. Regionally, the ASD group demonstrated significantly lower metabolic activity in the limbic and subcortical areas – including the amygdala, hippocampus, parahippocampal gyrus, caudate, thalamus, and cerebellum – along with parts of the temporal lobes, while showing higher metabolism in some primary sensory cortices (notably the calcarine fissure of the visual cortex and Heschl's gyrus in the auditory cortex). These PET findings suggest an atypical neurodevelopment-

tal trajectory of brain function in autism, where early-life hypermetabolism gives way to widespread hypometabolism as the child ages. Such metabolic patterns help establish baseline functional deficits in ASD brains, which can be valuable for tracking changes after therapeutic interventions (for example, to see if metabolic activity in these regions improves following stem cell therapy) [30]

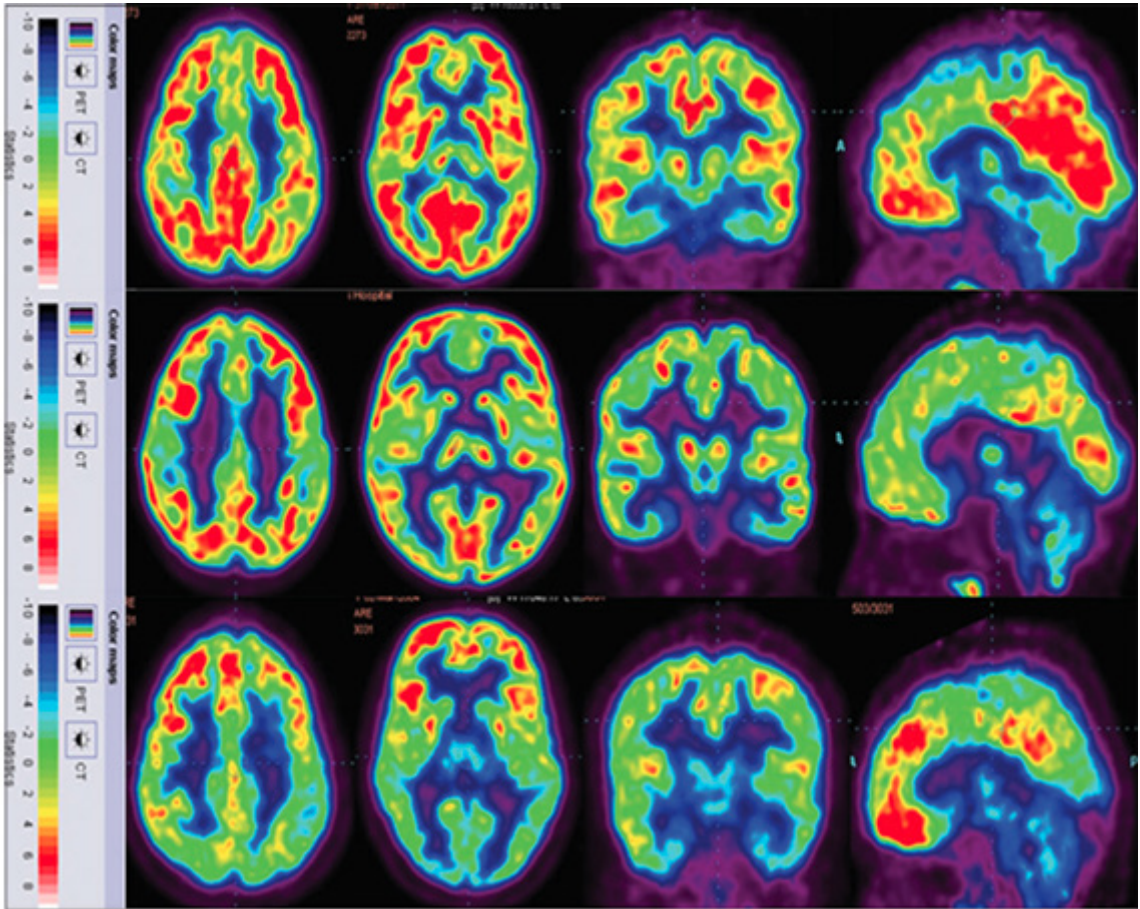


Figure 10: Representative FDG PET-CT brain scans of children with autism at ages 4 years (top row), 8 years (middle), and 12 years (bottom). Red, orange and yellow colours indicate higher glucose metabolism, while cool blue areas indicate hypometabolism. Notably, the 4-year-old shows relatively preserved metabolic activity with only a small hypometabolic region (hippocampus), whereas the 8- and 12-year-old scans reveal progressively larger hypometabolic areas in the cerebellum, thalamus, amygdala, hippocampus, and temporal lobes. This illustrates the age-dependent decline in brain metabolism observed in autism spectrum disorder (ASD) patients.

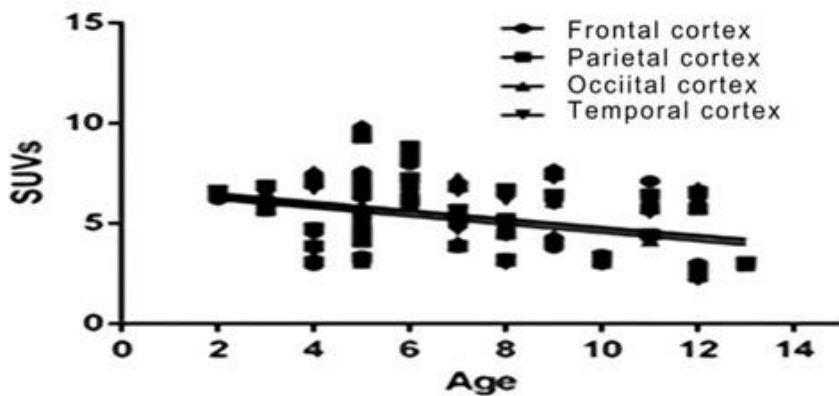


Figure 11: The trajectory of brain metabolism in children with autism shows a linear decline.

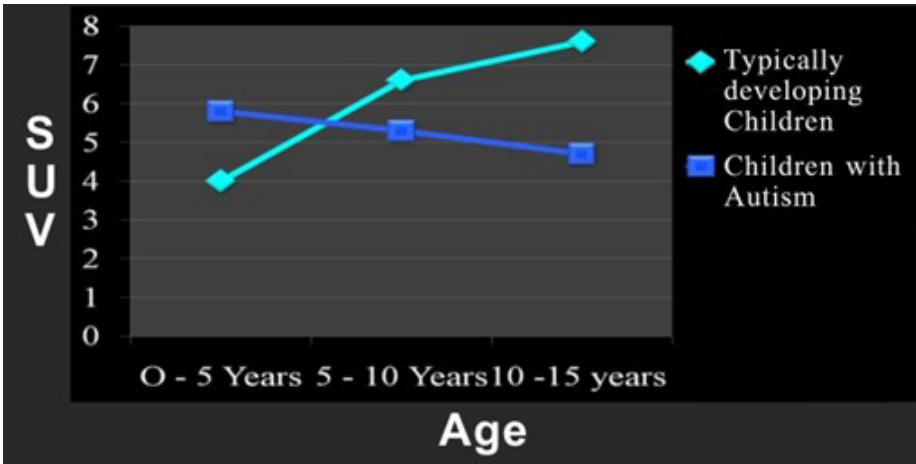


Figure 12: Comparison of the trajectory of brain metabolism in typically developing children and children with Autism

MRI study in stroke:

In stroke, MRI plays an essential role in identifying tissue that is irreversibly damaged and areas that may still be salvageable. Recent large randomized clinical trials, including RESCUE-Japan LIMIT and SELECT2, have shown that even patients with large strokes benefit from thrombectomy if imaging demonstrates sufficient viable brain tissue [31, 32]. This shift in practice underscores how diffusion and perfusion MRI can guide treatment selection and may also serve as valuable tools to monitor recovery in future stem cell therapy studies.

Our publications

PET-CT study in Stroke:

1. In a case of chronic stroke caused by ischemia in the territory of the 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 the brain in the regions of the parietal lobes. The standard uptake of FDG in the 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.

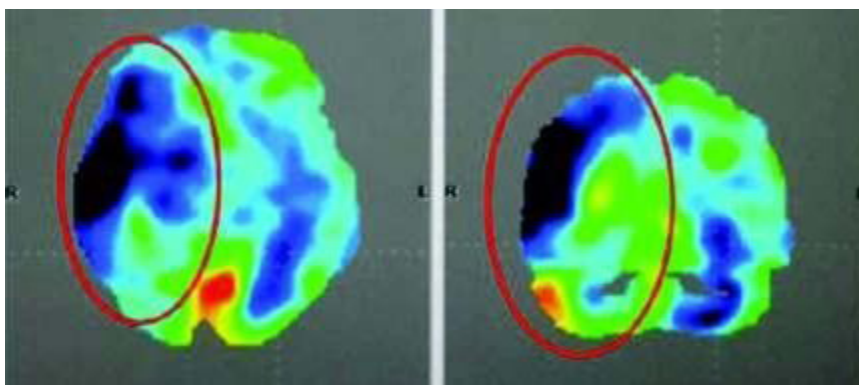


Figure 13A. PET-CT scan before Autologous BMMNCs transplantation suggestive of stroke in the region right MCA territory with black areas suggestive of gliosis, whereas as the blue regions suggestive of penumbra

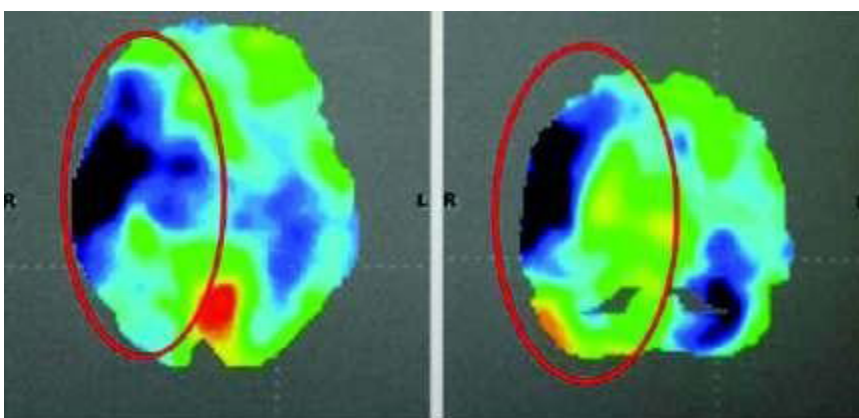


Figure 13B. PET-CT scan 1 year after Autologous BMMNCs transplantation showing improved metabolism in the parietal lobe penumbral regions seen as reduction in the blue coloured areas

2. A 58-year-old man with an ischemic stroke presented with poor control of the right upper and lower extremities, slurred speech, impaired cognition and behaviour, increased spasticity in the muscles of the upper limb, poor balance while walking and a 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 regions of the left frontal lobe, occipital lobe and basal ganglia.

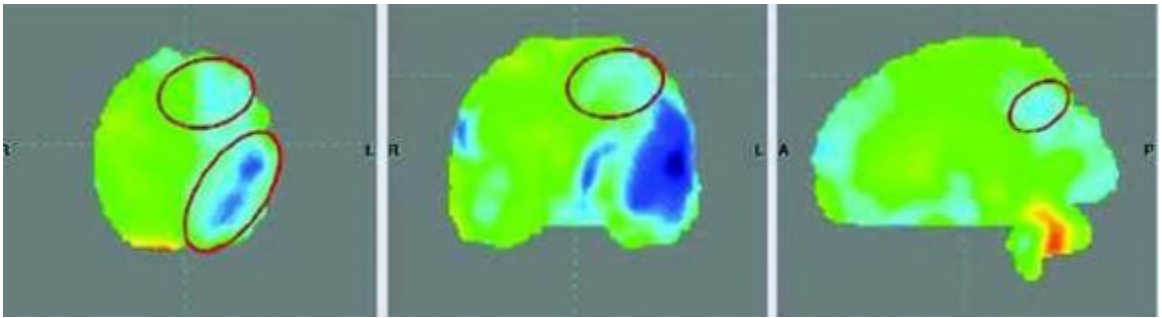


Figure 14A: PET-CT scan before Autologous BMMNCs transplantation suggestive of reduced metabolism in the regions of left frontal lobe, occipital lobe and basal ganglia

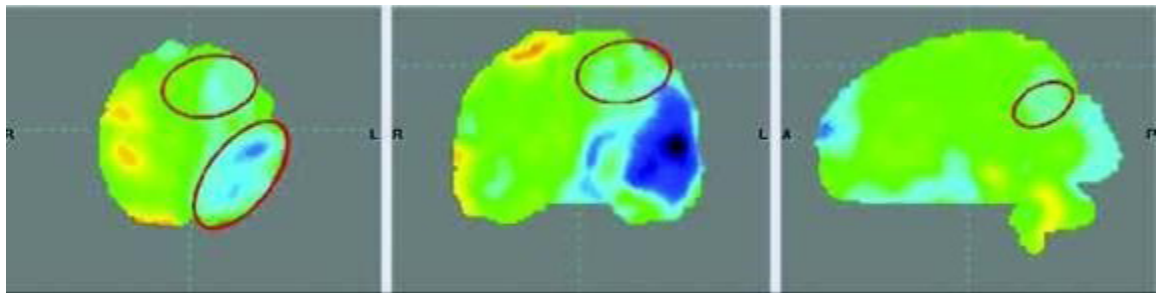


Figure 14B: PET-CT scan after Autologous BMMNCs transplantation suggestive of increased metabolism in the regions of left frontal lobe, occipital lobe and basal ganglia

PET-CT study in Hemorrhagic Stroke

Cellular transplantation in the case of chronic hemorrhagic stroke involving frontal, parietal lobes and cerebellum and brainstem was performed 1 year after the transplantation. There was increase in the metabolism of the brain in PET-CT scans. The increase in the metabolism was noted in the regions of Frontal lobe,

Parietal lobe and cerebellum. Clinically this increased metabolism was correlated with improved cognition, balance, motor function, functional independence and speech intelligibility as well as reduction in spasticity.

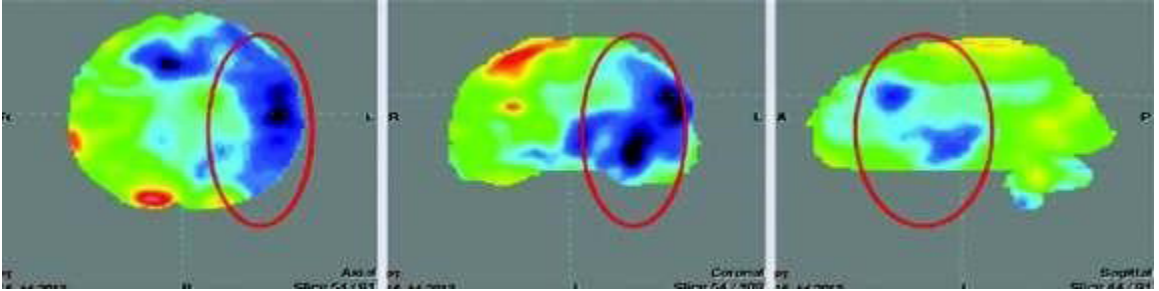


Figure 15A: PET-CT scan before autologous BMMNC transplantation suggestive of hemorrhagic stroke in the left parietal lobe with black areas suggestive of gliotic areas, whereas as the blue regions suggestive of penumbra

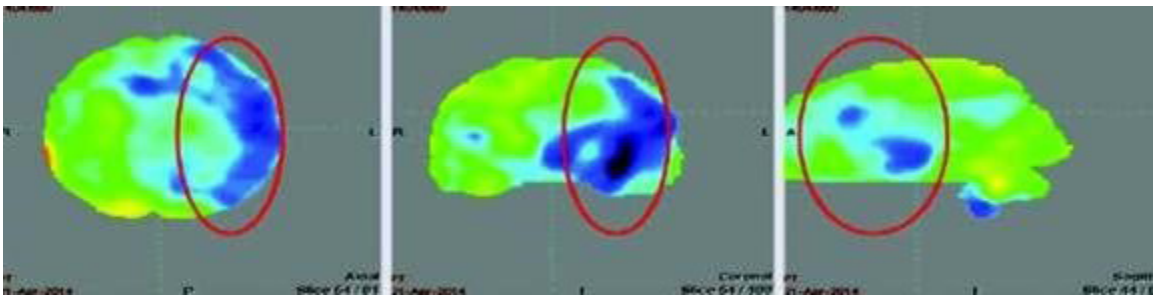


Figure 15B: PET-CT scan 6 months after autologous BMMNC transplantation shows reduced blue areas signifying improved metabolism of the penumbral regions

Functional MRI

Spinal Cord Injury

A patient with traumatic paraplegia due to a complete spinal cord injury at the level of the 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 the adductors of the hip, showing strength of grade 1 on manual muscle testing. He also had minimal sensory recovery. Bladder and bowel sensations had improved. This improvement is reflected in the functional MRI showing a greater

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.

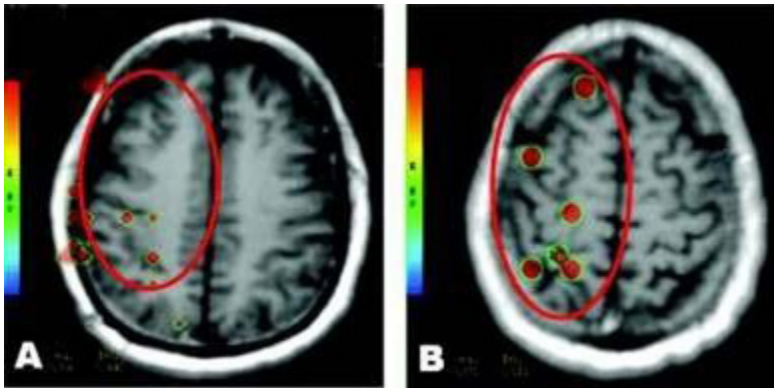


Figure 16: FMRI showing changes after stem cell therapy

PET-CT Study in Traumatic Brain Injury:

1. A 15-year-old girl presented to us with a history of RTA at the age of 8 years, 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 the bilateral parieto-occipital and right anterior inferior temporal regions. She underwent autologous bone marrow mononuclear cell transplantation with intensive multidisciplinary rehabil-

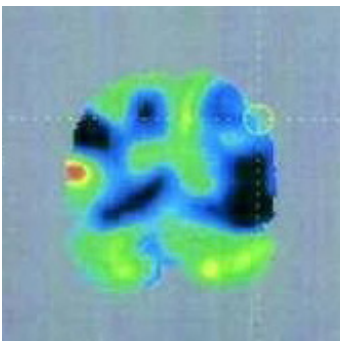


Figure 17A: PET-CT scan before autologous BMMNC transplantation showing gliotic areas in black with areas of reduced metabolism in blue seen in bilateral parieto-occipital & right anterior inferior temporal regions.

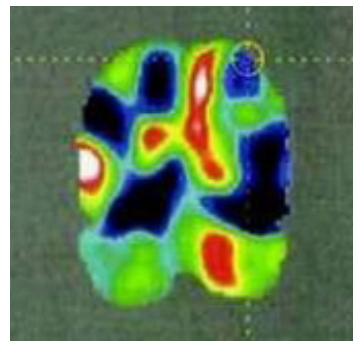


Figure 17B: PET-CT scan after AutologousBMMNCs showing improved metabolism in the regions of the anterior, middle and posterior cingulate gyrus, amygdala, hippocampus and parahippocampus.

itation. Post-SCT improvements were seen throughout, with maximum changes occurring by 6 months. Improvements were seen in her short-term memory, behaviour, 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 right-sided gross motor and fine motor coordination and reduced neglect on her right side with an overall improvement in her bilateral coordination. 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 the posterior cingulate gyrus. The parahippocampal 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.

2. A 34-year-old patient with a traumatic head injury was treated with autologous BMMNCs intrathecal transplantation. Due to the traumatic injury, he had developed right hemiplegia, increased muscle tone in the right upper and lower extremity, dysarthria, poor sitting and standing balance, an inability to walk without support and subnormal cognition. His PET-CT scan showed reduced metabolism in the region of the 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 the cerebellum.

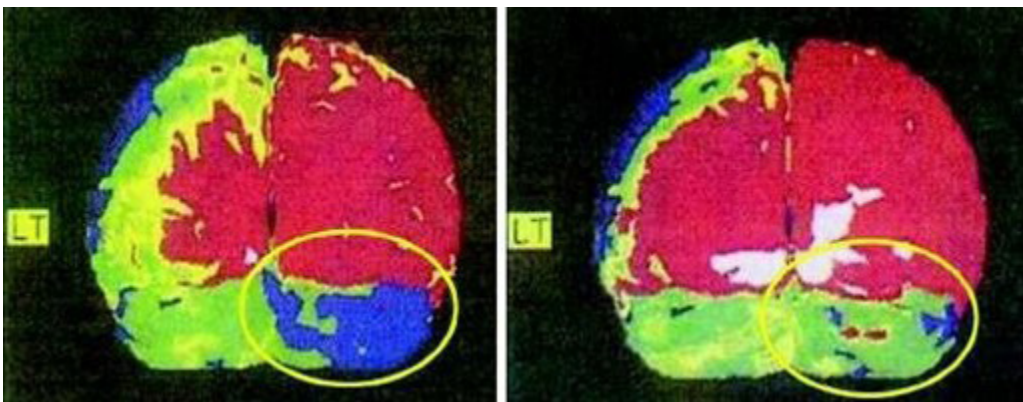


Figure 18: PET-CT scan showing improved metabolic activity, which is indicated by a decrease in blue areas after stem cell therapy.

PET-CT study in 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 handwriting, 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 19A). 6 months post-cellular therapy, he showed significant improvement in fine motor activities, gait and balance. He required only minimal help for his activities of daily living. PET-CT showed improved metabolism in all the areas of reduced FDG uptake (Figure 19B)

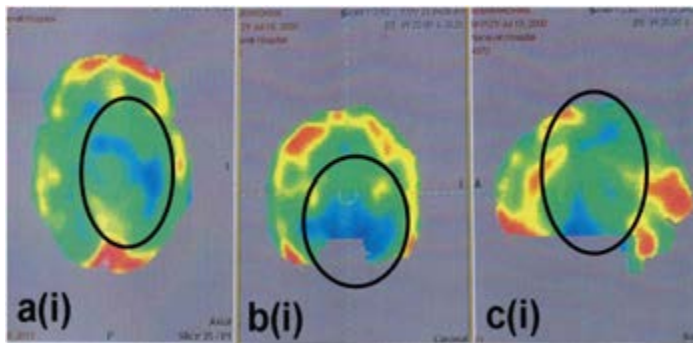


Figure 19A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of cerebellar lobes and mesial temporal structures

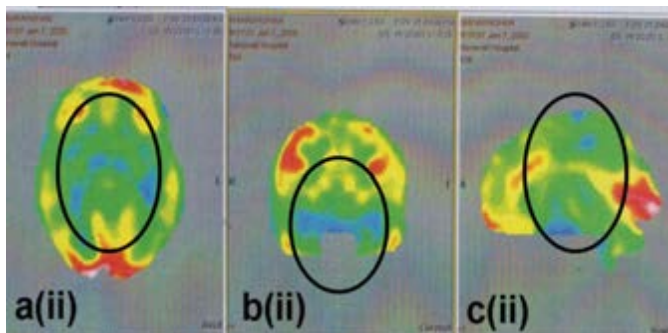


Figure 19B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of cerebellar lobes and mesial temporal structures seen as reduced blue and black areas

2. An 8-year-old child born of a consanguineous marriage, delivered by caesarean section, presented with a history of brain hypoxia due to a delayed cry after birth and also had a seizure 5 hours after birth. He was diagnosed with CP and the main symptoms were the presence of abnormal reflexes, fluctuating tone, poor voluntary control and poor cognition. His PET-CT scan findings suggested reduced metabolism in the regions of the hippocampus, basal ganglia and cerebellum bilaterally (Figure 20A). 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 the hippocampus, basal ganglia and cerebellum bilaterally (Figure 20B).

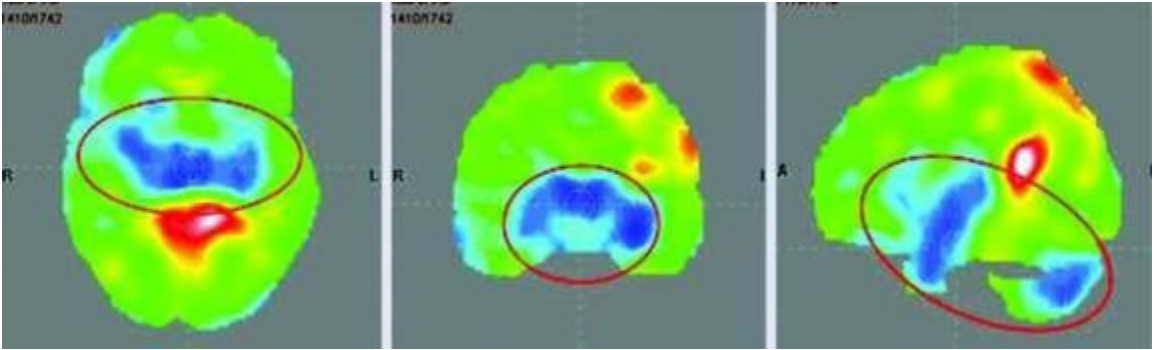


Figure 20A: PET-CT scan before cellular transplantation showing reduced metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally as indicated by the circles

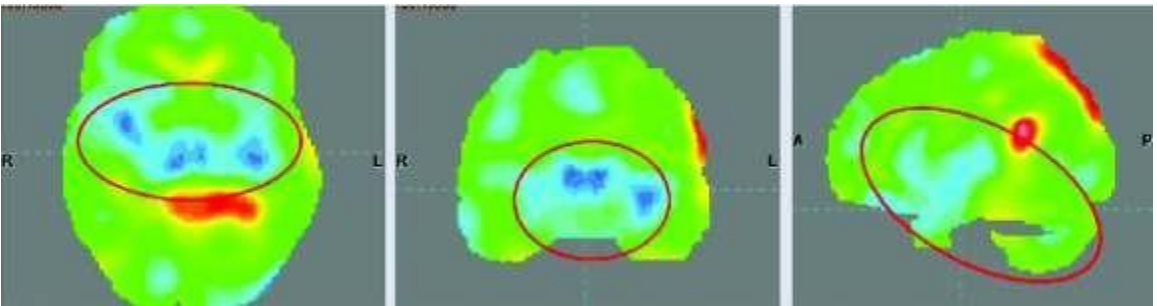


Figure 20B: PET-CT scan 9 months after cellular transplantation showing increased metabolism in the regions of hippocampus, basal ganglia and cerebellum bilaterally as indicated by the circles

3. A 12-year-old boy diagnosed with cerebral palsy was treated with autologous BMMNCs intrathecal transplantation. He presented with symptoms like increased muscle tone, poor voluntary control of bilateral lower extremities and fair fine motor activity of the 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 the hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes (Figures 21 A & B).

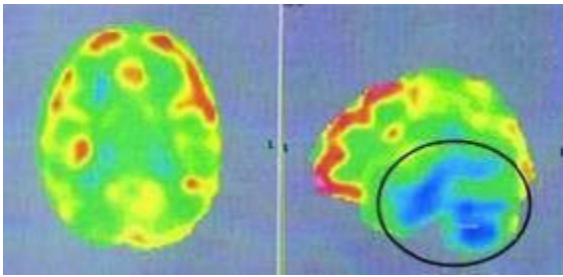


Figure 21A: PET-CT scan before cellular transplantation showing reduced metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes bilaterally as indicated by the circles

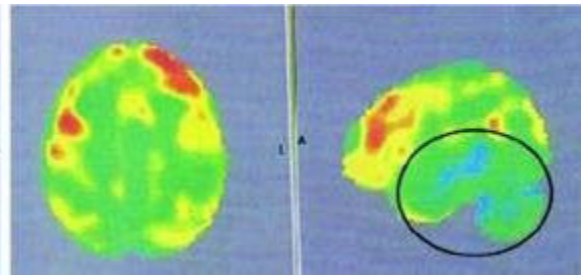


Figure 21B: PET-CT scan after cellular transplantation showing improved metabolism in the regions of hippocampus, basal ganglia, thalami, mesial temporal structures and cerebellar lobes bilaterally as indicated by the circles

PET-CT study in 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 an Intelligence Quotient (IQ) score of 44 with affected fine motor activities, balance, speech and higher functions. A PET-CT scan identified the frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus as the affected areas (Figure 22A). He was treated with cellular therapy of autologous BMMNCs intrathecal transplantation followed by multidisciplinary rehabilitation. Six months following therapy, he showed improvement in social behaviour, speech, balance, daily functioning and IQ score, which increased to 55. A PET-CT scan showed a significant increase in metabolic activity in all four lobes, mesial temporal structures and the left cerebellar hemisphere. The clinical improvements correlated with the changes observed in the PET CT scan (Figure 22B).

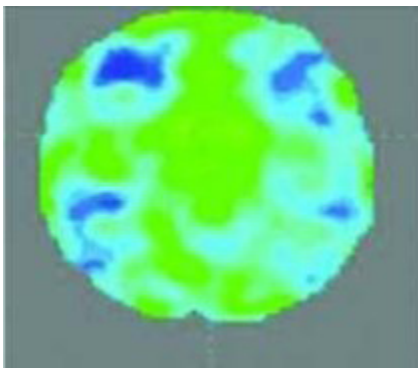


Figure 22A: PET-CT scan before cellular transplantation showing hypometabolism highlighted in blue and black colour in the regions of frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus

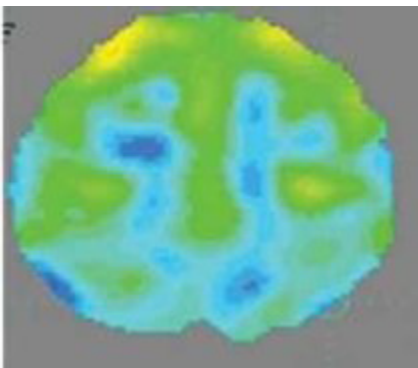


Figure 22B: PET-CT scan 6 months after cellular transplantation showing increased metabolism in the regions of the frontal, temporal, parietal, occipital, left cerebellar lobes, amygdala, hippocampus, and parahippocampus, indicated by reduction in the blue and black areas.

PET-CT study in Dopamine-responsive Dystonia:

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.

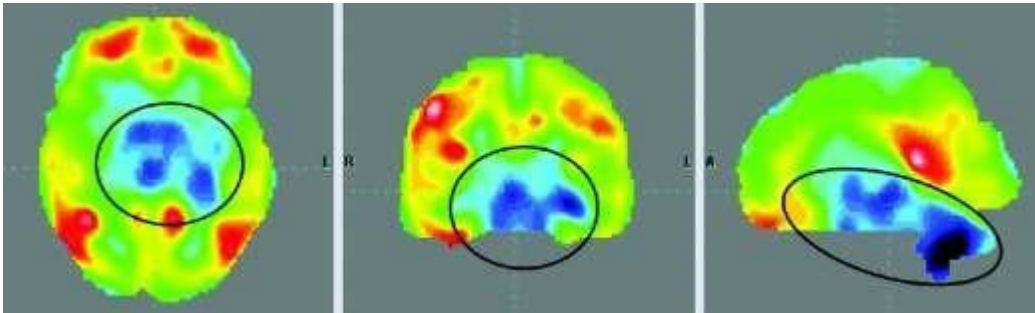


Figure 23A: PET-CT scan before stem cell therapy showing reduction in the metabolic activity of the cerebellar lobes and thalami bilaterally

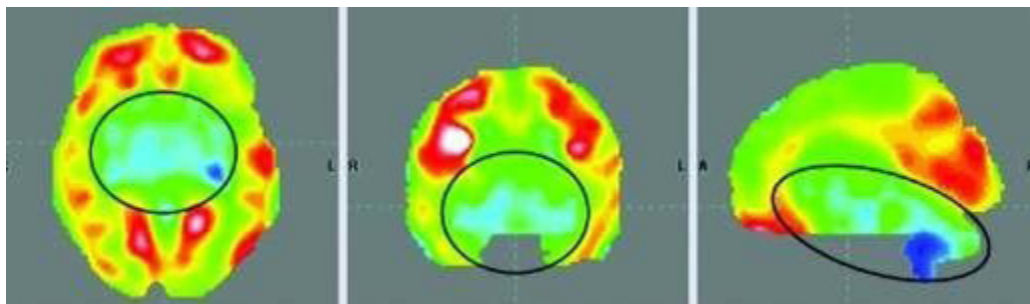


Figure 23B: PET-CT scan after stem cell therapy showing increased metabolic activity in bilateral cerebellar lobes and thalami bilaterally

PET-CT study in Dementia

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 the Mini Mental Status examination, she got a score of 10/30, indicating severe dementia. A PET scan showed global hypometabolism in the brain. Bilateral parietal lobes showed moderately reduced FDG uptake and bilateral frontal and temporal lobes showed mild reduction. She underwent autologous bone marrow-derived mononuclear cell transplantation. At the follow-up assessment, improvements were noted in terms of her cognition, behaviour and physical activities. On the MMSE, her scores improved from 13/30 to 16/30 at the 6-month 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 the PET-CT scan there was an increase in FDG uptake noted in the regions of the bilateral parietal, frontal and temporal lobes (Figures 24 A and 24B).

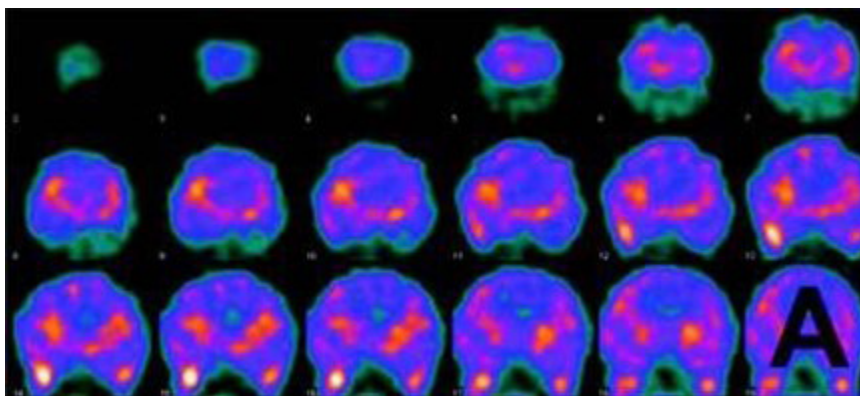


Figure 24A: Pre-stem cell. There is overall hypometabolism seen in the brain, with purple areas depicting areas of hypometabolism and orange areas as areas of normal metabolism

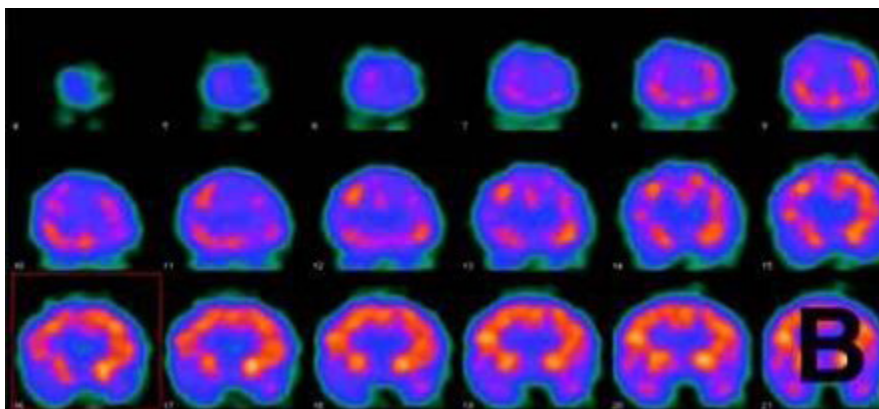


Figure 24B. *Post stem cell. As compared to Fig A, there is an increase in the orange area, which is the area of normal metabolism, and a reduction in the purple area. This suggests that after stem cell therapy, there is increased metabolic activity in the brain, thus improved neural activity.*

PET-CT study in Cerebellar Ataxia

An 18-year-old girl with progressive cerebellar ataxia was treated with autologous BMMNCs intrathecal transplantation. The symptoms were progressive, beginning at the age of 3 years. 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 the head and visual focusing deficits. She slowly regressed in her physical abilities

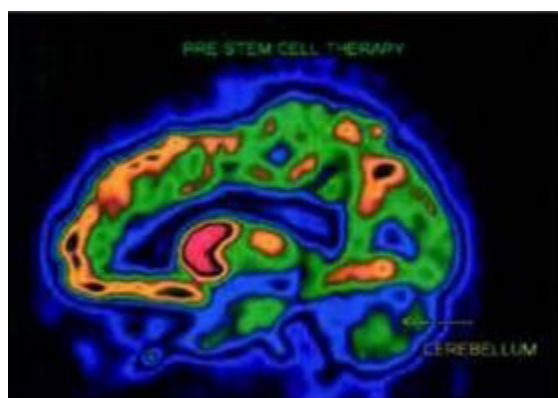


Figure 25A: *Pre-stem cell therapy there is hypometabolism seen in bilateral cerebellar lobes*

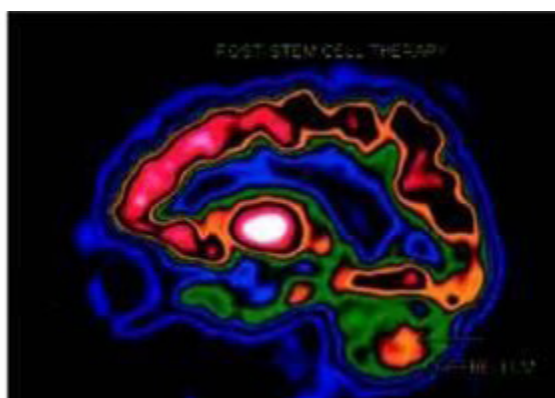


Figure 25B: *Post stem cell therapy there is increased metabolism seen in bilateral cerebellar lobes*

and was wheelchair-bound since the age of 15. 6 months after the 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 Figures 25 A & B.

MRI study in ALS:

Additionally, MRI has shed light on neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). While tract imaging shows deterioration of corticospinal circuits, quantitative susceptibility mapping has shown aberrant iron accumulation in motor areas of the brain. According to these findings, MRI may offer objective biomarkers for clinical monitoring, as they correspond with the severity and development of the disease (33, 34). These methods might potentially help with the assessment of cell-based treatments in the future.

Diffusion Tensor Imaging-MRI (DTI-MRI) in ALS:

DTI is a white matter-focused, MRI-based technique that sensitively detects microstructural changes in neural pathways. Key DTI metrics include fractional anisotropy (FA), which reflects overall fibre integrity; axial diffusivity (AD), indicative of axonal health; radial diffusivity (RD), a marker of myelin status; mean diffusivity (MD), representing overall water diffusivity; and mode of anisotropy (MO), which captures fibre geometry. Together, these measures provide a quantitative picture of white matter organization and degeneration.

In amyotrophic lateral sclerosis (ALS), DTI has shown significant promise as a biomarker of disease status. Baek et al. (2020) studied 96 ALS patients and 47 matched controls and reported widespread white matter degeneration, particularly affecting the corticospinal tract, corpus callosum, and cerebellar peduncles. Crucially, DTI changes correlated with the ALS Functional Rating Scale (ALSFRS-R) and rate of progression: lower FA and higher RD/MD were consistently associated with worse functional status and faster decline. Rapid progressors demonstrated more severe DTI alterations, highlighting the technique's ability to capture disease burden. These findings underscore the potential of DTI to serve as an objective, quantitative marker of both disease severity and progression in ALS, with strong applicability as an outcome measure in future clinical trials, including stem cell therapy studies. [35]

Structural imaging modalities Musculoskeletal MRI:

Radioimaging has advanced and various non-invasive techniques can give accurate information for diagnosing and monitoring MD. [36]. MRI has several advantages over other imaging techniques used in MD. It is not dependent on the operator and does not use ionizing radiations. There is no need to administer an intravenous contrast for image acquisition. Multiplanar acquisition in MRI makes it easier to be used for the patients with contractures, deformities and severe muscle weakness as seen in MD. MRI also has an inherently high soft tissue resolution and discrimination potential for fat, muscles, fluids, edema and bones. This makes MRI the imaging modality of choice for MD. Musculoskeletal MRI helps to differentiate between the soft tissue and muscles. This allows for grading of the severity of fatty infiltration and muscle atrophy in MD. MRI has been previously used successfully to assess the progression of disease in MD. [37,38].

Duchenne's Muscular Dystrophy

In a case report published in the American Journal of Case Reports, a patient with DMD was treated with Autologous BMMNCs intrathecally and intramuscularly, and the effects were studied over the period of 3 years using serial MRI-MSKs [39]. The patient had undergone four subsequent transplantations performed at 9, 21 and 33 months after the 1st transplantation. Clinical improvements and muscle strength measurements guided the timing of subsequent transplantations. There is a 5% increase in the fatty infiltration and a 3.9% reduction in the strength of the muscles every year in DMD, as suggested by various studies on the natural progression of the disease [40, 41]. 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 (Figures 26A and 26B), 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 a walker.

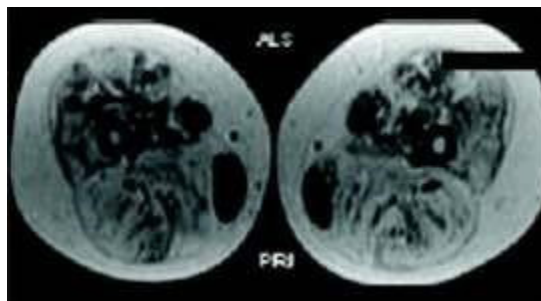


Figure 26A: T1 Weighted Axial Musculoskeletal MRI image before cellular transplantation

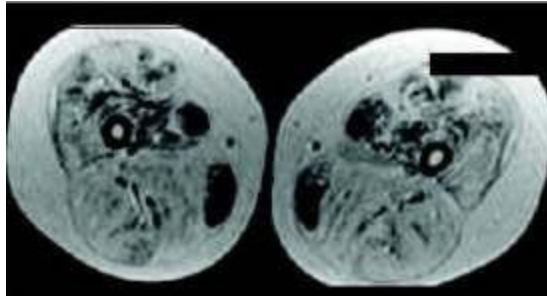


Figure 26B: T1 Weighted Axial Musculoskeletal MRI image 24 months after cellular transplantation showing no reduction in muscle mass and no increase in fatty infiltration suggestive of halting of disease progression in Duchenne Muscular Dystrophy

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 [42]. There was an increase in the muscle fibres of peronei, gastrocnemius, and long, medial, and lateral heads of the triceps with decreased fatty infiltration as observed on the MRI-MSK post 6 months of cellular transplantation (Figures 27A, 27B, 28A, 28B, 29A & 29B). Clinically, there was improved standing balance and the ability to walk with the help of push knee splints and unilateral human support. There was a 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 fibres. Post-cellular therapy, an increase in the muscle fibres and reduced fatty infiltration was an improvement that also correlated with the clinical improvements.

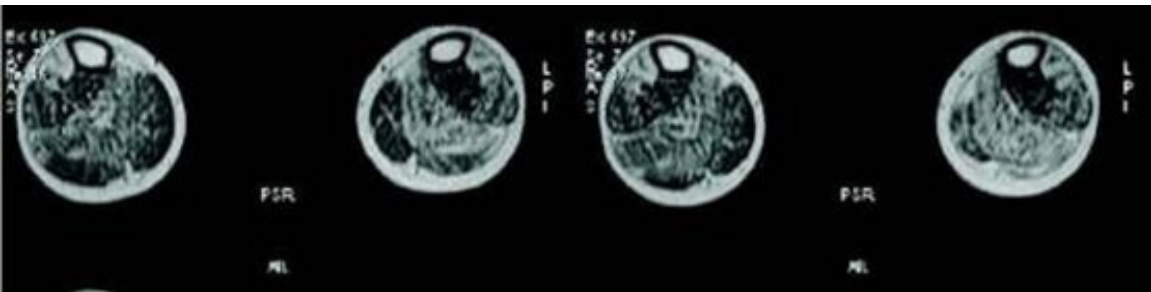


Figure 27A : T1-weighted axial musculoskeletal MRI images of the peroneous longus and Brevis before autologous BMMNC transplantation

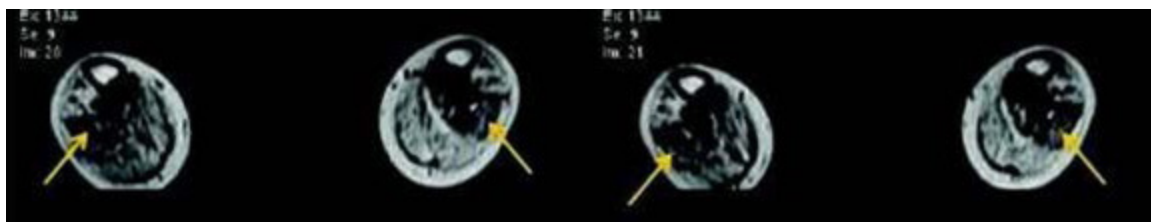


Figure 27B: T1-weighted axial musculoskeletal MRI images of Peroneus Longus and Brevis 6 months after autologous BMMNC transplantation, arrows showing muscle regeneration and reduced fatty infiltration

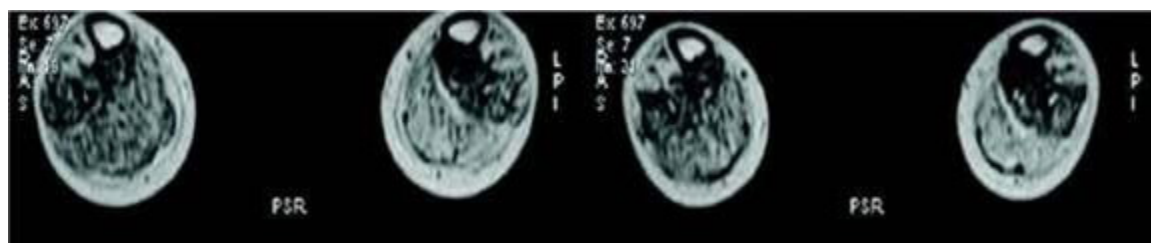


Figure 28A: T1-weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus before autologous BMMNC transplantation

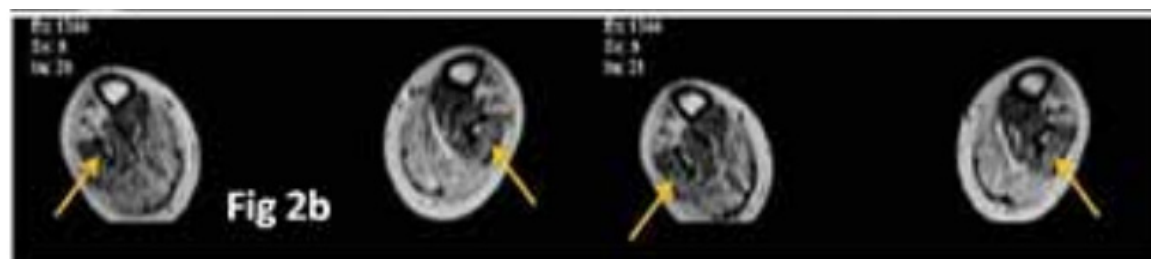


Figure 28B: T1-weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus 6 months after Autologous BMMNCs transplantation; arrows showing muscle regeneration and reduced fatty infiltration

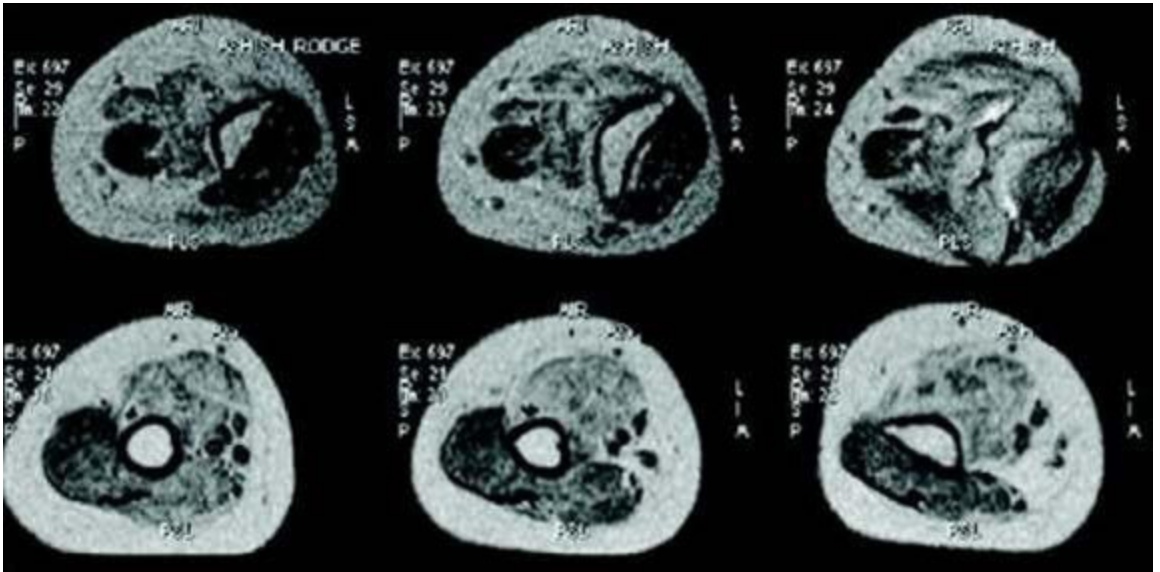


Figure 29A: T1-weighted axial musculoskeletal MRI images of the left and Right Long, Medial and lateral heads of the triceps before autologous BMMNC transplantation

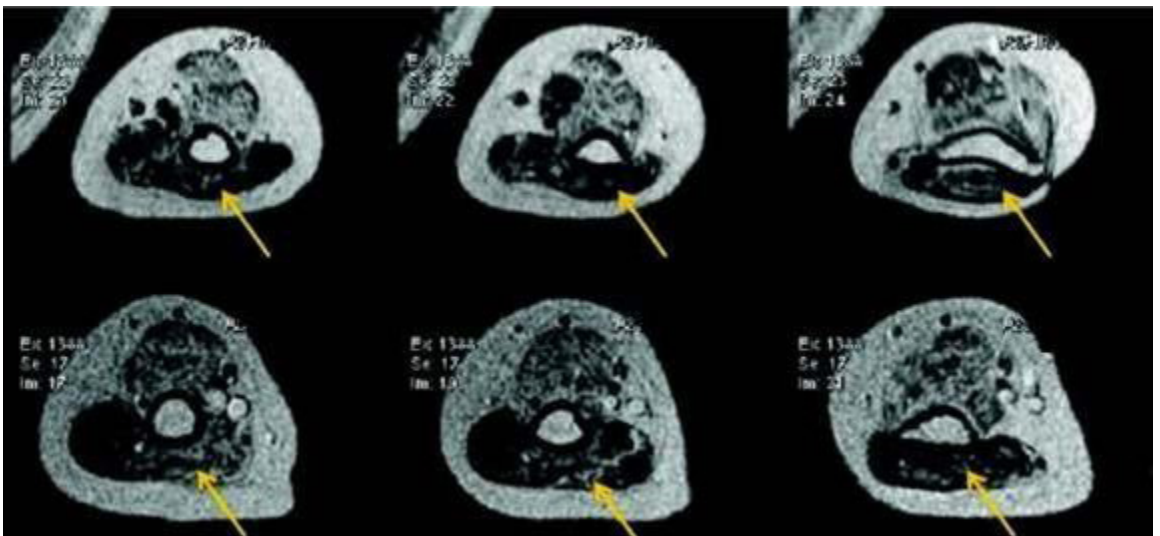


Figure 29B: T1-weighted axial musculoskeletal MRI images of the left and Right Long, Medial and lateral heads of the triceps 6 months after autologous BMMNC transplantation; arrows showing muscle regeneration and reduced fatty infiltration.

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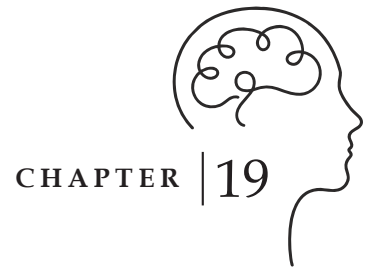
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"You can do anything if you have enthusiasm. Enthusiasm is the yeast that makes your hopes rise to the stars. Enthusiasm is the sparkle in the eyes, the swing in your gait, the grip of your hand, and the irresistible surge of will and energy to execute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

- Henry Ford



Importance of Neurorehabilitation - Concept of NRRT

Introduction

Neurorehabilitation is the clinical subspeciality that is devoted to the restoration and maximization of functions that have been lost due to impairments caused by injury or disease of the nervous system. The goal being to make patients functionally independent, neurorehabilitation requires a team of rehabilitation specialists such as nurses, physical therapists, occupational therapists, speech therapists, psychologists and others (1).

Importance of Neurorehabilitation:

Neuroplasticity is defined as brain's ability to adapt or use cellular adaptations to learn or relearn functions which were previously lost as a result of cellular death by trauma or disease at any age. Neuronal sprouting is thought to be a primary mechanism, allowing injured neurons, to reconnect in new ways and allowing intact, undamaged neurons to form new connections and enhance function. Motor learning will continue throughout life as long as the environment asks for change and the CNS has pliability and the desire to learn. The rehabilitation team promotes this learning and facilitates neural plasticity (2). The philosophic foundation of the 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 a specifically skilled type of exercise leads to increased angiogenesis in damaged cortical areas, whereas unskilled activities did not show this positive change. It is believed that, in humans, rehabilitation techniques would enhance neuroplastic changes.

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

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

Concept of Neuro Regenerative Rehabilitation Therapy (NRRT):

The concept of Neuro Regenerative Rehabilitation Therapy (NRRT) at NeuroGen promotes a multidisciplinary and holistic approach to bring about recovery of neural function with a close integration of neuroregenerative (including stem cell therapy), neuroprotective (medications) and neurorehabilitative therapies (physical/occupational/speech). Thus, it combines the best neurobiological repair technologies and neurorestorative techniques. The rehabilitation protocol is then individualized to the specific requirements of each patient emphasizing on functional recovery and independence in ADL.

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

Multidisciplinary rehabilitation in Various Neurological Conditions

1. Autism

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

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

- **Psychological Intervention:** A clinical psychologist plays an important role, as they may look at what effect a behaviour has on the child and may introduce a behaviour management plan accordingly.
- **Applied Behaviour Analysis:** An ABA analyst may use different strategies of ABA, like discrete trial teaching, pivotal response therapy, etc., to reduce the maladaptive behaviours and increase the appropriate ones.
- **Occupational Therapy:** This therapy focuses on teaching activities of daily living (ADL) like eating, bathing, grooming, etc., and more specifically, sensory/motor integration training would work on optimizing sensory processes and training for developing gross and fine motor skills.



Figure 1: Occupational Therapy:

- **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 teach the child to communicate through Picture Exchange Communication System (PECS).

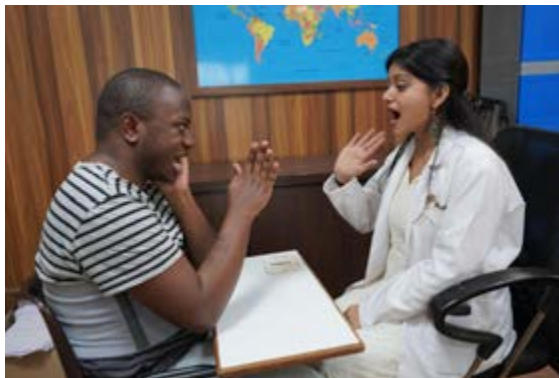


Figure 2: Speech Therapy

- **Physiotherapy:** A Physiotherapist can help in optimal development of motor skills and addressing any underlying weakness in both sensory and muscular systems in children with autism.



Figure 3: Physiotherapy

- **Aquatic therapy:** An aquatic therapist makes use of properties of water and hydrodynamic principles to address the motor and sensory problems observed in these children. Aquatic therapy helps to reduce hyperactivity, improve attention and concentration, improve sleep patterns, improve tone and improve strength of the muscles.



Figure 4: Aquatic therapy

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

2. Spinal Cord Injury

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

- Prevention of all secondary complications as a result of being bedridden.
- Restoration of functional independence to the maximum possible.

- Psychological counseling
- Social and Vocational Rehabilitation
- Family Education and Home adaptation (5)

1. Education:

Patient and caregiver education plays an integral part in rehabilitation. This includes preventive skin care, bladder-bowel training and safe ways to perform ADL tasks, nutritional guidelines, thermoregulation precautions, pulmonary management, cardiopulmonary resuscitation, equipment management and maintenance, transfer techniques, wheelchair mobility and transfer techniques, and leisure skills.

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

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

Intensity should range between 40% and 85% of maximal heart rate or within 13-15 on the 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., a helmet and padded gloves), and get proper rest between exercise sessions.

3. Preventing and Managing Pressure Ulcers and Skin compromise:

- Turning the positions at regular intervals, every 2-3 hrs.
- Pillows and rectangular foam pads to cover bony prominence should be used.
- Treatment like hydrotherapy, electromodalities and 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.
- Patients should be educated to maintain skin integrity.

4. Prevention and Management of Joint Contracture:

- Daily ROM exercises and proper positioning will prevent contractures.
- Use of splints for proper joint alignment techniques like weight-bearing,

ADLS and functional exercises prevents contracture.

- Splinting to prevent Joint Deformity:
- Education regarding the splint-wearing schedule, skin checks and splint care should be emphasized. (6).

5. Sexual Issues:

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

Psychological Aspect in Spinal Cord Injury

Spinal Cord Injury (SCI) leaves a major impression on the person's body and mind. A person who had been leading an satisfying life becomes immobilized, bowel and bladder incontinence and loss of sexual functioning and becomes dependent on others for every small necessity. The patient not only faces loss of body control but also experiences changes in self-worth, sense of independence, confidence, attractiveness, sexuality, and relationships 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 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 and peer role models, teach new coping skills, and decrease social discomfort. Likewise, multiple-family group psychotherapy is a powerful and effective tool for facilitating family adjustment to SCI. Family members experience similar emotional responses to the patient and similarly benefit from psychological intervention.

Role of Aquatic Therapy in Spinal Cord injury

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

3. Cerebral palsy:

Aims of Rehabilitation:

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

Principles of Treatment:

- a. Repetition and reinforcement are essential for learning and establishing of modified motor patterns.
- b. Maximize sensory motor experiences.
- c. Adequate consideration for developmental training and facilitation of purposeful activities: The therapist incorporates the principles of the neuro-developmental concept

Integrated approach for CP:

- a. Developing rapport with parents and patients:
- b. Normalising tone of muscles
- c. Stretching and Mobility
- d. Developing Postural Reaction: Postural reactions consist of righting reactions, protective extension and equilibrium reactions. These reactions are best developed by various exercises on a vestibular ball and tilt board.

5. Sensory Integration Therapy:

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

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

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

Psychotherapy

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

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

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

Treatment: Education and vocational preparation come into the foreground by school age. **Neurocognitive 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.

Counselling 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 behaviours. Aversion therapy i.e. to reward rather than punish on negative consequences can help enhance self-esteem. Expressive therapies are usually used with people who have difficulty verbalizing their feelings such as art, music, poetry, etc which could help freeing and empowering oneself.

Role of Aquatic Therapy in Cerebral Palsy

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

4. Muscular Dystrophy:

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

E.g., 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.

E.g., water exercises, where gravity is eliminated. (10)

Aims of Physical Rehabilitation

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

Aims of Functional Rehabilitation

1. Self-Care activities such as
2. Eating
3. Grooming
4. Bathing
5. Dressing

Mobility training:

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

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

Role of Aquatic therapy in Muscular Dystrophy

MD is a neuromuscular disorder with progressive muscle weakness due to contractile damage to the exoskeleton of myocytes. Therefore, day-to-day activities can also damage the muscle cells. There are different types of muscle contractions, out of which eccentric contraction is the most detrimental to the muscles. Patients with muscular dystrophy need to use these harmful contractions several times a day to fight gravity and maintain upright posture. In water, because of increased buoyancy and reduced gravity, these contractions are reduced, which means the same movement on land causes more harm to the muscles than in water. Therefore, aquatic therapy can provide greater benefits to these patients; immersion in water is responsible for secreting neuroprotective and anti-inflammatory cytokines which help to maintain the muscle strength further. Aquatic therapy can improve the strength of the muscles, provide a higher degree of freedom of movement, helps to reduce contractures and fibrosis, promote sitting and standing balance, facilitate ambulation even after they are unable to walk on land, and improve respiratory muscle strength and cardiovascular endurance.

5. Stroke:

Specific Measures for Stroke:

Rehabilitation approaches for stroke patients include neurodevelopmental treatment (NDT) and movement therapy in Hemiplegia. - Brunnstrom 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 behavioural 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:

Strategies to improve Sensory function:

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

Strategies to improve Motor Function:

- Strategies to improve Flexibility and Joint Integrity
- Strategies to improve Strength
- Strategies to manage Spasticity
- Strategies to improve Initial Movement Control
- Strategies to improve Motor Learning
- Strategies to improve Postural Control and Functional Mobility
- Strategies to improve Upper Extremity Function
- Strategies to improve Lower Extremity Function
- Strategies to improve Balance
- Strategies to improve Locomotion
- Strategies to improve Aerobic Function

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

Strategies to Improve Feeding and Swallowing:

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

Psychological Rehabilitation:

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

Attention training helped people with acquired brain injury and seemed to work best with younger patients less than a year after injury. Visuospatial training helped stroke patients with visuospatial neglect, the inability to respond or orient to something shown on the side opposite to the site of the injury. Visuospatial

training also tended to improve performance in other cognitive domains . Family counselling is a major factor for psychological rehabilitation in stroke.

Role of Aquatic therapy in Stroke

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

6. Motor neuron disease:

Specific measures for MND: The efficacy of therapeutic interventions is related to:

- Timing of interventions,
- Motivation and persistence of the patient in carrying out the program.
- Support from family members. A rehabilitation intervention plan depends on the following:
 - The rate of progression of the disease.
 - Presence of spasticity, bulbar involvement, and respiratory involvement causing hypoxia and fatigue.
 - Phase of Disease. Exercises are to be prescribed according to level of impairment, functional limitation and level of disability (11).

Phase I (Independent)

- **Stage 1:** In case of mild weakness, the advice is to continue normal activities. In case of clumsiness, do stretching exercises like Yoga. In the case of ambulatory patients, gentle resisted exercises without fatigue.
- **Stage 2:** In case of moderate selective weakness, stretching exercises to avoid contractures.



Figure 5: Exercises to improve grip strength

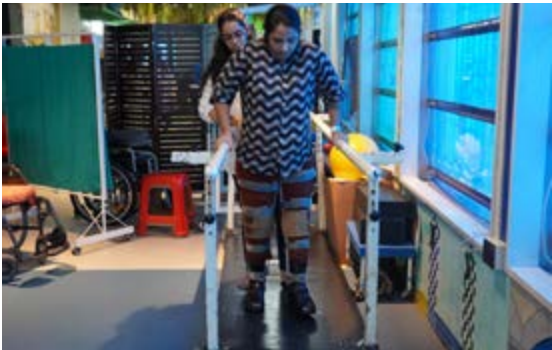


Figure 6: Gait Training with parallel bars



Figure 7: Activity for sitting balance



Figure 8: Gait Training with walker support



Figure 9: Quadruped for trunk balance



Figure 10: Strengthening of back extensors

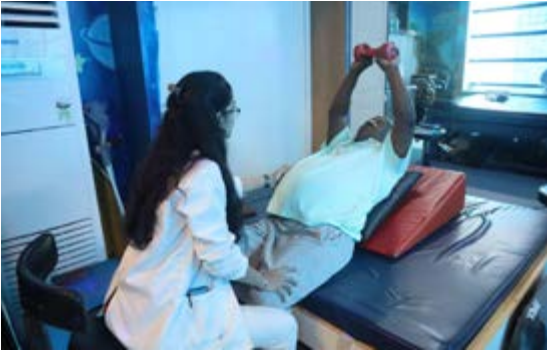


Figure 11: Strengthening of upper abdominals



Figure 12: Stretching of dorso-lumbar fascia



Figure 13: Hip Rotation exercise

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

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

In case of non-ambulatory cases, consider a wheelchair, standard or motorized.
Phase 2 – (Partially Independent)

- **Stage 4:** In case of pain and edema in hands and feet, consider modalities like massage, elevation and active exercises. In case of severe weakness in extremities, caution is to be taken to support the joints while doing rotations. In case of fatigability in ADLs, encourage isometric exercises to the level of tolerance and to consider slings or arm support, motorized chairs etc.
- **Stage 5:** In case of severe lower extremity weakness, teach family members proper techniques of transfer and positioning of patients' limbs and modifica-

tions 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, Duchenne's muscular dystrophy, myotonic dystrophy, hereditary motor and sensory neuropathy, spinal muscular atrophy and limb-girdle, Becker and fascioscapulohumeral dystrophy have found that exercises programs are beneficial and do not produce overuse weakness.

Role of Aquatic therapy in Motor Neuron Disease

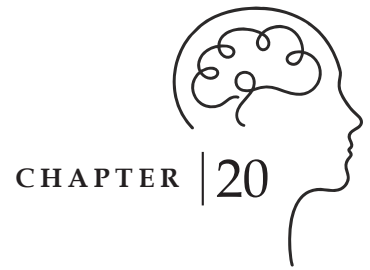
Aquatic therapy benefits the patients of motor neuron disease in multiple ways. It provides a higher degree of freedom of movement. It helps to reduce spasticity, improve posture, improve balance, improve voluntary control and improve respiratory as well as cardiovascular endurance.

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

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Integrative Therapies in Neurological Disorders: Mechanisms and Applications

Introduction

Integrative therapy is a holistic therapeutic approach which combines conventional medical treatments with complementary interventions to address complex, multifaceted disorders. In neurological and neurodevelopmental conditions, pathology often involves a plethora of factors such as hypoxia/ischemia, neuroinflammation, oxidative stress, gut dysbiosis, immune dysregulation, and neuromuscular dysfunction. A single intervention or treatment may not fully address these interlinked issues (Geng et al., 2015). Thus, integrative protocols have been proposed, which in this context include Hyperbaric Oxygen Therapy (HBOT), Ozone Therapy, Deep Tissue Mobilization (DTM) (a form of therapeutic massage), and Gut Cleansing Therapy (GCT). These interventions, when used alongside standard care or regenerative treatments like stem cell transplantation, aim to “force multiply” or reinforce recovery by simultaneously targeting multiple pathophysiological mechanisms. For example, autism spectrum disorder (ASD) involves cerebral hypoperfusion, neuroinflammation, mitochondrial dysfunction, gut microbial imbalance, and immune abnormalities (Geng et al., 2015). Similarly, injuries like stroke, traumatic brain injury (TBI), and spinal cord injury (SCI) feature ischemic damage and inflammatory processes, while degenerative conditions such as amyotrophic lateral sclerosis (ALS) and muscular dystrophy (MD) involve

chronic oxidative stress, excitotoxicity, and muscle pathology. Integrative therapies are designed to synergistically ameliorate these diverse pathological factors, thereby enhancing neuroplasticity, tissue repair, and overall functional outcomes. This chapter outlines the mechanisms of action of HBOT, ozone therapy, DTM, and GCT, and discusses how these modalities can be applied to seven conditions – Autism, Cerebral Palsy (CP), ALS, Muscular Dystrophy, TBI, SCI, and Stroke – with a brief overview of each disorder’s relevant pathophysiology. Finally, the role of integrative therapies in supplementing autologous bone marrow mononuclear cell (BMMNC) transplantation or other stem cell therapies is explored, highlighting evidence that these modalities create a more favourable internal environment for regenerative treatments.

Mechanisms of Action of Integrative Therapies

Hyperbaric Oxygen Therapy (HBOT):

HBOT involves breathing 100% oxygen at elevated atmospheric pressure inside a hyperbaric chamber. This significantly increases the amount of oxygen dissolved in plasma, thereby enhancing oxygen delivery to tissues even when blood flow is compromised. Under HBOT, oxygen tension in tissues rises, supporting mitochondrial oxidative phosphorylation and ATP production in hypoxic or metabolically impaired cells (Thom et al., 2005). Improved tissue oxygenation can salvage the ischemic penumbra in stroke or TBI and help revive shocked neurons. HBOT is also known to exert potent anti-inflammatory effects: it has been shown to inhibit

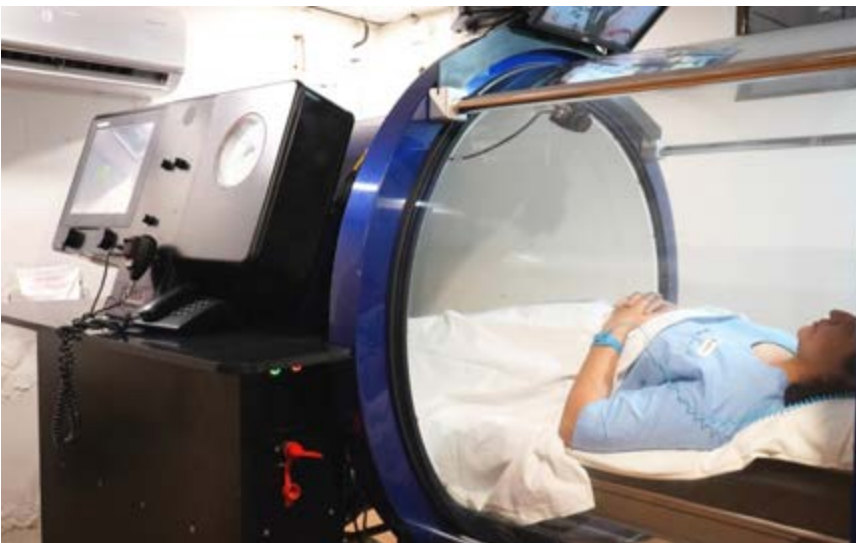


Figure 1: Hyperbaric Oxygen Chamber

the production of pro-inflammatory cytokines like TNF- α , IL-1 β and IL-6 by activated immune cells (Thom et al., 2005). This modulation of inflammation can limit secondary tissue damage after injuries. Additionally, HBOT induces mild oxidative stress that paradoxically triggers antioxidant defences (upregulating enzymes such as superoxide dismutase and glutathione peroxidase), thereby keeping overall oxidative damage in check (Thom et al., 2005). Notably, HBOT can stimulate the release of growth and repair factors. It promotes angiogenesis via increased vascular endothelial growth factor (VEGF) production and stimulates neurotrophic factors like brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which support neuronal survival and synaptic plasticity (Geng et al., 2015). These effects enhance tissue regeneration in the brain and spinal cord. HBOT has even been found to mobilize stem/progenitor cells from bone marrow into circulation by a nitric oxide-dependent mechanism (increasing circulating CD34⁺ cells several-fold) (Thom et al., 2005), which may contribute to endogenous repair processes.

Ozone Therapy:

Ozone (O₃) gas mobilised at low doses, is used medically for its bioactive properties. Ozone therapy can be administered via routes like blood ozonation, ear and rectal insufflation, or topical application. Mechanistically, ozone reacts with biological substrates to form reactive oxygen species and lipid peroxides, invoking an oxidative preconditioning or hormesis effect (Yin et al., 2022). A small, transient oxidative challenge activates the Nrf2 (nuclear factor erythroid 2-related factor 2) pathway, which in turn upregulates the body's antioxidant defences (e.g., glutathione, superoxide dismutase, catalase) (Yin et al., 2022). Through this redox modulation, ozone therapy helps restore a more balanced oxidative state, neutralizing excess free radicals implicated in neurodegeneration and ischemic injury. Ozone also improves tissue oxygenation; it stimulates 2,3-diphosphoglycerate in red blood cells, which facilitates greater release of O₃ from hemoglobin, thereby enhancing oxygen delivery to hypoxic tissues (Geng et al., 2015). In parallel, ozone has immunomodulatory effects: it can shift the cytokine profile toward an anti-inflammatory or immunoregulatory state. For instance, ozone exposure has been shown to increase levels of IL-10 and TGF- β (anti-inflammatory cytokines) while reducing pro-inflammatory mediators, effectively attenuating chronic inflammation (Thom et al., 2005). Some studies indicate ozone may encourage a switch of activated microglia/macrophages from a pro-inflammatory (M1) phenotype to a reparative (M2) phenotype in the central nervous system (Yin et al., 2022). Ozone therapy's mild oxidative stimulus also induces nitric oxide production, leading to vasodilation and better microcirculation. Lastly, ozone possesses direct antimicrobial properties and has been used to reduce pathogenic bacteria in the gut and bloodstream, which is pertinent in conditions involving gut dysbiosis or systemic infections.



Figure 2: Ozone Machine

Deep Tissue Mobilization (DTM):

DTM is a form of manual therapy that applies firm, sustained pressure and stretching to the deeper layers of muscles, fascia, and connective tissues. Unlike gentle massage, DTM specifically targets areas of tissue restriction, spasticity, or fibrosis. The primary effect is the mechanical breaking down of adhesions and remodeling of fibrotic tissue in muscles and fascia. This can restore elasticity and normal sliding motion between tissue layers, which is crucial for patients with contractures or chronic spasticity (e.g., in cerebral palsy or after stroke) (Harvey & Herbert, 2002). DTM also activates sensory receptors such as Golgi tendon organs in tendons and mechanoreceptors in fascia that induce neuromuscular reflexes to reduce muscle tone (Weerapong et al., 2005; Bialosky et al., 2009). By stimulating these proprioceptive fibres, deep pressure can trigger a spinal reflex that inhibits excessive muscle contraction, thereby relieving spastic hypertonicity. For example, sustained pressure on a spastic muscle can increase Golgi tendon organ firing, leading to reflexive relaxation of that muscle (Chaudhry et al., 2008). DTM further improves local circulation by the combination of pressure and release maneuvers which dilates capillaries and enhances lymphatic drainage in treated areas (Best et al., 2008). Increased blood flow delivers oxygen and nutrients to tissues while flushing out inflammatory metabolites (e.g., lactic acid), aiding recovery of muscle function. On a neurophysiological level, deep massage stimulates A-beta tactile fibres, which engage central pathways that can diminish pain perception and promote relaxation (Field, 2014). Indeed, DTM has been associated with increased parasympathetic activity and release of endorphins such as β -endorphin and en-

kephalins, contributing to analgesia and reduced anxiety (Field, 2014). By modulating both mechanical and neural aspects of the musculoskeletal system, DTM can improve range of motion, postural alignment, and sensorimotor integration, all of which are important in neurorehabilitation (Bialosky et al., 2009).

Gut Cleansing Therapy (GCT):

Gut cleansing is a type of detoxification therapy that encompasses interventions aimed at restoring healthy gastrointestinal function and microbiome balance. Approaches may include the use of gentle laxatives, enemas or colonic irrigation, probiotics, and dietary modifications to “reset” the gut environment. The rationale stems from the gut-brain axis – the bidirectional communication between the enteric system, immune system, and central nervous system. Many neurological disorders are now known to involve intestinal dysbiosis (imbalances in gut microbial communities) and increased intestinal permeability (a “leaky gut”), which allow inflammatory and neurotoxic byproducts (e.g., lipopolysaccharides from Gram-negative bacteria) to enter circulation (Camilleri, 2020; Sharon et al., 2016). GCT helps eliminate accumulations of toxins, pathogenic bacteria, and yeast from the gastrointestinal (GI) tract, thereby reducing the source of pro-inflammatory molecules that could exacerbate neuroinflammation. By clearing out harmful gut contents and possibly using prebiotics/probiotics, GCT fosters the regrowth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (Iovene et al., 2019; Goldenberg et al., 2017). A healthier microbiome produces more short-chain fatty



Figure 3: Benefits of Gut Detox

acids (like butyrate) which have neuroprotective and anti-inflammatory effects in the brain (Dalile et al., 2019; Stilling et al., 2016). Gut cleansing can also improve the integrity of the intestinal barrier (tight junctions), reversing leaky gut syndrome (Camilleri, 2020; da Silva et al., 2020). This means fewer toxins reach the bloodstream, easing the burden on the liver and immune system. Additionally, many patients with neurological disorders suffer from chronic constipation or poor gut motility due to neuromuscular impairment; GCT can relieve these GI symptoms, improving nutrient absorption (critical for tissue repair and brain function) and enhancing overall comfort (Del Giudice et al., 1999; Zaidel & Lin, 2003). By addressing the often-overlooked gastrointestinal component, GCT supports the other integrative modalities in reducing systemic inflammation and optimizing the internal milieu for healing.

Clinical Procedure and Potential Side Effects:

Hyperbaric Oxygen Therapy (HBOT):

Hyperbaric Oxygen Therapy (HBOT) is a well-recognized conventional line of treatment wherein patients inhale 100% oxygen in a specially built chamber at pressures greater than atmospheric pressure. Hyperbaric oxygen therapy (HBOT) relies heavily on Henry's law, which describes how raising the chamber's ambient pressure causes more oxygen to dissolve in the patient's blood plasma. [Ortega, M. A., et al., (2021). and Jones, M. W., (2024).]

After a comprehensive evaluation that includes a history and physical examination, the patient's suitability for Hyperbaric Oxygen Therapy (HBOT) is determined. Blood pressure, heart rate, breathing rate, and oxygen saturation are among the baseline vital indicators that are noted. After that, the patient is led to the monoplace chamber and given comfort. The process is described, with a focus on the pressurisation phase, when the patient may feel as though their ears are being compressed or blocked. After the patient enters the chamber, the pressure is progressively raised over ten to fifteen minutes, up to 2.0 to 2.5 ATA. Either directly (monoplace) or through a mask or hood (multiplace), the patient breathes 100% oxygen. Most sessions go between 60 and 120 minutes. Sessions may consist of short "air breaks" in between intervals of oxygen breathing to lower the danger of oxygen toxicity. To prevent barotrauma, chamber pressure is gradually lowered to atmospheric levels after treatment.

HBOT enhances tissue oxygenation, reduces cerebral edema, and modulates inflammation while restoring mitochondrial energy production. It promotes angiogenesis, neurogenesis, and antimicrobial defence, alongside beneficial effects on neurotransmission and cellular longevity, thereby supporting repair and recovery across multiple clinical conditions.

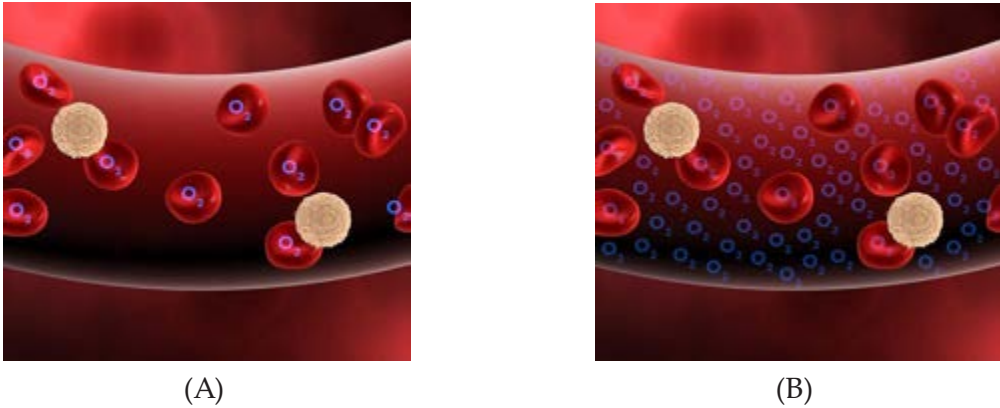


Figure 4: A) At normal atmospheric pressure, Oxygen is carried by hemoglobin (Hb) in red blood cells (RBCs). B) At high atmospheric pressure, Oxygen is dissolved in the plasma.

Possible adverse events:

The appropriate use of hyperbaric oxygen is one of the safest therapeutic procedures available to the modern practitioner. However, as with any medical therapy, there are a few risks associated with it if not done properly or with improper patient selection. The most common are highlighted here:

- Barotrauma (Tympanic membrane rupture)
- Pressure differences in the middle ear
- Sinus Pain
- Seizures
- Cataract formation
- Pneumothorax
- Dental Pain
- Oxygen Toxicity

HBOT has contraindications such as Untreated pneumothorax and recent exposure to chemotherapy drugs such as doxorubicin, cisplatin, or bleomycin are absolute contraindications. Acute asthma or severe COPD with air trapping, cold, sinus infections, respiratory tract infections, fever, uncontrollable convulsions, pregnancy (unless there is an emergency), congenital spherocytosis, pacemakers or epidural pain pumps, and extreme claustrophobia are among the relative contraindications. For a safe application, careful patient selection and monitoring are crucial.

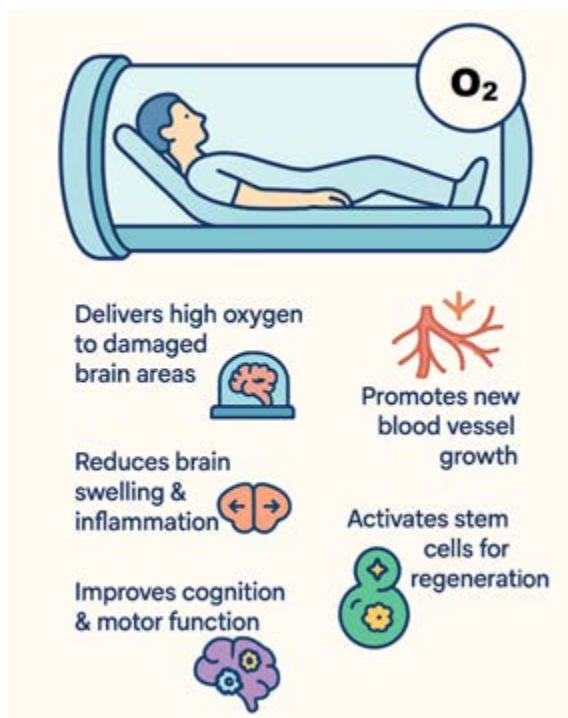


Figure 5: Benefits of HBOT

Ozone Therapy:

Ozone therapy is an integrated medical method in which the body is exposed to medical ozone, a controlled blend of oxygen (95%–99.5%) and ozone (0.5%–5%). This special blend is made in a licensed ozonator and administered to the body in a targeted and safe manner.

Procedure of Administering Ozone Therapy:

To guarantee patient safety and therapeutic effectiveness, a comprehensive clinical evaluation is carried out prior to starting ozone therapy. This includes checking for weight, medications, bleeding disorders, assessing total blood parameters to rule out anemia or thrombocytopenia, and screening for G6PD deficiency. Particular consideration is given to a history of seizures, particularly if auricular ozone is being explored, as it may not be appropriate in these circumstances. Additional contraindications are also carefully ruled out, including fever, rectal bleeding, ear discharge, recent ear surgery, and hyperthyroidism. The patient is cleared for ozone therapy only when it has been confirmed that certain contraindications do not exist. Patients with ALS do not receive ozone therapy. In case of any ear infection or ENT-related issue and any history of seizures and EEG abnormality, ear ozone is not allowed. Similarly, in case of any stomach discomfort or loose bowel movements, rectal insufflation is not recommended.

Non-invasive methods including ozone face cupping, auricular (ear) insufflation, and rectal insufflation are advised and favoured in paediatric populations since they are less invasive, safer, and linked with less discomfort. Furthermore, ozone bagging and funnelling are efficient methods for treating ulcers and wounds that do not heal, which are frequently observed in patients who are bedridden, have spinal cord injuries, or are neurologically impaired. For neurological diseases such as motor neuron disease, multiple sclerosis, cerebellar ataxia, SCA, etc., ozone therapy is not advised.

Ear Insufflation:

Medical ozone is administered to the external ear canals during ear insufflation. A modified stethoscope is used to inject ozone after the outer ear canal has been moistened with a cotton swab. The patient's ears are fitted with earpieces, and the chest piece is attached to the ozonator. To reduce ozone leakage, the patient is advised to hold the stethoscope at a 90-degree angle from their face. Depending on the diagnosis, each session usually lasts 3 to 5 minutes, and this treatment is very useful for disorders involving the brain, hearing, and eyes.

Rectal Insufflation:

Giving ozone through the rectum is called rectal insufflation. Ozone is applied systemically, and a determined amount of medical ozone is collected in an ozone-resistant bag based on the patient's body weight and the necessary concentration. The gas is then gradually given after a lubricated silicon catheter is carefully placed about an inch into the rectum. To minimize gas leakage, the catheter is removed in a swirling motion. To maximize absorption, the patient is instructed to hold onto the gas for five to eight minutes. Introducing ozone rectally can help balance the gut microbiome, Reduce inflammation, Improve oxygenation and circulation, Immune system modulation, Detoxification, Support for chronic conditions

Cupping:

It is a localized therapeutic approach that combines the use of medical ozone with cupping therapy. It improves the blood circulation over the targeted area. Cupping above the neck and face uses concentrations of 10 mcg/ml (as there are chances of leakage and patients may inhale large amounts of Ozone.) Ozone is applied to the cupped area during the treatment, which creates negative pressure by cupping and permits direct transdermal absorption. Given for non-verbal patients to improve blood circulation in facial muscles. This dual action improves lymphatic drainage, increases facial muscle tone, and improves local microcirculation. The administration of the technique usually takes one to two minutes.

Ozone funnelling/ bagging:

This technique is used for patients who have localized infections or wounds that don't heal. Ozone is given locally for 15 to 20 minutes while the afflicted limb or area is covered with a funnel or wrapped in an ozone-resistant bag. At lower quantities, this method can improve circulation and aid in wound healing; at greater concentrations, it can have antibacterial properties.

Other application pathways that may be employed based on conditions

A. Topical / non-invasive:

- Breathing ozone through oil: Inhaling ozone through oil entails bubbling medical ozone into high-density oil, which causes polyunsaturated fatty acids (PUFAs) to create ozonides.
- Scalp Bagging: To improve blood flow and provide antimicrobial benefits, the scalp is wrapped in an ozone-resistant bag and subjected to ozone gas.
- Vaginal Insufflation: A tube attached to the ozonator is used to deliver medical ozone vaginally. This approach promotes hormonal balance, helps control menstrual cycles, and is advantageous for a number of gynecological issues.
- Ozonated water/oils: Topical antibacterial and restorative properties of ozonated water and oils.

B. Invasive:

- Subcutaneous ozone injection: Using a 20 ml Luer lock syringe, a tiny amount of medical ozone is extracted, and under aseptic conditions, about 1 ml is injected at the location of localized pain. When used around joints or in capsule form, this technique reduces pain and inflammation.
- Intramuscular ozone injection: Using aseptic procedures, 5–10 ml of medical ozone is injected into the gluteal muscle after a little quantity is collected in a 10 ml Luer-lock syringe. It is used to treat myalgia, fibromyalgia, body aches, and other associated disorders.
- Minor Autohemotherapy: This method involves drawing 5–10 ml of ozone into a 20 ml Luer-lock syringe and then collecting 5 ml of peripheral vein venous blood into the same syringe. To ensure that the blood and ozone are sufficiently mixed, the syringe is slowly rotated between the hands for about a minute. After that, the ozonated blood is injected into the gluteal region. Minor autohemotherapy is used to treat a variety of ailments, but it is especially useful for allergic and autoimmune conditions.

- Major Autohemotherapy (MAH): Under aseptic conditions, 200 milliliters of venous blood are extracted and placed in a sterile, anticoagulant-containing bottle with 0.9% normal saline as part of Major Autohemotherapy (MAH). After then, the patient receives another intravenous infusion of the ozonated blood over a period of 15 to 20 minutes. Major autohemotherapy is used to treat a variety of ailments, but it is especially useful for cancer, oxidative stress, immunological dysregulation, chronic inflammation, and poor circulation.

Safety measures:

- It is not safe to breathe ozone.
- VENTILATION is a must; a door, open windows, and an exhaust are ideal.
- Prevention of inhalation of ozone: use a face mask.
- In case of ozone leakage, remove the person from the room and take him to an open space.
- Switch on the fan and open the doors and windows. Leave the room.
- If the patient is experiencing continuous cough spells, then vitamin C is given to neutralize the effect of ozone.
- The threshold is the minimum concentration of ozone required to overcome extracellular resistance, which is 10 micrograms/ml.
- Large doses of vitamin C must be avoided or spaced out during ozone therapy, as it can neutralize the effect of ozone.
- Vit E can be safely taken during ozone therapy.

Benefits of Ozone Therapy

1. Improved Cerebral Oxygenation and Perfusion
2. Immunomodulation
3. Oxidative Stress Regulation
4. Gut-Brain Axis and improve Dysbiosis
5. Detoxification Support
6. Behavioral and Emotional Regulation
7. Support for Speech and Language Development
8. Stimulation of Neuronal Metabolism and Mitochondrial Function

9. Neuroplasticity and Motor Function

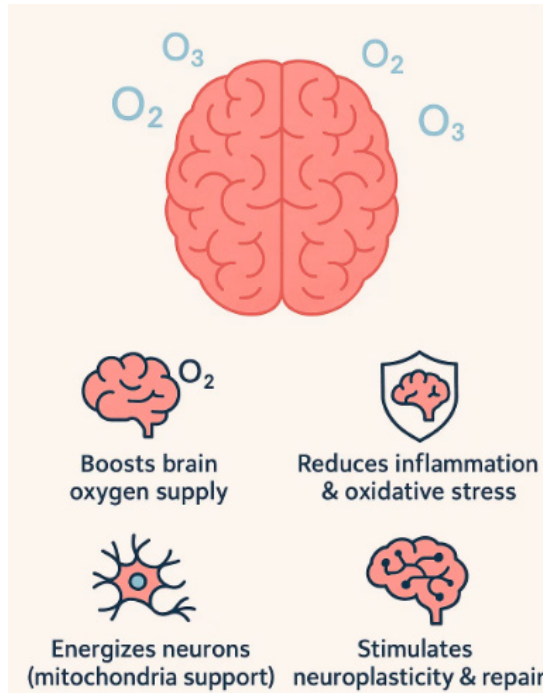


Figure 6: Advantages of Ozone Therapy

Possible adverse events:

- Ozone can burn and induce headaches, nausea, vomiting, and coughing if it gets into the mouth, nose, or eyes.
- When receiving ozone therapy through the rectum, a person may feel uncomfortable, cramp, and like they need to expel gas. These adverse effects are transient, though.
- As ozone therapy reduces inflammation and eliminates germs and viruses, so a person can feel some flu-like symptoms.
- Ozone can irritate the airways and lungs, which may result in chest pain, coughing, and shortness of breath.
- A gas embolism is a potentially fatal blockage of blood arteries that can occur when ozone gas enters the bloodstream directly.
- Ozone can cause allergic reactions like Skin rashes, itching, and swelling are some of the symptoms that some people may have when exposed to ozone.

- With Interactions to Medication Ozone therapy may have negative effects or lessen the effectiveness of several medications when taken with them.

Deep Tissue Mobilization (DTM):

In patients with neurological impairments, Deep Tissue Mobilisation (DTM) is a manual therapy approach that aims to improve joint mobility, reduce muscle hypertonicity, and restore functional movement patterns. Reduced tissue extensibility, contractures, and spasticity are common symptoms of neurological conditions like multiple sclerosis, stroke, cerebral palsy, and traumatic brain injury. By manually stretching and mobilising connective tissue, fascia, and deep muscle layers, DTM addresses these constraints and promotes structural and neuromuscular enhancements.

The process is using the fingers or thumbs to apply light yet firm pressure to particular acupoints. Every session starts with the identification of spots associated with the patient's condition, and then steady or circular pressure is applied for 30 seconds to 2 minutes. Relaxation techniques are frequently incorporated into sessions. Acupressure can be tailored to the patient's age, sensitivity, and health status and is generally safe and non-invasive.

Contraindications for Deep Tissue Mobilization:

Absolute Contraindications (should not be performed)

- Acute fractures or unstable joints
- Acute inflammatory conditions or infections
- Severe osteoporosis or bone fragility.
- Acute deep vein thrombosis (DVT) or pulmonary embolism Open wounds or severe skin lesions
- Severe cardiovascular disease Acute neurological instability
- Severe spasticity or hypertonicity
- Pregnancy
- Peripheral neuropathy or impaired sensation
- Bleeding disorders or anticoagulant therapy
- Severe cognitive impairment or non-cooperative patients



Figure 7: Benefits of Deep Tissue Mobilization

Applications of Integrative Therapy in Specific Disorders

Autism Spectrum Disorder (ASD):

ASD is a neurodevelopmental condition with a broad range of symptoms in communication, behaviour, and sensory processing. Beyond its genetic basis, many children with autism exhibit physiological abnormalities, including cerebral hypoperfusion, neuroinflammation, mitochondrial dysfunction, oxidative stress, gastrointestinal (GI) dysbiosis, and immune dysregulation (Rossignol & Frye, 2012; Iovene et al., 2019; Masi et al., 2022). For instance, chronic activation of the immune system in the brain (microglial activation) and elevated pro-inflammatory cytokines have been linked to ASD severity (Masi et al., 2022). Mitochondrial abnormalities can reduce ATP available for brain function, and gut microbiome imbalances are associated with GI symptoms and possibly behavioural changes in ASD (Iovene et al., 2019). These multisystem issues make ASD a prime candidate for an integrative approach. HBOT may counteract hypoperfusion and low oxygenation seen in certain brain regions of ASD. By dissolving additional oxygen into plasma, HBOT increases O_2 delivery to the brain, which can improve neuronal metabolism in under perfused areas (Geng et al., 2015). A pilot study

by Rossignol (2007) hypothesized that HBOT could improve ASD symptoms by ameliorating some underlying pathophysiology (e.g., better cerebral blood flow and reduced neuroinflammation). Inflammatory cytokines like TNF- α and IL-6, often elevated in autism, are suppressed under hyperbaric conditions (Geng et al., 2015), potentially relieving neuroinflammatory stress. HBOT has also been noted to enhance mitochondrial function and reduce oxidative stress markers in children with autism in some reports (Rossignol & Rossignol, 2006), although larger controlled trials are needed.

Ozone therapy in autism is proposed mainly to address immune dysregulation and gut issues. Ozone's activation of antioxidant systems can help neutralize oxidative free radicals that are found in excess in ASD (Rossignol & Frye, 2012). Moreover, ozone's immunomodulatory effect may calm down the Th1/Th2 imbalances and lower the production of inflammatory cytokines that affect the developing brain's connectivity (Masi et al., 2022). Importantly, ozone introduced via rectal insufflation could directly reduce pathogenic gut flora – ozone is known to have antimicrobial properties and has been used to treat infections in the GI tract. By potentially reducing “bad” bacteria (pathogens) and yeast, ozone therapy might improve gut barrier integrity and decrease the flow of neurotoxins from the gut to brain (the gut-brain axis).

Deep tissue mobilization can be beneficial in managing the sensory-motor aspects of autism. Children with ASD often have sensory processing disorders – some are hyposensitive and seek deep pressure, while others are hypersensitive to touch. DTM, applied by trained pediatric massage therapists or occupational therapists, can help an overreactive nervous system or provide needed sensory input to an underresponsive one. The deep pressure of DTM has a calming effect via the parasympathetic nervous system, often resulting in reduced anxiety and improved attention after sessions (Field et al., 2010). Regular massage therapy in ASD has been associated with better sleep quality and reduced stereotypical behaviors, possibly by increasing serotonin or oxytocin levels (Field et al., 2010). Parents often report that their child becomes more relaxed and socially receptive following deep pressure massage.

Gut cleansing therapy is another important component for ASD, given the high prevalence of GI problems (chronic constipation, diarrhea, food intolerances) in this population. GCT strategies such as probiotics, dietary interventions (gluten/casein-free diets), or even occasional gut detoxification can help restore a healthier microbiome (Iovene et al., 2019). Improvements in gut health may lead to better neurotransmitter production (for example, gut bacteria produce a large proportion of the body's serotonin) and reduce systemic inflammation that could be exacerbating ASD symptoms. A healthier gut environment also means improved nutrient absorption – critical nutrients like B vitamins, magnesium, and amino acids (often deficient in ASD kids) are better absorbed, supporting overall neuro-

logical function.

Rossignol and colleagues have conducted several clinical investigations of HBOT in autism. In an initial open-label case series, HBOT at 1.3 atm with 24% oxygen was associated with improvements in communication, social interaction, and cognition (Rossignol & Rossignol, 2006). This was followed by an open-label pilot study in 18 children, which reported reductions in oxidative stress and inflammatory markers alongside clinical improvements after 40 one-hour sessions at the same parameters (Rossignol et al., 2007). Most notably, in a multi-centre randomized, double-blind, controlled trial ($n = 62$; 33 HBOT vs. 29 control), 40 one-hour sessions at 1.3 atm and 24% oxygen resulted in significant improvements in overall functioning, receptive language, social interaction, and eye contact compared to controls (Rossignol et al., 2009). These findings, though they require replication, highlight that integrative methodologies similar to those described here have already been applied in practice, providing clinical precedent for their inclusion.

In summary, the integrative use of HBOT, ozone therapy, DTM, and GCT in autism aims to address the core pathophysiological disturbances by improving brain oxygenation, dampening down inflammation and oxidative stress, normalizing sensory responses, and healing the gut to complement behavioural therapies and other biomedical interventions for ASD.

Cerebral Palsy (CP):

Cerebral palsy refers to a group of permanent movement disorders caused by non-progressive injury to the developing brain, often from hypoxia or ischemia around birth. A common scenario is hypoxic-ischemic encephalopathy in a difficult labor or premature birth, where reduced oxygen/blood supply damages the motor cortex or basal ganglia. The initial hypoxia triggers a cascade: energy failure, glutamate excitotoxicity, oxidative stress, and cell death (Zhu et al., 2013). Injured areas often show apoptosis of neural progenitor cells and impaired myelination, limiting the brain's ability to repair itself (Zhu et al., 2013). Ischemia during a perinatal stroke or hemorrhage can also reduce growth factors like VEGF, hampering the development of new blood vessels and compounding long-term deficits (Zhang et al., 2000). Children with CP commonly have motor impairments (spasticity, muscle weakness) and can also experience seizures, cognitive challenges, or sensory deficits depending on the extent of brain injury. In treating CP, HBOT is utilized to improve oxygenation in chronically ischemic or metabolically impaired brain regions. By raising—for oxygen levels, HBOT supports any surviving but under-functioning neurons in perilesional areas and promotes angiogenesis. Increased oxygen availability can prevent further apoptosis of cells at risk by restoring ATP production and stabilizing the mitochondrial membrane in those cells (Geng et al., 2015). Some studies have reported improved SPECT scan perfusion in the cerebral cortex of CP children after HBOT, correlating with modest gains in

gross motor function. Additionally, HBOT may help reduce neuroinflammation in CP – for example, by inhibiting the NF- κ B pathway, which is a key driver of inflammation and secondary injury (Meng et al., 2016). There is evidence from animal models that hyperbaric oxygen can downregulate microglial activation and thereby lessen the release of inflammatory mediators after neonatal brain injury (Meng et al., 2016).

Ozone therapy in CP is less studied, but theoretically it can complement HBOT. Ozone can stimulate 2,3-DPG in red cells (Bocci et al., 2011), facilitating better oxygen unloading to tissues, which might be useful in regions of the brain with impaired circulation. Ozone's induction of antioxidant enzymes can also help reduce lipid peroxidation and DNA damage that occurred during the hypoxic insult. Furthermore, ozone might promote a more balanced immune response, which is relevant because many children with CP have an inflammatory state (due to concomitant issues like infections or from the injury itself). By boosting antioxidant defences and improving microcirculation, ozone therapy could support neuroplasticity in the developing brain.

Deep tissue mobilization is a mainstay in the rehabilitative management of CP. Children with spastic CP have increased muscle tone, hyperactive reflexes, and often muscle contractures due to the continuous state of contraction. DTM and related techniques (such as myofascial release or deep stretching) help modulate muscle tone. The pressure and stretching of DTM activate Golgi tendon organs, which reflexively inhibit the alpha motor neuron activity to the muscle, temporarily reducing spasticity (Aktas et al., 2020). Over time, regular deep muscle work can improve the resting length and elasticity of muscles, making them less prone to spasm. Breaking down fascial adhesions is also important, as spastic muscles often become “bound” by connective tissue, limiting range of motion. By improving circulation to the affected limbs,

DTM may enhance muscle growth and strength in children undergoing physical therapy by ensuring muscles are better oxygenated and nourished. The improved lymphatic drainage can reduce localized edema in stiff limbs and help flush out inflammatory substances that irritate nerves. Clinically, massage therapy in CP has been associated with improvements in muscle tone and even some gains in gross motor function when combined with conventional physiotherapy (Aktas et al., 2020).

Gut cleansing therapy can play a surprisingly significant role in CP management. Many children with CP suffer from gastrointestinal motility problems (due to neurologic impairment of gut innervation and the effects of immobility). Chronic constipation is extremely common and can lead to discomfort, poor appetite, and increased spasticity (via autonomic stress). GCT measures, including stool softeners, fibre, probiotics or occasional colonic enemas, can relieve constipation and im-

prove the gut microbiome. Restoring gut regularity reduces the buildup of toxins like ammonia or bacterial metabolites that can affect cognition and muscle tone. Moreover, recent research highlights that children with neurological impairment often have altered gut flora and a propensity for intestinal permeability (Del Giudice et al., 1999). By ensuring a healthier gut environment, a potential reduction in systemic inflammation can be achieved.

In summary, for cerebral palsy, an integrative regimen of HBOT, ozone, DTM, and GCT can address the multifactorial consequences of the perinatal brain injury. HBOT/ozone improve oxygenation and protect neurons, DTM alleviates the musculoskeletal sequelae of spasticity, and GCT manages the gut-related issues that can impact overall health and comfort.

Amyotrophic Lateral Sclerosis (ALS):

ALS is a progressive neurodegenerative disease affecting motor neurons, leading to muscle weakness, atrophy, and paralysis. Its pathophysiology is complex, involving motor neuron degeneration with a mix of cell-intrinsic and systemic factors. One hallmark is mitochondrial dysfunction in motor neurons where mitochondria show impaired oxidative phosphorylation and generate less ATP, contributing to neuron fatigue and vulnerability (Muyderman & Chen, 2014). Motor neurons in ALS also exhibit high levels of oxidative stress due to excessive production of reactive oxygen/nitrogen species and a decreased capacity to detoxify them (Ferraiuolo et al., 2011). Another key feature is excitotoxicity. ALS patients have a deficiency in the astroglial glutamate transporter EAAT2 (GLT-1), causing glutamate to accumulate at synapses and overactivate receptors on motor neurons (Rothstein et al., 1995). The resulting calcium influx triggers destructive enzymes and cell death pathways. Meanwhile, neuroinflammation is prominent – activated microglia and astrocytes in ALS secrete pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) that can exacerbate motor neuron injury (Geloso et al., 2017). Given these mechanisms, integrative therapies aim to support cellular energy metabolism, reduce oxidative and inflammatory stress, and maintain muscle function. HBOT can temporarily enhance oxygen availability to neurons and glia, which may improve their mitochondrial respiration. By increasing tissue oxygen tension, HBOT helps motor neurons carry out aerobic metabolism more efficiently, potentially slowing energy depletion in ALS (Geng et al., 2015). Interestingly, some preclinical studies indicate HBOT might upregulate certain mitochondrial biogenesis regulators (like PGC-1 α), thereby improving mitochondrial number or function (Zhang et al., 2016). Importantly for ALS, HBOT and ozone therapy together have been reported to shift microglial polarization from the M1 (neurotoxic) state to the M2 (neuroprotective) state (Wang et al., 2019; Scassellati et al., 2022). This means fewer inflammatory cytokines and more growth factors in the ALS spinal cord environment.

Deep tissue mobilization does not alter the disease course of ALS but significantly improves quality of life. As ALS progresses, patients experience muscle spasticity, cramps, joint stiffness, and pain due to both upper motor neuron (spastic) and lower motor neuron (cramps, fasciculations) phenomena. Regular DTM and stretching can reduce spasticity by the mechanisms discussed (Golgi-mediated reflexes) and also help prevent contractures in limbs that have become nearly immobile. By keeping muscles and joints as supple as possible, DTM maximizes the remaining mobility and can aid in activities of daily living. Additionally, the gentle but deep pressure of massage can provide pain relief; many ALS patients suffer from shoulder and neck pain as muscles weaken and joints sublux. Massage triggers endorphin release, which can mitigate pain perception (Field, 2014). It also improves circulation in the skin and muscles, which can help prevent pressure sores in patients with limited movement. Even the temporary relaxation of spastic muscles after DTM can make tasks like caregiving, dressing, and physical therapy exercises easier. Psychologically, the human touch and improved comfort from massage contribute to reduced anxiety and better sleep in ALS patients, which are meaningful symptomatic benefits.

Gut cleansing therapy might be an unconventional but useful adjunct in ALS. Constipation is common in ALS due to reduced mobility and, in late stages, autonomic dysfunction. Ensuring regular bowel movements via diet (high fiber, adequate hydration) or supplements and occasional enemas can prevent complications like impaction and abdominal pain. More intriguingly, emerging research suggests the gut microbiome may influence ALS progression (Blasco et al., 2020). Some gut bacteria produce compounds that can be neurotoxic or neuroprotective. For example, overgrowth of certain *Clostridia* might elevate toxin levels, whereas beneficial bacteria produce short-chain fatty acids like butyrate which have anti-inflammatory effects on microglia (Dalile et al., 2019). By cleansing the gut and replenishing with probiotics, we might tilt the balance towards a microbiome that favors reduced systemic inflammation. Lowering systemic inflammation could in turn reduce the pro-inflammatory milieu around motor neurons. While this connection is still being studied, the concept of “gut-brain” interaction in ALS is gaining attention.

Overall, the integrative approach in ALS – HBOT to support cellular bioenergetics and redox balance, DTM to maintain musculoskeletal health, and GCT to optimize gut health represents a multifaceted strategy to improve patient function and well-being alongside standard ALS therapies (like riluzole or edaravone).

Muscular Dystrophy (MD):

Muscular dystrophies are genetic disorders characterized by progressive muscle fiber degeneration and weakness. The most common form, Duchenne Muscular Dystrophy (DMD), arises from mutations in the dystrophin gene, leading to absence of the dystrophin protein that normally stabilizes muscle fiber membranes.

Other forms of muscular dystrophy include Becker Muscular Dystrophy (BMD), a milder allelic variant of dystrophin mutations, Limb-Girdle Muscular Dystrophies (LGMD) caused by defects in sarcoglycans and other glycoproteins, Congenital Muscular Dystrophies (CMD) often linked to laminin-211 (merosin) deficiency, and Facioscapulohumeral Muscular Dystrophy (FSHD) associated with aberrant expression of the DUX4 gene (Bushby, 2009; Gawlik & Durbeej, 2011). Though the genetic basis differ, these disorders converge on common downstream mechanisms of sarcolemmal fragility, impaired regeneration, fibrosis, and inflammation. The loss of these structural proteins makes muscle fibers fragile; normal muscle contractions cause micro-tears in the sarcolemma (cell membrane). The damaged membrane allows excessive calcium influx into muscle cells, which triggers deleterious pathways: calcium-dependent enzymes (calpains, phospholipases) become overactive and start digesting muscle proteins and lipids, and mitochondria become calcium overloaded, leading to dysfunction and increased reactive oxygen species generation (Zhao & Hoffman, 2010). Repeated cycles of muscle injury and inadequate repair (due to exhaustion of regenerative satellite cells and a fibrotic healing response) result in progressive replacement of muscle with fat and connective tissue. Chronic inflammation is also present where dystrophic muscle often has infiltrating macrophages that release cytokines and growth factors, initially to clean up damage but chronically contributing to fibrosis and oxidative stress (Tidball, 2005). Integrative therapies in MD aim to support muscle health, reduce oxidative/inflammatory damage, and maintain mobility. HBOT can help by improving oxygen delivery to muscles that are often relatively hypoxic. In dystrophic muscle, degeneration and fibrosis can compromise local blood flow, so increasing plasma oxygen content via HBOT ensures muscles receive adequate O₂ even if capillary density is reduced. Better oxygenation helps muscle fibers rely less on anaerobic metabolism, reducing lactic acid buildup and delaying fatigue. Additionally, by elevating tissue oxygen, HBOT may stimulate angiogenesis (formation of new blood vessels) in muscle, as seen by upregulation of VEGF in other ischemic tissues (Geng et al., 2015). More capillaries would improve long-term muscle perfusion. HBOT also has systemic anti-inflammatory effects; it can reduce NF-κB activity, thereby decreasing the production of muscle-damaging cytokines (Buras et al., 2006). Some case reports in DMD patients have suggested that HBOT might transiently improve muscle strength or endurance, though evidence is anecdotal and more research is needed.

Deep tissue mobilization (and physiotherapy in general) is critical in MD to combat contractures and maintain flexibility. As muscles weaken, imbalances between muscle groups and inactivity lead to tightening of tendons and joint contractures (e.g., Achilles tendon contractures in DMD causing toe-walking). DTM, through sustained stretches and deep pressure, can remodel these tight tissues and delay contracture formation (Bialosky et al., 2009). For instance, deep longitudinal stroking of the calf muscles and Achilles tendon area can help lengthen the connective

tissue and prevent severe clubfoot deformity in DMD. Massage also helps to flush out edema and lymph buildup in inactive limbs; improved circulation can reduce the chronic swelling some non-ambulatory MD patients experience in their feet/legs. Moreover, muscle fibrosis in MD creates painful adhesions; DTM can break apart some of these adhesions, potentially preserving a bit more muscle elasticity and reducing pain. The mechano-transduction from massage (stimulation of integrin receptors and stretch-activated channels) might even influence muscle cells and fibroblasts to alter their collagen deposition, theoretically slowing fibrosis if done regularly (Schwartz & DeSimone, 2008). On a symptomatic level, patients report that massage therapy alleviates muscle soreness and spasms and provides relaxation. Given that MD often also involves cardiac and respiratory muscles, any safe therapy that improves overall circulation and reduces physical stress is valuable.

Gut cleansing therapy is a less obvious but meaningful adjunct for muscular dystrophy patients. Chronic illness and immobility can predispose them to dysbiosis; additionally, some MD patients take corticosteroids (standard in DMD), which can affect gut flora and cause GI side effects. Ensuring a healthy gut means better absorption of nutrients that muscles need (protein, vitamins, co-factors). For example, vitamin D and calcium absorption are vital for muscle and bone health in DMD, and gut health influences this. GCT with probiotics might specifically increase beneficial microbes that produce vitamins like vitamin K or B vitamins. Reducing “leaky gut and cofactors). icial because translocated bacterial endotoxins can perpetuate systemic inflammation (Ryan et al., 2013). By cleaning out the gut and normalizing the microbiome, we reduce the background inflammatory noise, possibly easing the load on muscle regeneration processes.

In summary, integrative therapies in muscular dystrophy attempt to slow the degeneration cycle. HBOT improves muscle oxygenation and redox balance, DTM maintains flexibility and circulation, and GCT optimizes internal nutrition and reduces inflammation – thereby working alongside gene therapies or pharmacologic treatments in pursuit of better outcomes for patients with MD.

Traumatic Brain Injury (TBI):

TBI results from an external mechanical force injuring the brain, as in falls, car accidents, or sports. It initiates a complex pathological cascade often described in two phases: primary injury (the immediate mechanical damage) and secondary injury (delayed biochemical and physiological derangements). The primary injury can include contusion (bruising of brain tissue), lacerations, diffuse axonal injury (tearing of axons from shearing forces), and hemorrhage (tearing of blood vessels) (Masel & DeWitt, 2010). These cause instant cell death in affected areas and disruption of neural networks. The secondary injury unfolds over hours to days post-impact and involves processes like glutamate excitotoxicity (excess glu-

tamate release causing calcium overload in neurons), energy crisis (ischemia and mitochondrial dysfunction leading to ATP depletion), oxidative stress (excess free radicals from damaged mitochondria and infiltrating immune cells), and neuroinflammation (activation of microglia and influx of neutrophils/macrophages releasing cytokines) (Masel & DeWitt, 2010). Edema (brain swelling) and raised intracranial pressure can further compound the injury by compressing blood vessels and reducing perfusion. Clinically, TBI can lead to a host of deficits depending on severity and location, from cognitive and behavioral changes to focal neurological deficits, and often includes motor problems like weakness or spasticity. HBOT has been explored as a therapy for TBI to mitigate the secondary injury processes. By flooding the brain with oxygen, HBOT can reduce the volume of the ischemic penumbra (areas around the primary injury that are at risk due to reduced blood flow). Studies have shown that HBOT can reduce intracranial pressure and brain edema by causing vasoconstriction in the uninjured brain (reducing fluid leakage) while still improving oxygen delivery (since plasma O_2 is high) to injured tissue (Gonzales-Portillo et al., 2019). This effectively lessens brain swelling and improves oxygen supply simultaneously. Hyper-oxygenation also helps restore the function of energy-dependent ion pumps (like Na^+/K^+ -ATPase) in neurons and glia, which prevents the ionic imbalances that lead to cytotoxic edema and cell death. From an inflammatory standpoint, HBOT has been observed to decrease levels of inflammatory cytokines and inhibit neutrophil adhesion in brain microvasculature, thereby protecting the blood-brain barrier (BBB) (Veltkamp et al., 2005). It also promotes neuroplasticity in the subacute/chronic phase; patients who underwent HBOT months after TBI showed increased perfusion and metabolic activity in languid brain areas, corresponding with some functional improvements (Harch et al., 2012). The release of VEGF and BDNF under HBOT can encourage angiogenesis and synaptic remodeling, aiding neurorehabilitation (Yang et al., 2017).

Ozone therapy is not as widely applied in TBI as HBOT, but it has potential benefits in reducing oxidative stress and inflammation. Following TBI, reactive species surge, and the antioxidant defense gets overwhelmed. Ozone therapy's preconditioning effect elevates antioxidants which can then better neutralize the subsequent oxidative bursts (León et al., 1998). For example, giving medical ozone to animals shortly after a TBI has been shown to increase levels of antioxidant enzymes in the brain and reduce lipid peroxidation. Ozone also may stabilize mitochondrial membranes, making them less prone to initiating apoptosis. Another key benefit is ozone's effect on inflammation: in a rat model of cerebral ischemia, ozone therapy inhibited the NF- κ B pathway, resulting in lower expression of inducible nitric oxide synthase and pro-inflammatory cytokines in the brain, with an accompanying reduction in neuronal death (Zhu et al., 2022). Practically, ozone could be given via rectal insufflation in TBI patients to confer these systemic anti-inflammatory benefits.

Deep tissue mobilization plays a role in the rehabilitation phase of TBI. Many moderate-to-severe TBI survivors develop spasticity – increased tone in certain muscles, especially if there is damage to motor pathways. This can manifest as clenched fists, stiff extended legs, or foot drop, which impede mobility and self-care. DTM techniques (along with regular stretching) help manage spasticity by reducing the reflex hyperexcitability of muscles (Brain Injury Association of America, n.d.). For instance, deep pressure along the calf muscles and Achilles tendon in a patient with ankle spasticity can lead to a temporary reduction in tone, allowing better dorsiflexion movement. Over time, this may contribute to improvements in gait when combined with neurodevelopmental techniques. DTM also addresses the myofascial pain that TBI patients might have – some TBI patients, due to prolonged bed rest or abnormal postures, develop trigger points and neck/back pain. Releasing those through deep massage improves their comfort and can enhance participation in physical therapy. There is also evidence that massage therapy can improve sleep quality in brain injury patients (Field, 2014), which is crucial since sleep disturbances are common post-TBI and can hinder recovery. The stimulation from DTM might also provide sensory relearning for patients who have altered sensation; it helps the brain remap tactile and proprioceptive inputs which can aid in regaining coordination (Bove & Chapelle, 2012).

Gut cleansing therapy addresses the gut-brain axis issues in TBI. It's remarkable that after a brain injury, within days, patients can develop intestinal permeability ("leaky gut") and altered gut microbiota (Hang et al., 2003). This is partly due to the massive autonomic dysregulation and surge of catecholamines/cortisol in acute TBI, which can impair gut blood flow and function. A "leaky" gut allows bacterial endotoxins to seep into circulation, potentially exacerbating systemic and central inflammation. Early interventions to support the gut including oral nutrition with probiotics, avoidance of unnecessary antibiotics (which disturb flora), and possibly gut cleansing if ileus or GI bleeding occurs – can reduce such complications. In subacute/chronic TBI, GCT could involve dietary adjustments and supplements to encourage a healthy microbiome, aiming to reduce pro-inflammatory signals emanating from the gut. Some rehabilitation centers include bowel programs (regular timing, use of gentle laxatives if needed) as part of TBI care, recognizing that regulating the gut improves overall patient well-being and may even affect mood and cognitive function.

Preclinical work also supports the integrative rationale. In a controlled traumatic brain injury (TBI) model, HBOT not only improved neurological recovery but also reversed gut dysbiosis, restoring beneficial bacterial populations such as *Prevotella copri* and *Deinococcus*, which correlated negatively with neuroinflammation and neuronal apoptosis (Li et al., 2024). This highlights the potential of HBOT to influence the brain-gut axis, aligning with the role of gut-focused therapies in modulating neuroinflammation. In another study of soft-tissue trauma in rats,

both HBOT and ozone therapy independently reduced edema, inflammatory infiltrates, and oxidative stress markers (MDA, MPO activity), while enhancing histological tissue repair (Bakar et al., 2014).

In summary, TBI care benefits from integrative methods: HBOT and ozone help limit secondary brain damage and metabolic dysfunction, DTM aids in restoring physical function and comfort, and GCT addresses peripheral factors that can influence central recovery.

Spinal Cord Injury (SCI)

SCI involves damage to the spinal cord that results in loss of sensory, motor, and autonomic function below the level of injury. The pathophysiology parallels TBI in terms of primary mechanical injury followed by secondary cascades. Immediately after an SCI (from trauma like a vertebral fracture dislocation), there is necrosis of neurons and glia at the injury site and often hemorrhage within the cord. This is followed by spreading tissue damage due to ischemia (from blood vessel damage and edema raising intramedullary pressure), excitotoxic glutamate release, ionic imbalance, and inflammation (Ahuja et al., 2017). A hallmark of secondary SCI is the formation of a hemorrhagic necrotic lesion that can expand over the first week. Microglia and infiltrating macrophages clean up debris but also secrete cytokines that can kill neurons and oligodendrocytes (Oyinbo, 2011). The result is often a cavitation or cystic space at the injury epicentre and demyelination of surviving axons around it. Additionally, inhibitory molecules like chondroitin sulphate proteoglycans are released by astrocytes (forming the glial scar), which impede axon regeneration. Given these events, integrative therapies focus on reducing secondary damage and creating an environment conducive to neural repair. HBOT can be beneficial soon after an SCI by improving oxygen delivery to the ischemic penumbra of the injured cord. Animal studies have shown that HBOT after SCI leads to smaller lesion sizes and better neurological outcomes, likely by preserving the at-risk tissue (Wada et al., 2001). Oxygen-rich blood helps maintain ATP levels in neurons and glia that are partially compromised but not irreversibly damaged, preventing them from undergoing apoptosis. HBOT also appears to promote angiogenesis and blood flow in the injured spinal cord – for example, Zhang et al. (2016) demonstrated increased expression of HIF-1 α and VEGF in HBOT-treated SCI rats, correlating with more blood vessel formation. More blood vessels mean better perfusion and nutrient delivery during the healing phase. On the immunological front, HBOT can reduce neutrophil infiltration and lipid peroxidation in the injured cord (Harch & Neubauer, 2016). Clinically, some small trials in humans have noted improvements in ASIA sensory/motor scores with HBOT, though data are still preliminary.

Ozone therapy might also assist in the subacute phase of SCI by harnessing its antioxidant and anti-inflammatory properties. By giving medical ozone (for in-

stance, via the rectal route), we can induce systemic antioxidant responses that might lessen the oxidative damage in the cord. Elvis & Ekta (2011) reviewed ozone therapy and suggested it can stimulate antioxidant enzymes and inhibit inflammation, which in the context of SCI could translate to reduced secondary injury. Additionally, ozone might help combat secondary infections in SCI patients (like pressure ulcers or chest infections) due to its antimicrobial nature, indirectly improving outcomes.

Deep tissue mobilization is extremely important in long-term SCI management. After an SCI, especially in cases of paralysis, patients are at high risk of developing muscle contractures (due to imbalanced muscle pull and prolonged sitting/lying postures) and pressure sores (from constant pressure on skin due to immobility). Regular DTM and stretching of paralyzed or spastic muscles help keep the soft tissues supple and prevent fixed deformities (Harvey & Herbert, 2002). For example, in a patient with a cervical SCI who has tetraplegia, DTM to the shoulders, elbows, and wrists can maintain joint range and prevent frozen shoulder or elbow flexion contractures, which are common due to unopposed muscle tightness. Massage also improves circulation in dependent limbs, reducing edema and enhancing tissue oxygenation, which is crucial for preventing skin breakdown (Weerapong et al., 2005). Moreover, many SCI patients experience spasticity (especially those with injuries above T12); DTM can temporarily reduce spasticity and spasm frequency, providing relief and making caregiving (transfers, dressing) easier. There is also a sensory aspect: people with SCI often have areas of altered sensation or neuropathic pain. Slow, deep massage can modulate those sensations and sometimes reduce neuropathic pain by stimulating large-diameter afferents (gate control theory). Psychologically, the human interaction and relaxation from massage can alleviate some of the anxiety or depression that often accompanies life with SCI.

Gut cleansing therapy is an often overlooked but critical part of SCI care. After SCI, particularly high-level injuries, patients lose normal autonomic control of the bowel. This leads to slow transit, constipation, and a reliance on scheduled bowel programs to evacuate stool. A constipated, distended gut not only causes discomfort but can trigger autonomic dysreflexia in high SCI patients – a dangerous syndrome of sudden high blood pressure. Maintaining a clean, regular bowel is life-changing for SCI patients in terms of health and daily routine. GCT involves a c – anation of diet (high fiber, adequate fluid), medications (stool softeners, suppositories), and occasionally mechanical aids (enemas) to ensure regular clearance (usually every 1–2 days). Doing so prevents complications like megacolon or bowel obstruction. Additionally, SCI leads to changes in gut microbiota, often a decrease in diversity and an increase in pathogenic strains (Kigerl et al., 2016). This dysbiosis has been implicated in worse neurological recovery, possibly because it sustains systemic inflammation. By using probiotics or prebiotics as part of GCT,

we aim to restore a healthier microbiome. Kigerl et al. (2016) found that transplanting a normal microbiota into SCI mice improved their recovery compared to those with dysbiosis, highlighting how crucial gut health is even for spinal cord healing. Thus, GCT not only addresses constipation but might actually improve neurological outcomes by reducing the pro-inflammatory signals from the gut.

In conclusion, integrative management of SCI incorporates HBOT and ozone to salvage tissue and reduce inflammation, DTM to maintain physical integrity and function, and GCT to manage the gut and systemic metabolic state—all augmenting standard interventions like surgical stabilization and physiotherapy.

Stroke (Ischemic & Hemorrhagic)

Stroke is a cerebrovascular event resulting in brain cell death due to either lack of blood flow (ischemic stroke) or bleeding (hemorrhagic stroke). The vast majority (~85%) of strokes are ischemic, caused by thrombosis or embolism blocking an artery, whereas hemorrhagic strokes (~15%) result from vessel rupture leading to intracerebral or subarachnoid hemorrhage (Benjamin et al., 2019). Regardless of type, strokes produce an acute focal brain injury followed by cascades similar to TBI/SCI: in ischemic stroke, the cessation of blood supply leads to an ischemic core (where cells die within minutes from energy failure) surrounded by an ischemic penumbra of cells that are stressed but potentially salvageable. Excitotoxic glutamate release, calcium overload, and oxidative stress drive the expansion of damage in the hours after onset (Lakhan et al., 2009). Meanwhile, the brain's immune response gets activated; microglia and infiltrating leukocytes release cytokines and chemokines that can either help clear debris or inadvertently kill neurons (Iadecola & Anrather, 2011). The BBB often breaks down in stroke, especially after reperfusion, leading to vasogenic edema (swelling from leaky vessels) (Lakhan et al., 2009). In hemorrhagic stroke, the pressure from the hematoma and toxicity of blood breakdown products cause tissue injury, and secondary ischemia can occur around the clot. Long-term, strokes can leave patients with motor deficits (e.g., hemiplegia), speech/language impairments (aphasia), visual field cuts, cognitive deficits, etc., depending on the area of the brain affected. HBOT has been studied as an acute treatment for ischemic stroke, with the idea of rescuing the penumbra by supplying oxygen. By elevating arterial oxygen content, HBOT can deliver oxygen to tissues even when blood flow is limited; oxygen will diffuse farther from intact blood vessels under high pressure, potentially reaching cells on the brink of death (Zhou et al., 2014). Some experiments show that if given early, HBOT can reduce infarct size by sustaining aerobic metabolism in the penumbral zone until reperfusion occurs. Additionally, HBOT has been shown to reduce BBB permeability and hence brain edema in focal cerebral ischemia models (Velthkamp et al., 2005). This is extremely valuable because edema and increased intracranial pressure are major causes of early death in stroke – by mitigating edema, HBOT can improve outcomes. On the molecular level, HBOT decreases the expression of

adhesion molecules on endothelium, reducing leukocyte sticking and infiltration, which in turn lessens inflammation in the stroke region (Hadanny & Efrati, 2021). In the chronic stage of stroke, there is evidence (from SPECT and PET imaging studies) that HBOT can rekindle neuronal activity in metabolically sluggish areas around the infarct (those that didn't die but were hibernating). Patients who underwent HBOT months to years after stroke have demonstrated some neurological improvements, hypothesized to be due to enhanced neuroplasticity. HBOT may promote axonal sprouting and recruitment of alternate pathways for lost functions (Efesiobi et al., 2017).

Ozone therapy is less common in stroke care, but its principles can apply. Ozone could be helpful in the subacute phase to reduce oxidative damage from reperfusion. Paradoxically, by providing a controlled oxidative stimulus, ozone triggers the brain (and body's) own antioxidant defenses at a time when they are needed most. It also could improve blood rheology; in stroke patients, especially those with atherosclerosis, blood viscosity and red cell deformability are issues – ozone makes red cells more pliable and improves oxygen release, which might enhance perfusion in the microcirculation of the recovering brain (Bocci et al., 2011). Ozone's anti-inflammatory effect (suppressing NF- κ B and related cytokines) could help by limiting secondary injury and possibly promoting a shift to tissue repair processes sooner (Zhu et al., 2022). Some clinicians in Europe have used ozone as part of post-stroke rehabilitation protocols, noting improved endurance and lesser spasticity, although systematic data are lacking.

Deep tissue mobilization in stroke rehabilitation addresses the frequent consequence of spastic hypertonia. Many stroke survivors, after the initial flaccid phase, develop spasticity in various muscle groups (often flexors of the arm and extensors of the leg in typical hemiplegic patterns) (Gracies, 2005). This spasticity can cause pain and impede functional recovery by limiting joint motion. DTM, along with stretching and exercise, helps manage spasticity. By applying deep pressure and stretch to hypertonic muscles (e.g., the biceps, wrist flexors, calf muscles), therapists can induce a reduction in muscle spindle activity and enhancement of Golgi tendon organ firing, temporarily decreasing tone. Over time, this can lead to improved joint range – for example, a stroke patient with a clenched hand might gradually open the hand more fully with consistent deep massage and stretching of the forearm flexors. DTM also helps break down adhesions in soft tissues that form due to disuse or abnormal posturing, thereby preventing contractures (Weerapong et al., 2005). Another benefit is improved proprioceptive feedback: stroke can impair the sense of limb position, but stimulating the muscles and joints deeply with manual therapy sends a lot of signals to the brain that can help re-map sensory pathways (Bove & Chapelle, 2012). This can support motor relearning. Additionally, many stroke patients experience shoulder pain (due to subluxation and spasticity in shoulder muscles); deep massage can relieve that pain and im-

prove local circulation, which aids tissue health. By reducing pain and spasticity, DTM enables patients to participate more effectively in active rehabilitation like physiotherapy and occupational therapy.

Gut cleansing therapy is a novel but relevant consideration in stroke, especially because there is growing evidence of the gut-brain axis playing a role in stroke outcomes. After a stroke, particularly if there is hypothalamic or brainstem involvement, patients can have disrupted autonomic control of gut motility, leading to constipation or, conversely, in some cases, GI bleeding from stress ulcers. Early management of the gut (through stool softeners, bowel regimens, and nutritional support) is important for medical stability. More interestingly, stroke induces changes in the gut microbiome; studies have found dysbiosis in stroke patients characterized by reduced beneficial bacteria and overgrowth of potentially pro-inflammatory species (Zheng et al., 2022). This dysbiosis can leak pro-inflammatory metabolites that might worsen post-stroke inflammation or influence neurorecovery. GCT in stroke would involve measures like using probiotics to restore beneficial microbes, prebiotic fibers to feed them, and possibly eliminating any dietary factors that could promote dysbiosis (like excessive sugar or processed foods). By normalizing the gut flora, we potentially increase production of metabolites like butyrate, which crosses into the brain and has been shown to support brain repair and reduce neuroinflammation (Stilling et al., 2016). Additionally, avoiding constipation in stroke is crucial because straining can raise blood pressure (a risk for another stroke or for hemorrhagic transformation). Therefore, incorporating GCT as part of a comprehensive stroke care plan can contribute to reducing complications and possibly aiding neurological recovery through improved metabolic and immune status.

Clinical precedent also exists for integrative approaches in stroke. In a randomized, controlled trial of chronic stroke ($n \approx 74$), 40 sessions over 2 months of HBOT at 2.0 ATA, 100% O_2 , for 90 minutes, significantly improved neurological scores (NIHSS), activities of daily living, and quality of life compared to baseline, with no improvements during the control period; functional improvements were confirmed on SPECT imaging in previously dormant brain regions (Efrati et al., 2013). More recently, in a randomized study of acute ischemic stroke ($n = 62$), ozonated auto-hemotherapy initiated within 24 hours significantly reduced NIHSS, improved Barthel and cognitive (MoCA) scores, lowered neuronal injury markers (NSE, S100 β), and favourably modulated oxidative stress and hypoxia-related pathways (Cheng et al., 2025). Collectively, these trials demonstrate that HBOT and ozone, individually, can beneficially modulate the post-ischemic brain environment—further justifying their incorporation into comprehensive integrative stroke care.

Overall, integrative therapies in stroke serve to augment neuroprotective strategies and enhance rehabilitation – HBOT and ozone aim to salvage brain tissue and

create a neuro-friendly internal environment, DTM addresses musculoskeletal and sensorimotor sequelae, and GCT ensures systemic health factors (like gut-derived inflammation) are kept in check, all in concert with pharmacological treatments (e.g., thrombolytics, antiplatelets) and physiotherapy.

Integrative Therapies as Adjuncts to Cell Transplantation

One of the emerging frontiers in treating neurological disorders is cellular therapy, such as transplantation of stem cells (e.g., mesenchymal stem cells, neural progenitors, or bone marrow mononuclear cells). The goal of cell transplantation is to replace or support damaged neural cells, promote regeneration, and modulate the immune environment. However, the success of transplanted cells depends greatly on the host tissue environment. Often the injured CNS is hostile, with inflammation, scar tissue, and poor blood flow that hamper the survival and integration of injected cells. Here, integrative therapies can play a supportive synergistic role. For example, HBOT has been shown to enhance the efficacy of stem cell therapies in experimental models. Geng et al. (2015) demonstrated that in rats with spinal cord injury, combining HBOT with bone marrow mesenchymal stem cell transplantation led to significantly better locomotor recovery than stem cells alone. The combination group had more surviving transplanted cells (as evidenced by greater numbers of BrdU⁺ cells in the injured cord) and lower levels of inflammatory cytokines, indicating that HBOT improved the microenvironment for the graft. Similarly, Yin et al. (2022) found that HBOT combined with adipose-derived stem cells in acute SCI synergistically downregulated pro-inflammatory and oxidative stress pathways, translating to superior functional outcomes compared to either treatment alone. These studies suggest that HBOT's actions of increasing oxygen, promoting angiogenesis, and reducing inflammation help transplanted stem cells survive, engraft, and do their repair work more effectively. Another intriguing aspect is HBOT's ability to mobilize the body's own stem cells. As noted earlier, a course of HBOT can lead to an eightfold increase in circulating CD34⁺ stem/progenitor cells (Thom et al., 2006). In the context of cell therapy, this could amplify healing; the patient's own mobilized cells might home to injury sites and complement the transplanted cells' activity.

Ozone therapy may likewise enhance cell therapy. While direct studies are sparse, we can extrapolate ozone's reduction of oxidative stress could protect transplanted cells from hostile free radicals in post-injury tissue. Its immune-modulating effect might prevent the graft from being attacked by the host's immune cells and facilitate an anti-inflammatory, pro-healing milieu. Additionally, ozone-induced improvement in blood flow ensures transplanted cells get better oxygen and nutrients. For instance, in chronic limb ischemia, ozone preconditioning has been noted to improve stem cell graft outcomes by improving local circulation (Guo et al., 2018). Translating that to the brain/spinal cord, ozone could help overcome the poor perfusion in the "shocked" tissue, aiding cell graft survival.

Deep tissue mobilization supports cell therapies mainly in an indirect but crucial way: by maintaining and improving the patient's physical function and reducing complications. When a patient receives a cell transplant (say, an autologous BMMNC transplant for CP or stroke), they still need extensive rehabilitation to maximize functional gains. DTM is part of that rehabilitative process – it keeps muscles and joints in a state where they can respond to new signals, possibly from the transplanted cells forming new connections. If a child with CP receives stem cells and, as a result, gains some new neural function, that function could be squandered if their muscles are too tight or joints fixed; DTM and stretching prevent that, so the child can take advantage of any neural improvements. Moreover, by improving circulation and reducing pain, DTM might ensure the patient remains active and engaged in therapy post-transplant, which is vital because neural recovery is activity-dependent.

Gut cleansing therapy also plays a supportive role in cell transplantation. Transplanted cells are exquisitely sensitive to inflammation – systemic inflammation can impair their function or even cause rejection if they are allogeneic. Ensuring the patient's gut is not contributing to systemic inflammation (through leaky gut or dysbiosis) is therefore important to create an optimal internal environment for the cell therapy to work. Additionally, many protocols for stem cell therapy involve immunosuppressants or other drugs that can upset the GI system; GCT can mitigate those side effects, keeping patients healthier during the treatment course.

In summary, integrative therapies can be seen as “soil preparation” for the seeds (transplanted cells). By oxygenating tissue, reducing toxins and inflammation, and preserving musculoskeletal integrity, they make the brain or muscle more receptive to engraftment and functional integration of new cells. The concept of combination therapy is increasingly endorsed: rather than viewing cell transplantation in isolation, it is combined with interventions like HBOT to achieve outcomes greater than the sum of their parts. This comprehensive approach holds promise to significantly enhance recovery in conditions once thought to be irreparable, offering patients better odds at functional improvement and quality of life.

Conclusion

Integrative therapies bring a holistic dimension to the management of complex neurological disorders and injuries. By simultaneously targeting multiple pathophysiological pathways of hypoxia, inflammation, oxidative stress, neuromuscular dysfunction, and gut-derived factors, modalities such as HBOT, ozone therapy, deep tissue mobilization, and gut cleansing create a more favourable internal environment for healing and rehabilitation. These interventions do not replace standard treatments; rather, they complement them, acting as force multipliers to accelerate and augment recovery. The evidence, ranging from mechanistic studies to clinical observations, suggests that patients with autism, cerebral palsy,

ALS, muscular dystrophy, TBI, SCI, stroke, and other neuro conditions can attain improved outcomes when integrative therapies are incorporated into their care plans. For clinicians and researchers, this highlights the importance of a multi-disciplinary approach – one that bridges neurology, rehabilitation, nutrition, and complementary medicine to address the full spectrum of challenges posed by these disorders. As research continues, especially in combinations of integrative therapies with emerging treatments like stem cells, we move closer to a model of care that is comprehensive, personalized, and optimized for tissue restoration and functional recovery. The ultimate goal is to not only extend life but to improve the quality of life for individuals affected by these debilitating conditions, enabling them to reach their fullest potential in the journey of recovery.

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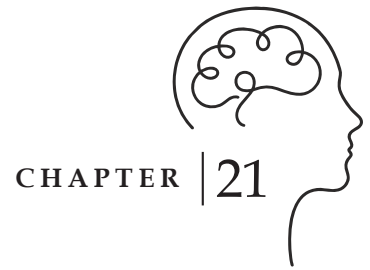
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Exosomes

Introduction

Exosomes were initially identified in the 1980s when two laboratories reported identical results within a week of one another on the ability of reticulocytes to endocytose transferrin that had been labelled with gold and transport it to the plasma membrane within tiny vesicles. They were initially thought to be cellular waste products. [1] Eventually, 'exosomes' was coined by Rose Johnstone in reference to vesicles expelled by exocytosis from a multivesicular body [2, 3]. Exosomes, microvesicles, and apoptotic bodies are the three primary subtypes of extracellular vesicles that are typically distinguished. Exosomes are vesicles formed from intracellular endosomes, whereas microvesicles and apoptotic bodies are vesicles derived by budding and pinching out of the plasma membrane surface. In recent years, an increasing amount of deep study has been conducted on exosomes. [2, 4].

Composition

A subset of extracellular vesicles with a diameter of 30 to 150 nm, exosomes are nanoscale and play a crucial role in cell-to-cell communication by delivering functional proteins, metabolites, and nucleic acids to recipient cells. [2, 5] Exosomes possess a lipid bilayer that is rich in unsaturated fatty acids, phosphatidylserine,

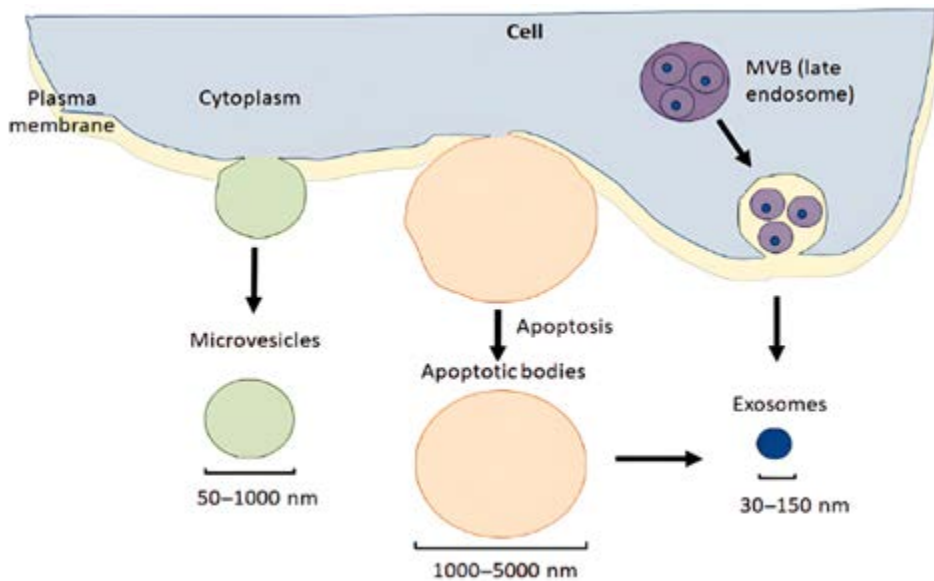


Figure 1: Types of Extracellular Vesicles

polyglycerin, sphingomyelin, cholesterol, and gangliosides. The density of exosomes varies in the range of 1.08 to 1.22 g/mL. [6] The lipid bilayer of the exosomes is specifically known to provide them with strength, firmness, and an armoured internal environment acting as a barrier, thwarting their enzymatic degradation and providing them higher stability as a carrier molecule. The inward budding of the plasma membrane to produce an early endosome is the first step in exosomal formation. All cell types release exosomes in both healthy and pathological conditions.

Sources

The components and characteristics of exosomes, which are released by particular organs and cells, are different. At the moment, the most significant exosome source is stem cells. [7]

They are present in breast milk, serum, amniotic fluid, synovial fluid, tears, lymph, bile, stomach acid, bronchial fluid, cerebrospinal fluid (CSF), plasma, urine, semen, saliva, and bile. [8] They can be produced from Schwann cells (SCs), mesenchymal stem cells, immune cells, fibroblasts, neural stem cells (NSCs), adipose-derived stem cells, endothelial progenitor cells, and umbilical vein endothelial cells (UVECs). [7, 9]

Mesenchymal Stem Cell-Derived Exosomes:

MSCs are multipotent stem cells that originate from different sources of adult tis-

sues, such as adipose tissue, skin, tissues of the orofacial area, and bone marrow. [10] Exosomes generated from mesenchymal stem cells (MSCs) have become a promising novel approach in regenerative medicine. They have been shown in numerous preclinical studies of skin, bone, and cartilage defects to improve tissue repair and regeneration. More specifically, MSC-derived exosomes use a variety of methods to provide their therapeutic benefits. By altering macrophage polarisation, they can reduce inflammation. In addition, MSC-exosomes stimulate angiogenesis, fibroblast migration, and proliferation during the proliferation phase. MSC-exosomes can also enhance the remodelling of the extracellular matrix. Consequently, exosomes produced from MSCs have presented a novel approach to the management of cutaneous wounds. [4] MSC-Exos have lower immunogenicity, greater safety, and do not immediately generate tumors, along with having biological activity that is comparable to that of MSCs. [11]

Adipose-derived stem cells:

Adipose-derived stem cells (ADSCs) are mesenchymal stem cells that have a high capacity for differentiation and self-renewal. By transporting payloads of bioactive compounds that may influence particular cellular targets and signalling pathways, exosomes generated from ADSCs (ADSC-exos) may replicate their functionalities. [12] A distinctive feature of ADSC is higher lipid content, which contributes to their enhanced paracrine signalling capacity, mediated by exosomes. They promote tissue repair through increased cellular communication, improved membrane fusion, and better interaction with target cells. [9]

Bone marrow-derived mesenchymal stem cells:

BM-MSCs, or bone-marrow-derived mesenchymal stem cells, are one of the most extensively researched postnatal stem cell groups and are thought to be used more frequently in cancer and cell-based therapy. An alternate source of mesenchymal stem cells (MSCs) for tissue engineering and personalised medicine is offered by these stem cell types, which can differentiate into blood cells, heart cells, and osteogenic cells, among other lineages. [10] The use of exosomes produced from BMMSCs (BMMSC-exos) as a potential treatment strategy has been studied in animal models of various diseases. These investigations have demonstrated that exosomes' anti-apoptotic, antioxidant, and anti-inflammatory properties may decrease tissue fibrosis and encourage tissue regeneration. [7] For wound healing, BMSC-Exos outperform exosomes derived from ADSC and UCMSC in enhancing the proliferation of dermal fibroblasts. [9] Since BM-MSCs have several drawbacks, including an invasive procedure, declining proliferation and differentiation with age, restricted access, the potential for donor site destruction, and low self-renewal, UC-MSCs derived from a baby's umbilical cord may be a viable substitute. [4]

Neural stem cells (NSCs):

Neural stem cells (NSCs) are self-renewing, differentiating, and proliferating cell populations that may improve neurological performance by stimulating vascular regeneration along with neuroregeneration and repair. Additionally, there currently exist two types of neural stem cell sources, each with unique advantages and disadvantages. The first consists of endogenous neural stem cells, which are mostly found in the lateral ventricle's subventricular zone (SVZ) and the hippocampal dentate gyrus's subgranular zone (SGZ). The second consists of exogenous neural stem cells that have developed from embryonic tissue. NSC exosomes are responsible for essential biological processes, including material transport and cellular communication. [13] NSC-Exos have also become a promising focus. [9] NSC-produced exosomes can improve neuroplasticity, speed up neuroregeneration and motor function recovery following brain damage, and have therapeutic effects by modifying the activity of distant target cells as well as nearby neurones and glial cells. Exosomes secreted by NSCs play an important role in the promotion of angiogenesis in brain injury. [13] Because of their anti-inflammatory, neurogenic, and neurotrophic properties, as well as their ability to interact with the brain tissue's microenvironment, NSC-derived exosomes have been shown to be effective in the treatment of a number of neurological disorders. [14]

Human-induced pluripotent stem cell:

The term "induced pluripotent stem cell-derived exosomes" (iPSCs) refers to fibroblasts that have the ability to differentiate into pluripotent stem cells. The iPSCs have the ability to differentiate into any type of cell in the body and exhibit regenerative properties. [7] iPSC-Exos have been reported to serve as tissue engineering agents and therapeutic agents in bone diseases. Emerging evidence has shown that exosomes derived from iPSCs exhibit therapeutic effects for bone diseases such as bone defects, osteonecrosis and osteoarthritis (OA). [15] The first instance of human iPSC-derived MSCs (hiPSC-MSCs) being used to heal rat cutaneous wounds was documented by Zhang et al. [16]

Others:

There are multiple sources to derive exosomes from; some of the different sources are mentioned below. There are multiple activation states of macrophages (e.g., M1 pro-inflammatory and M2 anti-inflammatory), and their exosome contents and activities vary as well. The pro-inflammatory properties of M1 macrophages and their exosomes may have a negative impact on tissue regeneration. Exosomes produced from regulatory T cells (Treg) (Treg-Exos) have a scientific base in regenerative medicine and beneficial immunomodulatory capabilities. The body's response to injuries, the manufacture and remodelling of the local extracellular matrix, and the development of new tissue all depend on fibroblasts, and their exosomes (FC-Exos) are essential for these activities. Primary fibroblasts are more strongly linked to wound healing than MSCs and can be extracted from the skin

with less invasive techniques. Schwann Cell-Derived Exosome have attracted a lot of interest in the therapy of neurological disorders. Exosomes derived from dental pulp stem cells (DPSC-Exos) possess higher drug-loading efficiency than many other MSC-derived exosomes. [9]

Modifications:

Depending on whether they have undergone artificial modification, exosomes are classified as either natural or engineered. [17] Surface engineering, cargo loading, and genetic modification are the three primary methods for altering exosomes in order to improve their stability, specificity, and therapeutic potential. Surface engineering is altering the exosomal membrane to enhance uptake by particular cell types, circulation time, and targeting capabilities. Numerous scientists have compiled different surface engineering techniques, which can be divided into chemical and biological methods, cellular-level modification, and modification following exosomal isolation. [9]

The term “cargo loading” describes the process of incorporating therapeutic effector molecules – like medications, RNA, or proteins – to exosomes for particular uses. It is classified into one of two categories: active loading or passive loading. In order to improve cargo integration, active loading typically entails using methods like electroporation, sonication, or extrusion. Incubating exosomes with the intended cargo to enable diffusion-based incorporation – which involves co-cultivation, transfection, and incubation – is commonly referred to as passive loading. [9] Because of its hydrophilic centre, the exosome can also be loaded with several water-soluble medications. [18]

Changing donor cells to express certain proteins or RNAs that are then integrated into exosomes is known as genetic modification. It can be separated into genetic engineering-based surface display and parent cell modification. Donor cells can generate exosomes enriched with certain proteins or RNAs by transfecting them with genes encoding targeted therapeutic molecules. Exosomes containing these genetic components can be secreted when cells are engineered to express therapeutic mRNA or siRNA, enabling targeted gene therapy applications. [9]

Isolation Techniques:

The first step in investigating exosomes is to discover a productive and effective method for extracting and purifying them since they have a great deal of promise for usage as medication therapy carriers and as diagnostic markers for a variety of illnesses. Exosomes can be isolated from various cell culture or bodily fluid sources using various separation techniques because of their diverse size ranges and variable protein and nucleic acid contents. The several isolation methods used to separate exosomes are shown in the subsections that follow. [6] Effective research

on exosomes can only be conducted based on high-quality separation products. Therefore, the first step in doing research on exosomes is choosing the right separation method. [19]

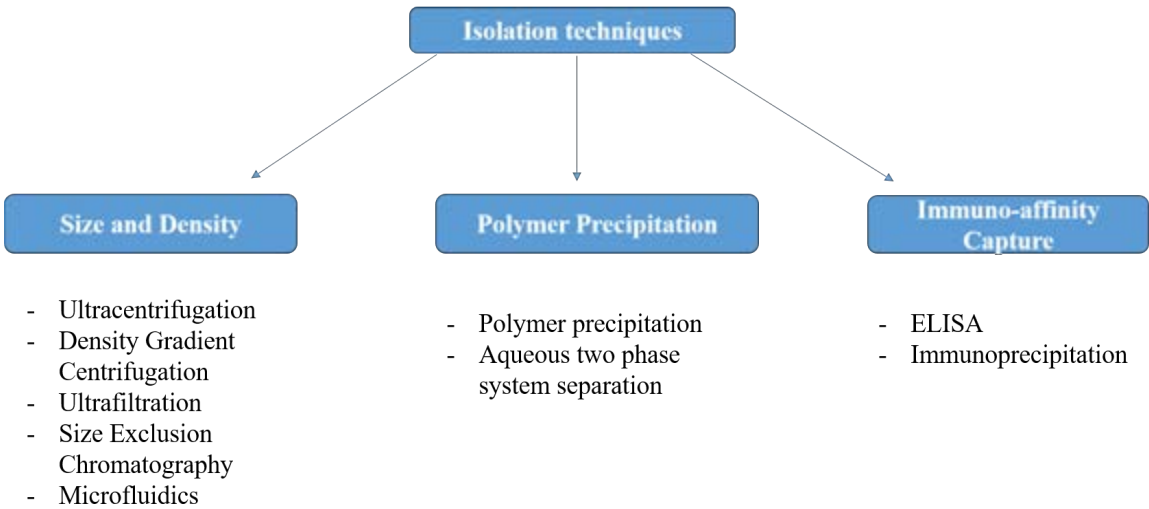


Figure 2: Graphical abstract portraying the techniques involved in the isolation of exosomes

A.Size- and density-based

i. Ultracentrifugation:

Currently, the most widely used technique for separating exosomes is ultracentrifugation. Ultracentrifugation (UC) works on the basis of the density and size discrepancies between the sample’s impurities and exosomes. [20] This is the fundamental idea behind the ultracentrifugation (UC) step: various particles in a sample have different sedimentation coefficients, which causes them to settle in distinct layers that may be collected individually. [6] The primary steps of ultracentrifugation are as follows: First, a series of continuous low-medium speed centrifugations to remove enormous extracellular vesicles and dead cells, along with cell debris; subsequently, an increased centrifugal force of $100,000 \times g$ for separating exosomes; the exosomes were subsequently rinsed with PBS to eliminate contaminants like contaminating proteins. [17] UC can be divided into two categories: density gradient centrifugation and differential centrifugation. [7] The UC technique is a “gold standard” for exosome isolation because of its high throughput and widespread application in exosome extraction these days. Despite having a number of advantages over other approaches, the system has certain drawbacks. [6] However, the entire separation procedure is time-consuming and has poor/unstable reproducibility. Lastly, exosomes may be damaged by high-speed centrifugation, which would lower their biological activity. [20]

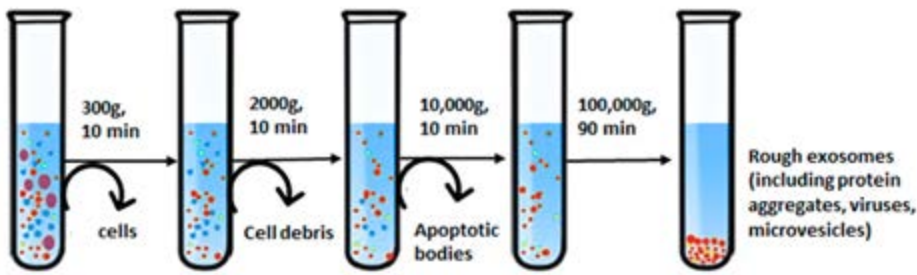


Figure 3: Graphical representation of ultracentrifugation.

ii. Density Gradient Centrifugation:

An enhanced separation method based on ultracentrifugation is density gradient centrifugation. The use of two or more separating media with varying densities, such as sucrose and iodixanol, is how density gradient centrifugation differs from ultracentrifugation. Additionally, the sample is placed on top of the separating medium for ultracentrifugation after large impurities have been removed by low-speed centrifugation. Higher isolation purity is this method's advantage. But its clinical use is limited by the need for pre-preparation, laborious operation, and a protracted centrifugation time. [20] The approach is traditional and appropriate for omics studies. [19]

iii. Ultrafiltration:

Typically, the ultrafiltration technique separates samples selectively using ultrafiltration membranes with varying molecular weight cutoffs (MWCO). This technique frequently aids in isolation in exosome research. [17] Exosomes are often separated from the filtrate using membrane filters with pores as small as 0.22 μm . Prior to the filtration stage, cells, debris, and bigger vesicles are eliminated using membrane filters with pore diameters of 0.80 μm . It is a more expensive method since it necessitates a chromatographic column of comparatively higher quality for separation. The performance of the separation process is hampered by the separated exosomes' vulnerability to pore blockage and damage by external pressure. [6] Exosome isolation via ultrafiltration is a substitute for traditional ultracentrifugation since it drastically decreases the processing time and requires no specialized equipment. [7]

iv. Size-exclusion chromatography (SEC):

SEC is a method for separation based on differences in molecule size, which is like the principle of ultrafiltration. The essential step is that the larger particles cannot penetrate the gel pores when the sample is introduced to the column that contains porous beads (such as Sephadex, Sepharose, Sephacryl, or BioGel P), and the elution speed is accelerated. Recent studies have suggested that an improved level of

exosome purity can be obtained by combining SEC and ultrafiltration. According to the separation results, SEC was able to isolate more pure exosomes. SEC is a straightforward, sustainable, and cost-effective technique for handling large-scale samples. [20]

v. Tangential flow filtration (TFF):

Tangential flow filtration (TFF) is a modern version of ultrafiltration that uses ‘tangential flow,’ in which the media flows parallel to the membrane, in contradiction to the perpendicular flow in UF’s stagnant filtration. When it comes to time efficiency, scalability, and the requirement for costly equipment, TFF is superior to ultracentrifugation. Based on these features, a number of research studies have described a scalable technique for combining TFF and SEC to purify EVs with low protein contamination. [21]

vi. Microfluidics techniques:

The ability to precisely regulate and manipulate small fluid quantities makes microfluidics crucial for biological and medical research. Exosomes can also be separated using microfluidic devices according to surface markers and size. The two types of isolation mechanisms that these microfluidic techniques fall under are passive and active. Compared to active approaches, passive microfluidic exosome isolation techniques are easier to use and less complicated because they do not require an external actuation mechanism. Filtration, inertial focusing, immunoaffinity, lateral displacement, and viscoelastic flow are the primary passive microfluidic isolation techniques. The other passive techniques are label-free and rely on the physical characteristics of the exosomes, with the exception of the immunoaffinity-based isolation techniques. Dielectrophoretic and acoustic isolation techniques are the most common approaches in the active approach. Both of these techniques are dynamically adjustable and allow for continuous-flow exosome isolation. There is a significant research effort to actively improve exosome isolation strategies, despite the fact that active approaches are more demanding and complex. [22]

B. Polymer Precipitation:

i. Polymer Precipitation:

Polyethylene glycol (PEG) is typically used as a medium in the polymer precipitation process, and the exosomes undergo extraction during centrifuging to decrease their solubility. [17] The solubility properties of the sample components can be changed, and a high-viscosity environment can be produced by adding a water-soluble precipitant to the sample solution. Exosomes are hydrophobic lipid bilayer vesicles. [23] Polymer precipitation techniques are easily scalable to huge preparation quantities because they do not require complex equipment. By com-

binning multiple detection systems for the examination of exosome (or protein/genetic material) contents, this technique also enables rapid disease diagnosis. [24] Conversely, the recovery rate and purity are a little low, false positives might occur, and the polymer generated is tough to eliminate, making it unsuitable for further functional experimental study. [17]

ii. Aqueous two-phase separation (ATPS):

A two-phase system is created when a more hydrophilic and denser solution (like dextran) and a comparably more hydrophobic solution (like PEG) are combined. PEG makes up the upper phase of this system, while dextran forms the lower phase. Proteins and other macromolecular complexes preferentially concentrate into the PEG phase, whereas exosomes preferentially accumulate in the dextran phase. This technique offers a quick, low-cost, and potential exosome isolation method to streamline exosome-based applications. [24] Compared to simple polymer precipitation, the aqueous two-phase system for EXs separation (ATPSs) is a more sophisticated precipitation technique that produces harvested EXs that are purer. [25]

C. Immunoaffinity Capture

Immunoaffinity chromatography is a technique for purification and separation that uses the particular affinity of ligands and antibodies to extract desired materials from heterogeneous mixtures. The sole technique for differentiating between vesicle subgroups or separating out specific subgroups from the sample continues to be immunoaffinity techniques. Immunoaffinity capture-based EX isolation is based on two principles: 1) Immunoprecipitation 2) Enzyme-linked immunosorbent test (ELISA). Additionally, it can be applied to both qualitative and quantitative exosome determination. [17]

i. ELISA:

ELISA, or enzyme-linked immunosorbent assay, requires antibodies against a target antigen that has been immobilised on the surface of a microplate. While immobilised EXs are identified by a different antibody with a detection tag, unbound elements are removed by washing. ELISA can also be used as a quantitative method since a calibration curve can be measured once the quantitative standards are available. [17, 26]

ii. Immunoprecipitation:

The preferred method for immunoprecipitation is to use submicron-sized magnetic or polymeric beads to fix antibodies or recombinant proteins to solid-state matrices. Given its vast area, this approach shows very good sensitivity and great yield efficiency. Additionally, this technique makes it possible to process huge

initial sample quantities, preconcentrate EXs, and perform surface immunoanalysis on them. Hence, this approach is appropriate for use in diagnostics in the future to find disease-specific markers. [25] However, the disadvantages are that it takes a lot of time, has high separation costs and has stringent reagent and storage conditions. For researchers that do not require high purity or particular exosome subclasses, this approach is not the best option. [20]

Several commercial kits, such as the exoEasy Maxi kit (QIAGEN), MagCapture™ Exosome Isolation Kit PS (Wako), and Minute™ Hi-Efficiency Exosome Precipitation Reagent (Invent), are presently available in the marketplace and depend on the aforementioned classical isolation technology. In conclusion, the method of isolating EXs is still not entirely resolved and still varies depending on the EXs' source or intended use. [25]

Detection methods for exosomes:

Since exosomes have so many important uses in medicine and health science, it is crucial for clinical research to properly identify and detect them. Numerous detection methods rely on the identification of biomolecules, including proteins, lipids, and nucleic acids. Commonly used detection methods that are performed are the aptamer-based separation method, surface-enhanced Raman scattering technology, nanoparticle tracking analysis, dynamic light scattering (DLS), flow cytometry, transmission electron microscopy (TEM), and surface plasmon resonance imaging (SPRi)-based detection.

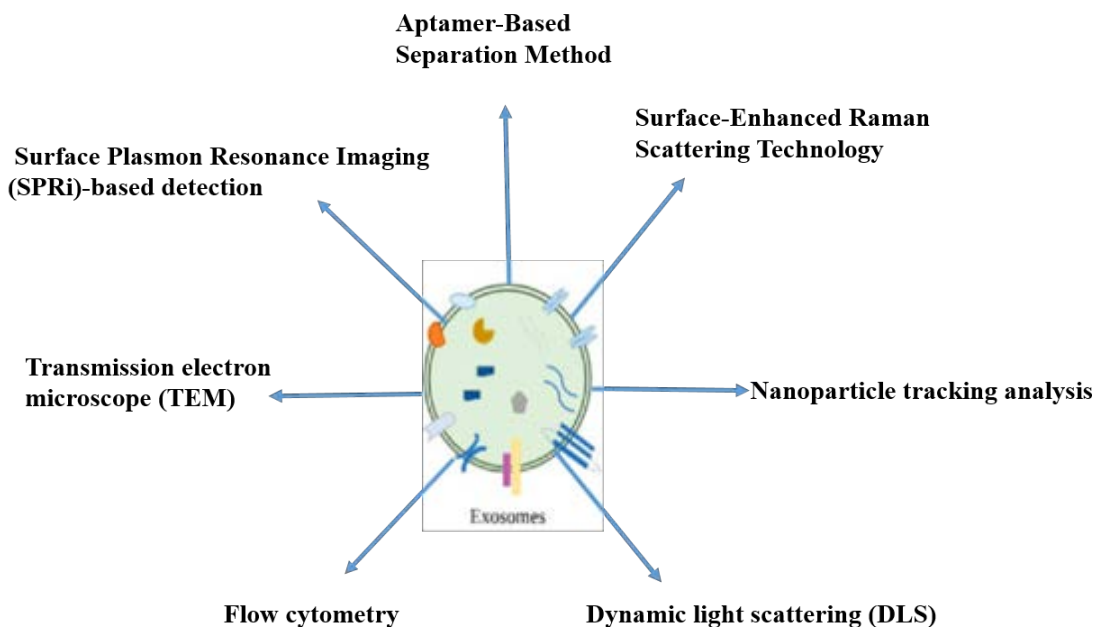


Figure 4: Graphical representation of the detection method of exosomes.

i. Aptamer-Based Separation Method:

Similar to antibodies, aptamers are short, single-stranded nucleic acid molecules which bind to targets with similar affinity and selectivity. Aptamers have numerous benefits over antibodies and were found through a library selection procedure known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Aptamers are synthetically manufactured with minimal costs, great homogeneity, and, if needed, well-defined chemical changes. Moreover, aptamers have low immunogenicity and toxicity and far better stability and shelf life than antibodies, particularly when stored lyophilised. Aptamers have gained popularity as affinity reagents for the separation of small molecules, proteins, and cells due to these advantageous properties. [28]

In a study by Zixuan Zhou et al., they considered CD63 and PTK7 as surface biomarkers as aptamer-targeting proteins and as ELISA proteins in the subsequent exosome characterization experiment since they are different from other EVs. [28] In another study by Melissa Ling et al., they modified aptamer-mediated cell selection for use with resins in affinity chromatography. A constant-flow cell isolation system can be integrated into closed-system manufacturing. In order to maximize cell separation and purity, they assessed the following parameters: resin coupling techniques, aptamer density, blocking buffer composition, pre-clearing phase inclusion, aptamer link length, and resin volume. [27]

ii. Surface-Enhanced Raman Scattering Technology:

SERS has been used recently to identify and distinguish exosomes from various cell types. [29] SERS detection methods can be categorised into two main variations: direct and indirect techniques. When using direct SERS, the signal readout is obtained by acquiring the analyte's intrinsic SERS spectrum, which is rich in structural data indicative of the molecular composition as well as structure. Simple, low-cost plasmonic materials can be used to do this label-free examination. Conversely, indirect methods are intended to track the extrinsic SERS signal of molecular markers in order to identify and measure the target species. The most popular indirect approach uses SERS-encoded nanoparticles (also known as SERS tags) in conjunction with surface ligands, allowing selective recognition (such as aptamers or antibodies) as optical probes that work similarly to fluorescent labels. Labelled methods have several advantages, including high-throughput screening, multiplexing, robust quantitative response in complex media (like biofluids), ultrasensitivity, and the ability to be integrated into small devices for automated testing. However, they usually require complex and thorough fabrications of relatively expensive SERS substrates. [30]

iii. Nanoparticle tracking analysis:

Nanoparticle tracking enables exosomes and microvesicles to be sized as well as

quantified through the use of light microscopy and software, which analyzes the particles' Brownian motion. [31] The size of the particle can be determined by examining its motion, even if the exosomes and microvesicles are smaller than the microscope's diffraction-limited resolution. Nanoparticle tracking analysis has rapidly established itself as the industry standard for measuring exosomes and microvesicles. [32]

iv. Dynamic light scattering (DLS):

Dynamic Light Scattering (DLS) analyses the intensity of scattered light over time to determine the average size of numerous particles. Along with microvesicles and exosomes, DLS is a widely used laboratory technique for characterising the size and concentration of a wide range of particles, such as proteins, polymers, and nanoparticles. The DLS is designed to quantify particles between 0.3 nm and 10 μm in size for exosomes and microvesicles (Malvern). Only a few big particles (like platelets) can alter the DLS's precision; hence, meticulous sample preparation is necessary for DLS studies on exosomes. DLS cannot be utilised to profile molecularly specific data about the exosomes or microvesicles since it is not susceptible to molecular labelling. [32]

v. Flow cytometry:

In flow cytometry, the primary method for high-throughput evaluation of cells, every vesicle or exosome moves independently through a laser spot, and the emitted scattered and fluorescent light is recorded. The challenge in using flow cytometry on exosomes and microvesicles is the challenge of recovering such faint signals because of their 100 $\times$ smaller size compared to cells. The inability to resolve particles smaller than 300 nm is therefore a drawback. Flow cytometry has the advantage of being able to resolve individual exosomes and quantify numerous surface indicators per exosome. New flow cytometry devices (such as the A50-Micro-PLUS and Apogee) are increasing the detection limit to 100 nm and beyond, which could increase the flow cytometers' usefulness for exosome analysis. [32, 33]

vi. Transmission electron microscope (TEM):

TEM is a popular technique for characterising exosomes. After being fixed, samples are dried in order to prepare them for TEM imaging. The main technique for detecting exosomes using Transmission Electron Microscopy (TEM) is negative staining, which entails applying the exosome sample to a grid coated with carbon, fixing it, dehydrating it, staining it with a heavy metal-containing solution (such as uranyl acetate) to provide contrast, letting it air dry, and then imaging it with a high-resolution electron beam. In addition to the methods mentioned above, which can give surface indicators' size, concentration, and analysis, TEM imaging makes it possible to resolve the shape and heterogeneity of exosomes. TEM can be used in combination with immunogold labeling to detect particular exosomal

proteins. [23, 32]

vii. Surface Plasmon Resonance Imaging (SPRi)-based detection:

The SPRi technology offers a label-free, real-time way to find exosomes. Antibodies are adhered to the sensor chips in this technique. [6] A study by Fan et al. demonstrated the application of SPRi biosensors carrying numerous recognition sites, each containing diversified biological affinities of antibodies for multiple identifications of exosomes that had greater sensitivity. Non-small cell lung cancer (NSCLC) cells provided the sample. Gold nanoparticles – AuNPs – were used to improve the SPRi biosensor system's performance. [6, 34]

Storage:

Three primary storage methods – cryopreservation, freeze-drying, and spray-drying – are used to maintain the repeatability of research on exosomal structure, content, and functionality while making exosomes portable and manageable.

To preserve the functional stability of biological particles, a storage method called cryopreservation lowers the temperature below a threshold required for biochemical reactions. Temperatures of 4°C, -80°C, and -196°C are commonly utilized, although frequent freezing and thawing may have an adverse effect. However, this method's susceptibility to frost injury is a significant drawback. [9] In order to overcome this shortcoming, one or more antifreezes with adequate concentrations are sometimes added selectively to increase the duration of shelf life. [9, 17]

Freeze-drying stabilizes the exosomes and facilitates reconstitution by removing water from the exosome samples under low pressure. Exosomes can be kept for a long time at room temperature after being lyophilised. Pre-freezing, sublimation drying, and analytical drying are the three primary phases of freeze-drying technology. [17]

Spray-drying converts the liquid exosome suspension into a dry powder by rapidly drying it with hot gas, offering easy transportation. But high temperatures potentially denature sensitive proteins on the exosome surface, making it less suitable for exosomes with heat-sensitive components. In contrast to lyophilization, spray drying is a continuous process that can achieve one-step milling, which is more cost-effective and extensible and allows for product particle size adjustment. [9]

Applications / Functions:

A variety of administration methods are available, including intravenous, intravitreal, intranasal, and local injections. The most popular administration technique is intravenous since it has the best bioavailability of all the other routes and does not

have first-pass metabolism or absorption barriers, allowing for long-term therapy. [35] Their payloads can contain proteins, nucleic acids, and metabolites that reflect the traits and physiological state of the donor cell. Exosomes are efficient carriers for delivering proteins, genetic material, and other medications to target cells because of their lipid bilayer, which allows them to easily penetrate the blood-brain barrier. [11,36] Exosomes contain an abundance of biomarkers for the diagnosis and prognosis of disease. They are mostly used to treat cancer, although they have also made considerable headway in the treatment of TB, heart disease, and disorders of the central nervous system. [17] Exosomes serve as drug delivery carriers, therapeutic targets, imaging aids, tissue-repairing agents, and diagnostic biomarkers. They can also be utilised for the production of vaccines. [37, 38]

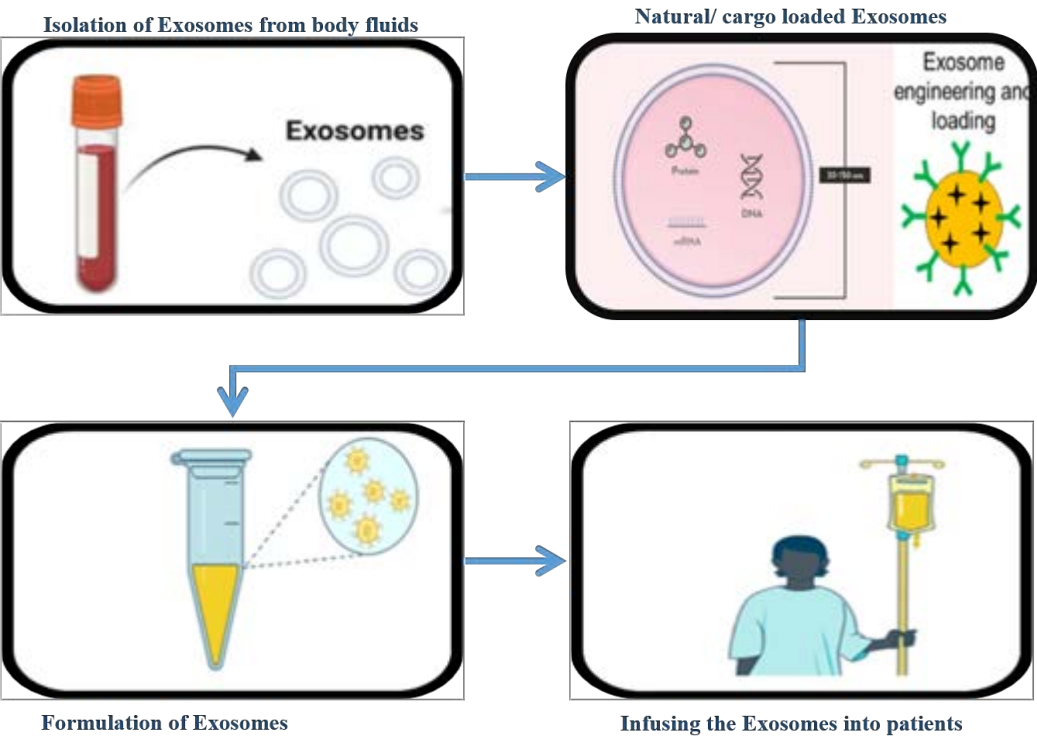


Figure 5: Overview of exosome therapy showing isolation, drug loading, formulation, and patient infusion.

Exosomes' inherent ability to regulate intricate intracellular pathways has increased their potential to be useful in the treatment of patients with autoimmune disorders, chronic inflammation, and other chronic degenerative diseases. [36]

Exosomes may have a broad impact because they can interact with cells in various parts of the brain. In fact, Zangh et al. showed that EVs produced by neural stem cells (NSCs) at various nervous system locations were implicated in a number of

characteristics of brain activity. This may be because exosomes can pass through the blood-brain barrier, promoting the proliferation and differentiation of neurons while inhibiting inflammation in the brain tissue. [36] Exosomes have become a viable alternative to cellular therapies because of their potential to increase safety and therapeutic efficacy in several regenerative applications. [39] The application of exosomes has become a novel and cell-free therapeutic paradigm and been given high expectations due to their convenience in clinical practice. [4]

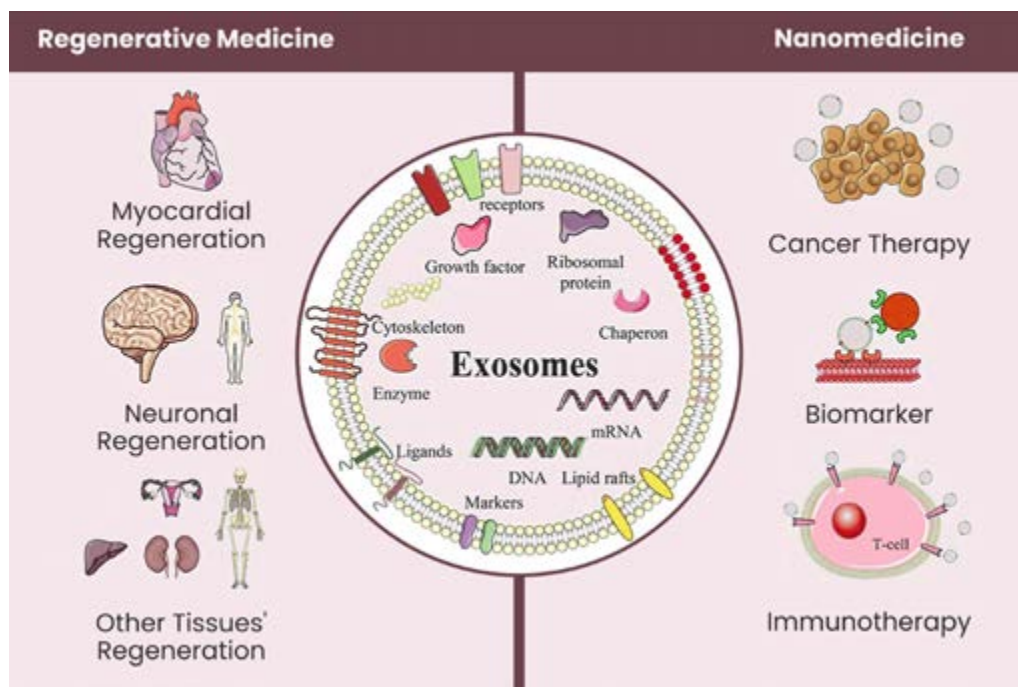


Figure 6. Roles of exosomes in nanomedicine and regenerative medicine

Exosomes in Specific Neurological Disorders

Numerous conditions affecting the central nervous system are classified as neurodegenerative illnesses. Research on exosomes shows promise for their use as a treatment and biomarker for neurodegenerative diseases. Though exosomes obtained from other types of stem cells, like neural stem cells, seem to be helpful in treating neurodegenerative illnesses in cell and animal models, MSC-derived exosomes have been widely used in several research and clinical trials. [2]

Autism Spectrum Disorder (ASD):

Autism spectrum disorder is a term used to describe neurodevelopmental, life-long diseases characterized by difficulties with social and communication skills as well as repetitive, stereotyped patterns of behaviour and activities. [36] According to recent research, neuroinflammation plays a key role in the pathophysiology of

ASD. [40] The treatment of behavioural disorders now has hope because of recent developments in stem cell therapy. Mesenchymal stem cell (MSC) transplantation has been demonstrated to be effective in treating autism spectrum disorder (ASD) in both clinical and animal research. Nonetheless, the signals and metabolites released by the MSCs were principally linked to the therapeutic effectiveness of the MSC transplantation. For instance, MSC-secreted organelles called exosomes contain bioactive chemicals that are critical to the therapeutic effects of treating ASD.

- **Pre-clinical Studies:**

An intranasal administration of MSC-exo improved behaviour in a study using Shank3B KO mice, a mouse model of autism. Specifically, experimental mice showed an increase in ultrasonic vocalisation and a decrease in repetitive activities. It was discovered that MSC-exo could penetrate the blood-brain barrier (BBB) and build up in these mice's cortex and cerebellum. GABA Rb1 receptor RNA expression was also elevated in the frontal brain. Although more research is needed to determine the precise underlying mechanisms, these findings imply that the therapeutic effects might be mediated by the exosomes released by MSCs. [41]

Similarly, valproic acid (VA)-treated mice with autism-like traits showed behavioural improvements in their offspring as well. In a study, Liang and colleagues used human umbilical cord (hUC)-MSC-derived exosomes (by the intranasal method) to regain social abilities and cure repetitive stereotyped behaviours and other unusual characteristics associated with autism in the offspring of mice treated with VA. It was discovered that MSC-exo effectively entered the brain tissues by the intranasal route. The study led to the conclusion that the anti-inflammatory properties of the exosomes may contribute to the therapeutic benefits of MSC-exosomes. [42]

- **Clinical Studies of MSCs/Exosomes in ASD**

In Yong-Tao Lv et al.'s phase I/II study, the effectiveness and safety of UCMSCs and human cord blood mononuclear cells (CBMNCs) in autism were investigated. There were a total of thirty-seven children between the ages of three and twelve who were divided into 3 groups: (1) CBMNC (n = 14; rehabilitation treatment + CBMNCs), (2) control group (n = 14; rehabilitation therapy only), and (3) combination group (n = 9; rehabilitation therapy + CBMNCs + UCMSCs). Weekly intravenous and four intrathecal stem cell infusions were given. The Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI), and Aberrant Behaviour Checklist (ABC) were the instruments used for assessment. All groups showed a significant drop in ABC scores at 24 weeks; the combination group saw the largest decline (59.9%) compared to the CBMNC (38.0%) and the control (17.4%). The combination group reported the greatest improvements in CGI subscales and CARS scores (37.9% reduction in CARS) when compared to CBMNC (20.0%) and control

(13.7%). There were no notable abnormalities found in the laboratory. According to this study, combination therapy demonstrated a high level of therapeutic efficacy, and both CBMNCs and UCMSCs are safe. [43]

Sun et al. conducted an open-label, phase I research in which 12 children with ASD, ages 4 to 9, received intravenous (IV) infusions of human cord tissue mesenchymal stromal cells (hCT-MSCs), a third-party MSC product made from umbilical cord tissue from unrelated donors. The VABS-3 Socialization Subscale, PDDBI Autism Composite, and CGI-I were the instruments utilised for the assessments. Four children at 6 and 12 months showed improvement in all three criteria, while six out of 12 children improved in at least two measures. Agitation during infusion was a very frequent side effect; five children produced anti-HLA antibodies but showed no clinical symptoms. According to this study, it was safe and possible to provide mesenchymal stromal cells from human cord tissue intravenously to young autistic children. Half of the children's autism symptoms improved, and 40% of them acquired detectable but clinically silent antibodies against the human leukocyte antigen after the infusion. [44]

A case report of 2 of two children, ages five and six, who were injected with multiple intravenous (IV) injections of umbilical cord mesenchymal stem cells (US-MSCs). The improvements observed in Case 1 were in physical ability, emotional reactivity, and sociality through peer association. Verbal and nonverbal communication, eye contact, and a decrease in anomalous behaviours were all noted improvements in case 2. CARS scores dropped in both situations as the ASD symptoms considerably improved. There were no reports of any severe adverse effects. [45]

Clinical data indicates that MSC-based treatments, such as UCMSCs and cord blood-derived mononuclear cells, are safe and may be effective in reducing the main symptoms of ASD. Repeated infusions and combination therapy might be more beneficial. To validate these results, further thorough, controlled trials are required.

- **Spinal Cord Injury (SCI):**

A devastating damage to the central nervous system, SCI is marked by a high rate of impairment and fatality and can result in sensory and motor disorders. SCI is pathophysiologically divided into primary and secondary injuries. [35] In recent years, stem cell therapy has demonstrated promise in the treatment of SCI because it can effectively reduce inflammation, block astrocyte scarring, and stimulate axon regeneration. The current unclear and inconsistent research results have limited the further study of exosome therapy to some extent and also impeded the pace of clinical translation of exosome therapy, despite the fact that exosomes derived from stem cells have made significant progress in the repair of SCI in animal

experiments. [46]

NSCs have the capacity to repair SCI and can develop into neurons, oligodendrocytes, and astrocytes. Although implantation efficiencies are extremely low, localized transplantation of NSCs has also been demonstrated to have a therapeutic effect on SCI. [47] For people with SCI, the restoration of motor and sensory abilities is essential. According to the findings of a meta-analysis, exosomes generated from various stem cells can greatly enhance the motor function of animals suffering from spinal cord injuries. [46]

- **Pre-clinical studies:**

Recent research showed that exosomes generated from SCs are internalized by endothelial cells and promote their migration, proliferation, and tube-like network development via delivering integrin- β 1. Exosome therapy dramatically decreased tissue damage, increased vascular development, and boosted motor recovery in a mouse model of SCI. Crucially, these effects were significantly reduced by inhibiting integrin- β 1 in these exosomes, highlighting their vital function. [48]

Based on recent studies, mesenchymal stem cell-derived exosomes, or MSC-exosomes, may be the secret to innovative new therapies for people with spinal cord injuries. MSCs are multipotent, self-renewing stem cells that can differentiate in multiple directions and release a lot of exosomes. By encouraging angiogenesis and axonal development, controlling inflammation and the immune response, preventing apoptosis, and preserving the integrity of the blood-spinal cord barrier, these MSC-exosomes are essential for the repair of SCI. They can also be used to deliver medications or genetic material to specific cells, and their relatively small size makes them able to permeate the blood-brain barrier. [49]

- **Clinical Studies:**

In Akhlaghpasand et al.'s open-label, single-arm phase I clinical trial All 9 patients with subacute SCI between October 2020 to November 2021 were screened. Exosomes were separated by ultracentrifugation after HUC-MSCs were recovered from human umbilical cord tissue. Assessment tools included the American Spinal Injury Association (ASIA) scale, the modified Ashworth scale (MAS), the lower urinary tract function questionnaire, the neurogenic bowel dysfunction (NBD) assessment using the NBD score, and the functional assessment using the Spinal Cord Independence Measure (SCIM-III). Allogeneic HUC-MSC exosomes were safely and well tolerated when intrathecally injected. There were no unfavourable study events noted. At 12 months following injection, there were also notable improvements in the SCIM III total score (P-value = 0.027), the NBD score (P-value = 0.042), the ASIA (P-value = 0.039) and light touch (P-value = 0.038) scores, and the baseline. Exosomes may be linked to possible improvements in patients' clinical and functional outcomes. Larger phase II/III clinical trials with sufficient power

are desperately needed in the future. [50]

Traumatic Brain Injury (TBI):

TBI is the primary cause of fatal injuries for young people in developed nations [8]. The primary cause of TBI is brain trauma brought on by an external mechanical force. Cognitive, bodily, and emotional disruptions might result from this trauma, either temporarily or permanently [9, 11]. Although there are many pharmacological therapies for TBI that can lessen the damage to the nervous system [8], none of them are good enough to repair the neurological deficit that results. Rehabilitation and certain palliative medications are the main therapies used following traumatic brain injury, as survivors may experience severe impairments in their sensory and motor abilities [2, 36, 51]. Researchers have concentrated on using SCs as a possible therapeutic approach for the treatment of TBI. [52] Adipose tissue-derived MSCs (AD-MSCs), bone marrow-derived MSCs (BM-MSCs), and umbilical cord-derived MSCs (UC-MSCs) are among the cell types that have been explored for post-TBI therapy, along with induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs) (adult and embryonic) [36]. Preclinical studies looking at TBI models have shown that all of these cell types can enhance neurological outcomes and recovery. [53]

- **Pre-clinical studies:**

Zhang et al. (2020) showed that intravenous delivery of exosomes, which carry therapeutic cargo such as microRNAs and proteins to promote neurogenesis and angiogenesis and reduce neuroinflammation and neuronal cell loss, shows promising results for functional recovery in rat models of Traumatic Brain Injury (TBI). When exosomes, especially those made from mesenchymal stem cells (MSCs), are administered, the brain's indicators of inflammation and cell death decrease, and sensorimotor and cognitive function increase. Although there are still issues with standardization and optimization for clinical translation, this cell-free method has an advantage over cell transplantation since it is more stable and can more successfully traverse the blood-brain barrier. [54] Following systemic exosome delivery, a prior study also found decreased inflammation, enhanced motor recovery, increased spatial learning, and remodeling of brain and vascular tissue. These results provide credibility to MSC-exosomes' potential as a durable, cell-free, blood-brain barrier-penetrant substitute for stem cell transplantation. [55]

Cerebral Palsy (CP):

One of the most common causes of physical disability in children is cerebral palsy (CP). Risk factors for cerebral palsy include things like intrauterine growth restriction, perinatal infections, and preterm birth [5]. Notably, CP is associated with acute intrapartum hypoxia-ischemia [8]. Eighty per cent of CP cases are thought to have no known cause. Numerous strategies have been employed in recent de-

acades, such as physical and occupational therapy to enhance motor function [9], bone, muscle, and nerve surgery to aid in training and rehabilitation [11] and drugs to treat sleep disorders, seizures, and muscle relaxation. [56] Exosomes' therapeutic potential for the treatment of cerebral palsy is still being researched; however, several preclinical trials have shown encouraging results.

- **Preclinical studies:**

The effectiveness of exosomes made from human amniotic mesenchymal stromal cells (hAMSC-Exos) in a rat model of cerebral palsy (CP) brought on by prenatal hypoxic-ischemic damage was examined in a recent preclinical investigation. According to TUNEL and caspase assays, administering hAMSC-Exos through the tail vein significantly enhanced neurobehavioural function and decreased neuronal apoptosis. These findings demonstrate how hAMSC-Exos may be used therapeutically to support neuronal survival and functional recovery after hypoxic-ischemic damage. [56] The combination therapy produced the most noticeable improvements in neuronal architecture and function. Another study using a murine model showed that hUC-MSC-derived exosomes and mouse nerve growth factor (mNGF) preserved synaptic structures and increased levels of synaptic proteins PSD-95 and synaptophysin. [57]

A preclinical study used a rat model of cerebral palsy brought on by hypoxic-ischemic brain injury to assess the therapeutic potential of exosomes made from human umbilical cord mesenchymal stem cells (hUC-MSC-Exos). By reducing neuronal death and promoting neuronal regeneration and myelination, systemic treatment of hUC-MSC-Exos markedly enhanced motor and cognitive performance. According to molecular findings, the exosomes' neuroprotective benefits were mediated by the transfer of proteins and microRNAs that improve synaptic plasticity, reduce neuroinflammation, and encourage neurovascular remodelling. These results suggest that hUC-MSC-Exos, which target several pathogenic processes at once, have potential as a cell-free, minimally immunogenic treatment for cerebral palsy. [58]

Muscular Dystrophy:

Duchenne muscular dystrophy (DMD), which affects both the heart and skeletal muscle, is caused by a genetic dystrophin deficit that results in disability and early mortality [59], resulting in weakening and gradual deterioration of muscular fibres. There is no one-size-fits-all treatment available in the clinic, despite the fact that a variety of therapeutic approaches have been the subject of intense investigation. These include methods to improve muscle function by focusing on various pathological aspects involved in DMD or to restore partially functional dystrophin expression through strategies like gene replacement, exon skipping, stop codon read-through, and genome-editing therapies. [60]

- **Preclinical studies:**

Using a dystrophic animal model, this study examined the potential therapeutic benefit of exosomes in treating Duchenne muscular dystrophy (DMD). To assess their effect on muscle pathologies, exosomes – small extracellular vesicles known to transport proteins, lipids, and nucleic acids – were given. The results showed that exosome therapy increased total muscle contractile function, decreased muscle damage, and improved sarcolemmal (muscle cell membrane) integrity. Exosome-mediated transport of bioactive chemicals that aid in membrane repair, lower inflammation, and encourage regeneration signaling was thought to be responsible for the positive effects. These results demonstrate the potential of exosomes as a cell-free treatment approach for muscular dystrophies; however, more research is necessary before they can be applied in clinical settings. [60]

The therapeutic potential of exosomes made from cardiosphere-derived cells (CDCs) in the treatment of Duchenne muscular dystrophy (DMD) was examined in a study by Marbán et al. CDC-derived exosomes were given to mdx mice, a commonly utilised model for DMD, by the researchers. The outcomes showed that these exosomes successfully enhanced the mice's skeletal and cardiac muscle performance. The exosomes' capacity to transport bioactive compounds that reduce inflammation and encourage tissue healing was thought to be the cause of the therapeutic advantages. According to this study, exosome-based treatments may be a viable way to cure DMD. [61]

Amyotrophic lateral sclerosis:

ALS is the most prevalent form of motor neuron disease and the third most prevalent neurodegenerative disease, after Parkinson's and Alzheimer's diseases. Exosomal miRNAs have been studied by multiple groups of researchers to understand potential use as biomarkers for one more neurodegenerative disease, amyotrophic lateral sclerosis (ALS). [6] Mesenchymal stem cell (MSC) therapy has the potential to treat ALS and other neurodegenerative disorders, according to preclinical and clinical research. Due to anatomical limitations, living MSCs cannot transfer signalling information to recipient cells at doses significantly higher than MSC EVs, which are more stable than MSCs. Because MSC EVs can move to inflammatory sites and cross the blood-brain barrier, they are thought to be essential in the treatment of neurological disorders. [62]

- **Preclinical studies:**

The current study examined the possible neuroprotective impact of adipose-derived stem cell-derived exosomes (ASC-exosomes) on the human SOD1 gene with a G93A mutation (SOD1(G93A)) in animals, the in vivo model most frequently used to investigate ALS. To investigate the possible beneficial effects of ASC-exosomes on SOD1(G93A) animals, specific motor tests were used to gauge the course of sickness in mice treated with exosomes and PBS. The effects of intravenous

and intranasal exosome administration were also investigated. Motor tests and examinations of lumbar motoneurons and glial cells, the neuromuscular junction, and muscle were used to track the impact of exosome delivery on the course of the disease. The findings showed that repeated administration of ASC-exosomes enhanced motor function, protected muscle, neuromuscular junction, and lumbar motoneurons, and reduced the activation of glial cells in treated SOD1(G93A) mice. Additionally, exosomes can settle in ALS-affected areas of the animal brain. These findings help by offering more insight into the potential application of ASC-exosomes as a treatment for ALS in humans. [63]

- **Clinical Studies:**

In Crose JJ et al., an open-label, dual-site, single-investigator pilot safety study of ten subjects was performed. A baseline physical examination and an evaluation of their Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALS-FRS-R) scores were performed on each participant. Out of these 10 participants, seven of the participants had ALS of the spinal type and three of the subjects had bulbar-type ALS. Over the course of the three-month trial, there was an average drop of 3.1 points for each of the ten participants. Thirty percent of individuals' ALS functional rating scale-revised (ALSFRS-R) scores did not decrease, and there were no significant adverse events or IP-related adverse events. It seems that HBM-MSV EVs are safe for ALS patients. According to this preliminary research, a controlled study of EVs for the treatment of ALS is warranted. [62]

Stroke:

Stroke is a predominant cause of global impairment, and cerebral damage severely impacts patients. Despite the fact that brain injuries and strokes are the top causes of disability globally, there are currently no drugs available that facilitate neurological rehabilitation and promote brain recovery. There is growing evidence that the beneficial outcomes of this type of cell-based therapy are mediated by exosomes that are released from the cells that are delivered. The microRNA cargo in these exosomes is primarily responsible for the therapeutic effects. The clinical value of using exosomes for stroke treatment has received widespread attention in recent years due to their unique properties, such as low immunogenicity, toxicity, and biodegradability; ability to cross the blood-brain barrier (BBB); and important role in cell communication. [64]

- **Pre-clinical study:**

Mesenchymal stromal cells (MSCs) secrete exosomes, which have the protective effect. As exosomes are believed to promote brain healing and have mending properties, such as neurovascular remodeling, anti-inflammatory, and anti-apoptotic activities. [65] A meta-analysis by Cherian et al. (2022) systematically examined the impact of administering exosomes derived from different source cells in

the management of ischemic stroke models. For the meta-analysis, 17 homogeneous studies were selected. The ischemic stroke models treated with placebo and exosomes were compared. On days 7 and 28, the exosome-treated stroke model demonstrated a significant reduction in brain infarct volume and better functional recovery. Subcellular exosome therapy is therefore a promising treatment that should be investigated further in animal ischemic stroke models in order to draw firm findings regarding its safety and recommended dosage. [66]

- **Clinical study:**

Dehghani et al. (2022) included 5 malignant middle cerebral artery infarct (mMCAI) patients with a mean age of 62 years who were recruited between January, 2019, till September, 2020. All patients had mMCAI with hemiplegia, hemifacial paresis, three with right hemisphere infarct and two of them had left hemisphere infarcts. After undergoing a decompressive craniectomy, each patient received a one-minute dose of exosomes via a frameless stereotactic guided cannula in the infarct penumbra region. Ninety days was the median follow-up period following implantation. In terms of mRS, one patient's impairment remained stable after three months, whereas two patients improved by two grades, from 5 to 3 and 4 to 2. Additionally, these patients' NIHSS decreased from 18 to 8 and from 11 to 2. There were no post-interventional negative effects in these five ischemic stroke patients. It is suggested that local injectable exosome therapy after mMCAI can be safe and that it will be used in the future as a preventative, restorative, and supportive treatment for individuals with acute ischemic stroke and post-ischemic impairment. [67]

Challenges and Limitations:

Since this field of research has advanced significantly over the years, exosomes have expanded our options for diagnosing and treating a number of fatal illnesses. The specific mechanism of biogenesis is still unknown, despite the fact that treatments are being developed. The methods used for exosome isolation are laborious and challenging to apply in a therapeutic context. Exosomes are diverse and widely distributed organisms. The mechanisms that sort cargo into exosomes and release cargo into cells following exosome internalisation are particularly unclear. Once we find a universal isolation strategy, we may correlate clinical outcomes all throughout the world. Until we have a consistent separation and characterization procedure in place, scientists cannot validate promising findings. The exosome's repeatability varies greatly due to methodological complexity and variance, which makes it challenging to interpret the data. Standard operating procedures (SOPs) are therefore required for the isolation, storage, characterization, and analysis of exosomes. Exosomes will have tremendous therapeutic application possibilities with a deeper understanding of their synthesis and function; current research has already looked into exosomes as a natural gene/drug delivery mechanism and

biomarker. To sum up, in order to progress this area of study, we must have an effective and reliable isolation technique. [38]

Future Directions:

Even though studies on exosomes have exploded in the past ten years, there is still much to learn about these microscopic extracellular vesicles that affect illness. As an instance, for exosomes to be successful in upcoming clinical trials, their yield must be enhanced without compromising their therapeutic activity. Furthermore, since exosomes reflect their donor cells, it is crucial to take into account their contents. The reason why exosomes separated from the same cell line have different diameters is a crucial factor that must be taken into consideration. In addition, improving exosomes' capacity to reach their target organ is essential to ensuring that disease states receive the exosomes when they are administered systemically. Lastly, understanding how exosomes target particular tissues and how they are eliminated is fundamental [2]. Further research into the physiological and pathological circumstances, as well as the mechanisms interacting with exosome release and the disruption of exosome-mediated cell-cell communication, will be necessary in the years to come. These findings could serve as the foundation for an entirely new class of personalized treatments.

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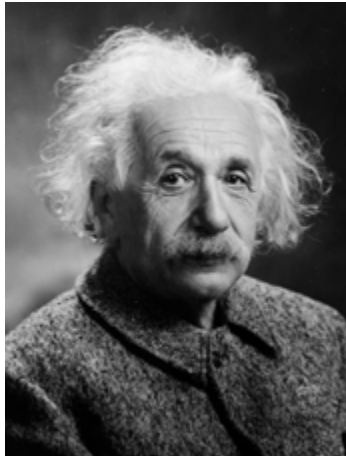
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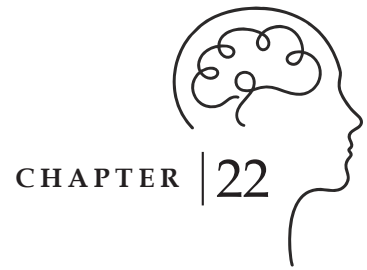
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"Every error is an opportunity to learn, just don't commit the same mistake again. That is stupidity. But commit as many new mistakes as you are capable of. Don't be afraid, because its the only way nature allows you to learn."

- Albert Einstein



Complications

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

Cell-related adverse events:

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

- a. Embryonic and Fetal Stem Cells
- b. Adult Stem Cells

- c. Umbilical Cord Stem Cells
- d. Induced Pluripotent Stem Cells

Below are the major cell related adverse events reported with different cell types. It is important to note that not all the complications are associated with all cell types. There are some adverse events like teratomas which have been reported only with the use of embryonic and fetal stem cells.

1. Tumorigenicity/ Teratomas

Embryonic and Fetal Stem Cells

Apart from ethical problems related to human embryonic stem cell derivations, nude mice experiments for various disorders, including brain injury, brought out the problem of teratoma formation after embryonic stem cell transplantation. To achieve human embryonic stem (ES) cell-based transplantation therapies, allogeneic transplantation models of nonhuman primates have been useful. A model based on cynomolgus ES cells genetically marked with the green fluorescent protein has been described by researchers from Jichi Medical Centre, Japan. Primates provide a close mammalian representation to the humans. The cells were transplanted into the allogeneic fetus because the fetus is supposed to be immunologically premature and does not induce immune responses to transplanted cells. In addition, fetal tissue compartments are rapidly expanding, presumably providing space for engraftment. However, the researchers found that 3 months after transplantation, a fluorescent teratoma, which was obviously derived from transplanted ES cells, was found in the fetus. Hence, it was understood that, though the transplanted cynomolgus ES cells can engraft in allogeneic fetuses, the cells may, however, form a tumor if they “leak” into an improper space (1).

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

Hence, it has been established in many mammalian models that although ES cells may provide treatment for degenerative disease in the future, their unlimited self-renewal and high differentiation potential poses the risk of tumor induction after engraftment.

Thus, a lot of caution and diligent research will be required before using various human ES cell lines for cell transplantation as a therapeutic option for patients with degenerative disease. In the literature review.

Ablating the immune cell niche with immunosuppressive drugs to prevent im-

mune rejection following allogenic transplantation, has the potential to create a tumorigenic microenvironment. A recent paper demonstrated tumor formation from the donated cells following transplantation of donor derived fetal neural tissue into an ataxia telangiectasia patient [3].

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

2. Seizures

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

Seizures could be hypothesized to arise post transplantation due to increased production of Brain derived Neurotrophic factor (BDNF), Vascular endothelial growth factor (VEGF) and Nerve growth factor (NGF) by BMMNCs. However the exact mechanism remains unknown [7-9]. Also these disorders present with seizures as a co-morbidity [10,11]. The percentage of children that developed seizures as an adverse event was very small (Table 1). However, this adverse event is preventable by using an antiepileptic prophylactic regimen (Table 1). After the use of antiepileptic prophylactic regimen (Table 1) the percentage of seizures as an adverse event reduced significantly.

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

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

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 cells (BMMNCs) intrathecal transplantation. Seizures occurred as an adverse event in 8 (6.10%) of the children. There was a correlation between the electroencephalograph (EEG) examination and the occurrence of seizures, with seven children showing a pre-existing epileptogenic condition. The history of seizures was not so strongly correlated, and only four children had a history of seizures and 3 children did not have any history of seizures. Seizures were considered an adverse event in the event of a 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 the following factors: EEG examination findings, the history of seizures and the current medical treatment for the seizures. The antiepileptic regimen is given in detail in Table 2.

Table 2: Prophylactic antiepileptic regimen

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

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

Immunogenicity:

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.

Allogenic:

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

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

Procedure related adverse events:

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

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.

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

Giddiness, vomiting and neck pain are some other occasionally occurring adverse events. But these are usually always self limiting and respond to medical management and rest. Similarly, other surgical methods, such as intraspinous, intracerebral, intrarterial and intravenous injections have possibilities of side effects or complications, specific to the respective procedures. It is beyond the scope of this book to describe the adverse events associated with all other types of stem cells, though umbilical cord stem cells may be associated with immunological reactions and infections. Induced Pluripotent Stem Cells (IPSCs) have not reached clinical applications due to associated complications of genomic instability, viral vector infections and mutagenesis.

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

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

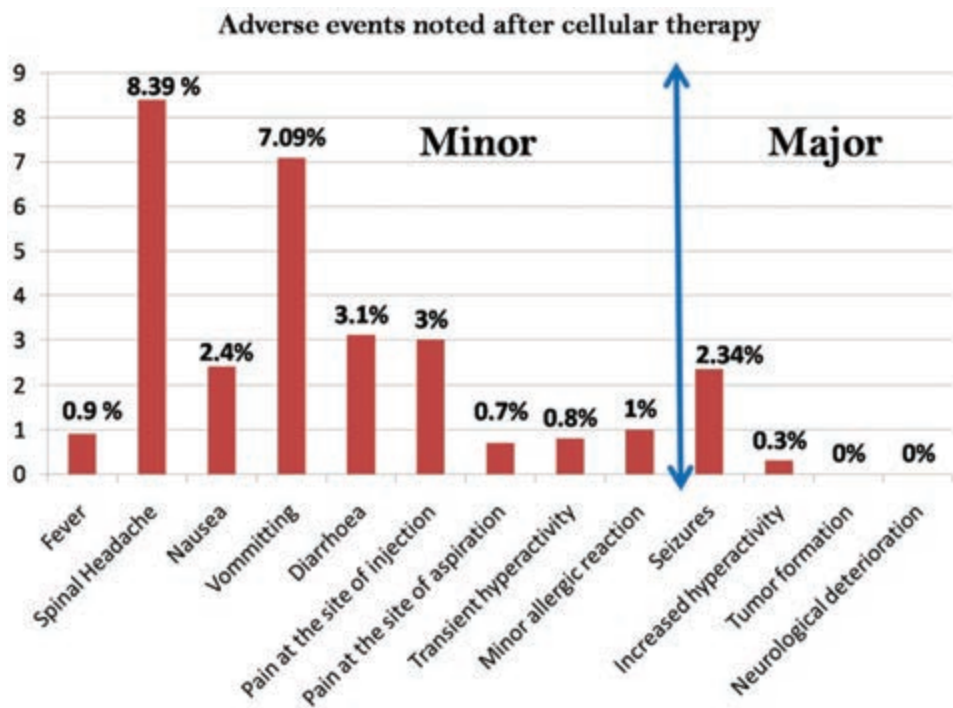


Figure 1. Percentage of adverse events observed post transplantation

Minor adverse events:

Fever was noted in 0.90% of the patients, which resolved during the hospital stay by administration of antipyretic medication. Spinal headache was observed in 8.39% of the patients during hospitalization. Patients with mild headache were advised to lay flat in supine position for as long as possible and to increase the oral fluid intake to 4 to 5 liters. In patients where the pain did not resolve or the patients who had severe headache or the patients who had headache along with nausea or vomiting or patients who had poor command following and cognition, I.V fluids were given. It was noted that the spinal subsided within 72 hours of administration of I.V fluids.

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

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

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

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

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

Major adverse events:

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

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

Literature Review:

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

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

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

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

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

of difficulty in locating lesion. Moreover, transient pain was noticed in patients, and was relieved by medication [78].

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

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

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

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

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

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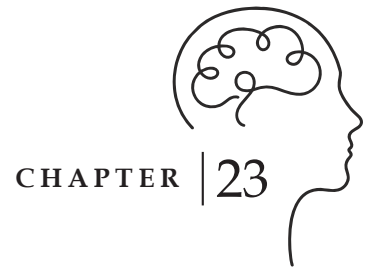
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"Stem cell research, with appropriate oversight, should be directed by scientists, not politicians."

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



Regulations for Cell / Stem Cell Therapy

Regenerative medicine is a rapidly evolving field of medicine, possibly the most promising and most challenging to regulate. There has been a colossal increase in the information regarding regenerative medicine in the past decade. Stem cell science has moved from laboratory to clinic with published evidence, exploring possible treatment and cure for numerous incurable diseases and disabilities. Positive clinical outcomes have reinforced the belief that regenerative medicine may become a cornerstone of future healthcare. As this area of medicine advances toward clinical translation, there arises an urgent need for robust, evidence-based regulatory frameworks. However, availability of multiple types of cells, multiple routes of administration and multiple possibilities for dosage and unlimited combinations of these makes regulating this field particularly challenging.

Major reason for regulations worldwide not being clear about cell/stem cell therapy:

Fundamentally there are two types of treatments:

1. Treatments using Drugs and Products that are manufactured by the company and sold to doctors and hospital.
2. Procedures done by doctors in their own hospital.

Stem cell therapy is also divided into these two categories of products and procedures.

There are several companies that manufacture stem cell derived products and sell them to doctors. These products as per Indian law require approval from CDSCO which is the Indian equivalent of the US FDA. The CDSCO, the US FDA and FDAs of all other countries do not give approval to procedures. They only approve stem cell derived products.

Several government regulatory documents and court orders within India and the United States, state also state this. The first country to make this distinction was Japan which made 2 separate acts for drugs and therapies for treatment of diseases. While PMD Act regulated the products, Act on Safety of Regenerative Medicine (ASRM) was designed to promote safe regenerative medicine therapies.

Regulators in India also, through various regulatory documents, have been attempting to make this distinction.

In majority of the countries all over the world an attempt is being made to impose the regulations for stem cell products on stem cell procedures. Randomised controlled Clinical trials can be done for products and it is difficult if not practically impossible to do them. For procedures, therefore till such time this very important distinction is made the field of regenerative medicine will not get the wider acceptability.

Global trends in the regulation of stem cell therapy

Majority of the countries have very cautious and skeptical approach towards regulating stem cell therapy and have very stringent regulations but there are some countries like Japan, Korea and USA that have quiet progressive approach of regulating stem cell therapy. Some of the other countries like Australia, Canada, European countries have strict regulations but have provisions for clinical use for terminally ill patients suffering from incurable diseases. However, in India there are regulatory pathways defined only for approval of stem cell derived products and not for surgical procedures involving use of autologous minimally manipulated cells.

Challenges in defining regulatory pathways

The practice of medicine broadly consists of two aspects (a) the use of medical drugs and products and (b) surgical procedures. Over the last 50 years or so medical drugs and products have been tightly regulated by the FDAs or equivalent in various countries. Surgical procedures however have never been regulated. They evolve based on the outcome of the procedure and surgeon's clinical discretion. The main challenge with regulations for regenerative medicine is that cells/stem

cells can be delivered to patients either as a product manufactured by companies and sold to doctors or as surgical procedures performed by doctors in their own hospitals. People in general loosely use the term stem cells are “not approved”. It should be noted that only cell/stem cell product can be approved and there is no approval pathway for autologous cellular procedures.

Countries with Permissive regulations

South Korea [5]

Evolution of Regulatory framework in South Korea

The regulation of regenerative medicine and stem cell-based therapies in South Korea has evolved significantly over the past two decades. Initially, oversight was under the Korea Food and Drug Administration (KFDA), later renamed the Ministry of Food and Drug Safety (MFDS).

Korean guidelines make a clear distinction between the levels of manipulation of the cells. 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) (1) 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.”*

In 2020, South Korea implemented the Act on the Safety of and Support for Ad-

vanced Regenerative Medicine and Advanced Biological Products (ARMAB Act).

The Act defined “Advanced Regenerative Medicine” to include cell therapy, gene therapy, tissue-engineered products, and combination therapies. It also covered human-derived materials used in their manufacture.

1. Dual Regulatory Tracks:

- Advanced Regenerative Medicine (ARM): Governs the clinical research and eventual treatment provision at medical institutions.
- Advanced Biological Products (ABP): Governs the development, manufacturing, and approval of commercial cell and gene therapies (like CAR-T cells) under the MFDS.

This law also Introduced expedited approval procedures for advanced biopharmaceuticals for severe or rare diseases to accelerate commercialization.

A key amendment to the ARMAB Act was passed which took effect in February 2025, which significantly expanded patient access beyond just clinical research.

The most critical change was the shift from restricting advanced regenerative medicine to clinical research only, to allowing treatment provision for patients outside of traditional clinical trials, under certain conditions.

- **Risk-Based Classification:** The amendment established a risk classification system for regenerative medicine treatments (e.g., high, moderate, low risk).
- **Approval for Treatment:** Advanced regenerative medicine treatments classified as high- or moderate-risk can be provided by designated institutions after a review and approval of the treatment plan by the national review committee.
- **Expansion of Research Scope:** The revised law also expands the scope of clinical research to all diseases, not just severe, rare, or intractable conditions, provided safety management requirements are met.

This amendment essentially actualized the original intent of the ARMAB to allow patients with serious conditions to receive promising cell and gene therapies as a treatment option, not just as a part of a research study, drawing parallels to expanded access or compassionate use programs in other jurisdictions, but with a unified, government-backed regulatory structure.

Japan [7,8,9]

Evolution of regulatory framework in Japan

Till 2014 Japan had no statutory body for regulating regenerative medicine separately. All the drugs and regenerative medicine products were regulated under

same regulations. In 2014 Japanese Health authorities realized the need for separate regulations for regenerative medicine and implemented the changes in their current regulatory system. Japanese Diet passed 'Regenerative medicine promotion act' which made it necessary to amend existing laws to promote growth and safe implementation of generative medicine. In view of this Pharmaceuticals Affairs Law (PAL) was amended Pharmaceuticals and Medical Devices Act (PMD Act). This amendment made a provision for separate regulation of regenerative medicine products by companies and set standards and regulatory criteria for the manufacturing and sell of the same. It was very progressive of Japanese regulatory evolution to recognize difference between drugs and regenerative medicine as well as difference between regenerative medicine product and therapies for treatment of diseases. While PMD Act regulated the products, Act on Safety of Regenerative Medicine (ASRM) was designed to promote safe regenerative medicine therapies. ASRM safeguarded the patients from unsafe cellular therapies.

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

Highlights of the Japanese regulations

(A) Regulations for the product

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

(A) Regulations for the product:

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

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

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

Act on safety of regenerative medicine (ASRM)

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

Low risk regenerative medicine therapies (Class III):

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

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

Medium risk regenerative medicine therapies (Class II):

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

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

The Japanese guidelines have made a provision for a middle level regulatory body for faster approval process. This committee has an authority to conditionally approve the treatment and marketing using the cell based products; but the provisional plans are required to be submitted to department of health, Labour and welfare. Once the conditional approval is granted the institute must conduct

High risk regenerative medicine therapies (Class I):

The approval is through the “Certified Special Committee for Regenerative Medicine” which is from outside of the institute as that in Class II but the Ministry of health, labor and welfare (MHLW) will impose a certain period of restricted implementation. During this period the MHLW will confirm the safety by hearing opinions of the Health Science Council. The Ministry can order change of the plan if there is nonconformity to the standards of safety and the institute will have to adhere to these changes for the conditional market approval.

Thus the Japanese government has been very permissive in promoting the regenerative medicine. The classifications that are made are based on the safety of the cell products and not the efficacy. The approval is granted with proven safety and presumed efficacy, imposing further testing to establish safety satisfying the stan-

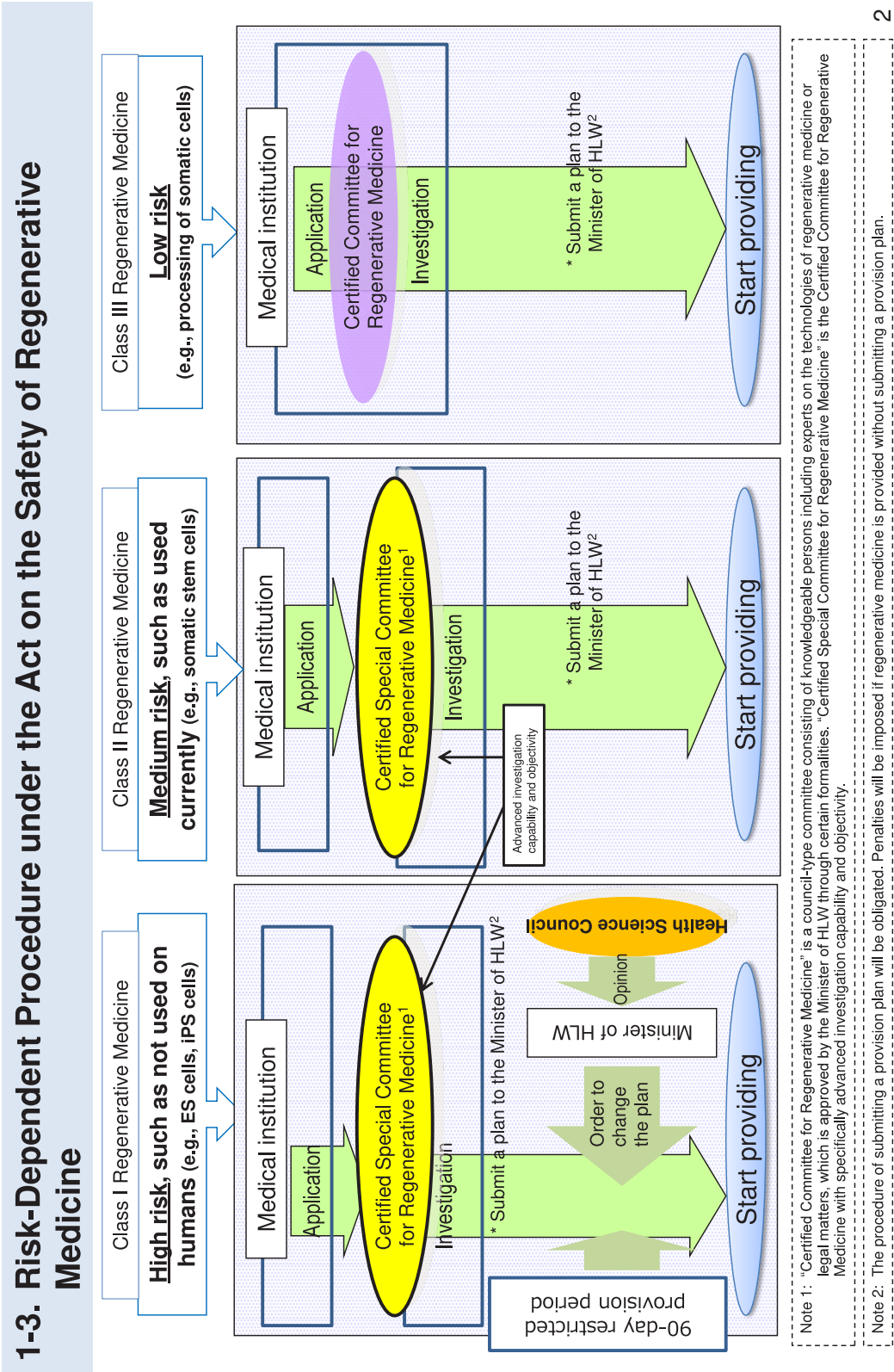


Figure 1: Categorization of regenerative medicine

dards of evidence based medicine. Regulatory bodies from other countries should consider following the Japanese model of regulations for regenerative therapies.

USA

Evolution of regulatory framework in USA

The original guidance regarding use of tissue products was drafted and approved in 1996. In the subsequent year (1997) a separate code of federal regulation (CFR) 1271 was drafted to regulate these products. (3) These were classified under Human cells and tissue and cellular and tissue based products HCT/Ps in this CFR which made a clear distinction between a 'drug' which is a chemical molecule from these biological products. The products were further classified as biological products or medical devices based on difference criteria and a separated set of regulations was drafted for both. These guidelines took into consideration the differences not only between the type of cells but also between the procurement procedures and routes of administration that may significantly alter the safety and efficacy profile of the cells. Although the classification was primitive and inadequate, it was based on the available body of evidence and existing trends and concepts for monitoring development of new therapeutic drugs. The products were classified into; minimally manipulated cells, defined as, cells that do not alter their relevant biological characteristics (due to the technique and/or chemicals used to procure them) and more than minimally manipulated cells. The regulations also differentiated between the route of administration as homologous and non-homologous use. Homologous use was defined as the repair, reconstruction, replacement, or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic function or functions in the recipient as in the donor.

These products were regulated by 2 governing laws, first, Public health services act (PHS) which mandated that any new biological product for licensing will be required to produce the data from clinical study or studies that demonstrate the safety, purity and potency of the cells. (4) However, the guidelines did not define what type of studies or number of patients will be considered appropriate for demonstrating this.

The 21CFR 1271.15 clearly stated that minimally manipulated cells used for non-homologous use will be exempted from the regulatory requirements of FDA for marketing approval if the cells and procured and transplanted in the same surgical procedure. Another provision in the 21CFR 601.40 allowed for the accelerated approval for serious or life threatening illness. This was applicable to certain biological products that had been studied for safety, efficacy and provided meaningful therapeutic benefit to the patients over existing treatment. In accordance with these guidelines various autologous regenerative medicine products that were used for homologous use were approved and licensed in USA between 1997

to 2011.

Between the years 2011 and 2015 in the view of growing clinical evidence for stem cell therapy, Right to try act was designed. Although the regulations prevented generalized marketing of regenerative medicine products up till 2015, after the introduction of Right to try act marketing of experimental drugs for the terminally ill patients was allowed on a case by case review basis.(5)

The act quoted, *“Notwithstanding the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.), the Controlled Substances Act (21 U.S.C. 801 et seq.), and any other provision of Federal law, the Federal Government shall not take any action to prohibit or restrict the production, manufacture, distribution, prescribing, dispensing, possession, or use of an experimental drug, biological product, or device that (1) is intended to treat a patient who has been diagnosed with a terminal illness; and (2) is authorized by, and in accordance with, State law.”*

Another law passed by the state of Texas in USA named Charlie’s Law. Charlie’s law legalizes provision of investigational (unproven, but safe medical therapies) stem cell treatment in certain patients with chronic diseases or terminal illness.

The law categorically defines investigational stem cell treatment as adult stem cell treatment, that is being used in clinical trials but is unavailable for use in general.

This is a very progressive step by the state of Texas which has definitely brought back hope for patients suffering from incurable illnesses. USA as well as other countries should follow in these directions to bring hope to the patients who can’t wait for the conventional evidence based medicine to provide answers and who are suffering from years in absence of any cure or treatment to alleviate their symptoms provided by modern medicine.

Recently, certain states have enacted their own laws to expand patient access under defined conditions. Utah and Florida have introduced state-level frameworks permitting the clinical use of specific non-FDA-approved stem cell therapies. Utah’s 2024 law allows licensed practitioners to offer placental or perinatal stem cell treatments with mandatory patient consent. Florida’s 2025 legislation permits the use of stem cell therapies subject to ethical sourcing, FDA-registered facilities, and stringent informed consent and advertising regulations.

Although president George bush had banned the federal funding for the research on embryonic stem cells and by using embryonic cell lines in 2001, President Barack Obama subsequently lifted this ban. Currently embryonic stem cell research is eligible for federal funding. To obtain federal funding to conduct research using stem cells, a sponsor must submit its application to the NIH. Guidelines for applying to the NIH can be found on the Federal Register (Vol 65, No 166/Friday, August 25, 2000/Notices). Under the auspices of the Obama administration, the National

Institutes of Health plans to expand federal funding for stem cell lines that meet following ethical requirements: the embryo used is discarded after IVF; informed consent is obtained from the donors; the couple must not receive compensation (neither financial nor medical benefits) or be coerced or threatened. Older stem cell lines created in the spirit of the new regulations will be considered for federal funding, whereas embryos created solely for research purposes will be excluded [12].

Regulatory framework in Europe:

European medical agency (EMA) has drafted a separate legislation for regenerative medicine products which is known as act on advanced therapy medicinal product (ATMP).⁽⁹⁾ Various regenerative medicine products are put into this newly designed category for such products. This legislation recognizes the difference between the drugs and stem cell products. Another law formulated by EMA, called Hospital Exemptions act (HE) allows a practitioner or an institute to offer stem cell therapy as a form of treatment for terminally ill patients. ⁽¹⁰⁾ The law states that,

“Advanced therapy medicinal products which are prepared on a non-routine basis according to specific quality standards, and used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient, should be excluded from the scope of this Regulation whilst at the same time ensuring that relevant Community rules related to quality and safety are not undermined.”

Recently in the year 2016 EMA also formulated a PRIority Medicines (PRIME) program to support development of medicines for unmet medical needs. ⁽¹¹⁾ Under this scheme promising therapies and medicines that are important for public health will be given additional support and accelerated regulatory approval. The products that are in the stage before Phase II as well as Phase III trials can be a part of this scheme.

These schemes highlight patient's right to seek treatment for a disease that has no cure. If there is no treatment available, then regulatory bodies should not prevent patients from taking benefit of safe but unproven therapies. This concept was known as compassionate use in Europe earlier which has now evolved in the legislation explained above. In Australia and Canada as well such laws exists which allow marketing and provision of safe but unproven therapies and drugs to patients that suffer from incurable disorders after taking their informed consent and by reporting any possible adverse events noted. In Australia this is known as Special access scheme and in Canada it is known as special access program. ^(12,13)

Some common themes and concepts emerge from these new and more permissive regulations. It is important to implement these in the guidelines and regulations of

countries that have less permissive regulations.

Regulatory framework in Australia:

The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health and Ageing, and is responsible for regulating medicines and medical devices. They have excluded procedures using autologous cells from the TGA regulations.

Therapeutic Goods (Excluded Goods) Order No. 1 of 2011 states that: *Item 4(q) of the Order specifically excludes from TGA regulation HCT that are:*

1. *Collected from a patient who is under the clinical care and treatment of a medical practitioner registered under a law of a State or an internal Territory; and*
2. *Manufactured by that medical practitioner, or by a person or persons under the professional supervision of that medical practitioner, for therapeutic application of a single indication and in a single course of treatment of that patient by the same medical practitioner, or by a person or persons under the professional supervision of the same medical practitioner.*

Products (goods) covered by item 4 (q): The Item only covers HCT that are collected from a patient and used in that same patient. That is, the products are ONLY for autologous use

Regulatory framework in Bahamas

The Bahamas has a detailed regulatory framework for stem cell therapy, established by the Stem Cell Research and Therapy Act, 2013, and further updated by the Longevity and Regenerative Therapies Act (LARTA), 2024. This legislation aims to create a safe and ethical environment for both research and treatment, attracting medical tourism while preventing scientifically unfounded procedures.

The LARTA is a Government Unit that grants ethics approvals and licenses for longevity and regenerative therapy programs. The LARTA Agency was established to guarantee The Bahamas' transformation into a global hub for safe, ethical and accessible longevity and regenerative therapies.

New concepts that have emerged from the recent regulations (Figure. 2)

1. Conditional marketing approval
2. Risk Stratification
3. Post-Hoc efficacy analysis
4. Presumed efficacy
5. Patients right to seek treatment

- 6. Distinction between cellular therapies
- 7. Distinction between a stem cell product and medical service



Figure 2: New concepts emerging from the recent regulations

Conditional approval

Conditional approval first introduced by Japan and later also implemented by USA and South Korea is revolutionary concept that allows for faster marketing of promising stem cell therapy products. In the last century the most promising medical research was done by individual doctors in their field of practice who kept patient care at the center of their research. But industrialization of pharmaceutical sciences and stricter regulations implemented for getting marketing approval made it impossible for individual practitioners to develop promising therapies and medicines. Conditional approval allows for promising therapies to be marketed for a stipulated time at an earlier stage of Phase I or pilot trials which are sufficient to prove the safety of the therapy and suggest efficacy of the same.

The concept of conditional approval has shifted the control of medical innovation

back in the hands of individual doctors practicing and researching to provide better patient care.

Risk Stratification

Risk stratification means grouping the stem cell therapies and products based on their risk to human life and health. Such stratification helps to differentiate between less harmful and more harmful cellular therapy products. South Korea and Japan have based their regulatory requirements on risk stratification. With low risk needing only institutional clearance, medium risk needing outside institutional clearance and high risk requiring clearance from MHLW.

Post-Hoc efficacy analysis

Concept of Post-Hoc efficacy analysis means that true efficacy of the product or therapy can be determined post marketing. This is the most dramatic shift in the current medical regulations that do not permit marketing of unproven drugs and therapies. However, based on this principal therapy or product can be permitted for marketing based on studies showing definite safety but preliminary efficacy analysis. The roots of this concept are in the basic principle of compassionate use, facilitating early availability of potentially lifesaving experimental medication which are safe but unproven.

This is a revolutionary concept that has already been implemented in Japan since November 2014 and products have already received approval under this legislation.

The basis of post – hoc efficacy analysis lies in the concept of Practice based evidence which allows for gathering information regarding efficacy of a particular therapy after using it clinically as a form of treatment and recording the clinical outcomes in the patients treated. Unlike evidence based medicine, the concept of practice based evidence gives the flexibility to offer a treatment after the safety is established and offer it as a treatment while simultaneously studying the effects on clinical outcome.

Presumed efficacy

It has been debated earlier that the modern standards for efficacy testing are too idealistic and may in turn slow down the progress of medical science. Although the regulations are for safeguarding the patients they fail to determine when a therapy will be considered as proven. The current regulations ask for Phased clinical trials that take up to 6 to 8 years before a new product can come in the market and have a cost estimate of about 5 million dollars. Current research and statistical methods are more suited for a drug or a molecule that has finite chemical reactions in the body, however in biological products there are infinite possibilities for in-

teractions and therefore it may take decades before a conclusive efficacy analysis can be done.

Japan in their regenerative medicine regulations for the first time proposed a concept of 'Presumed efficacy'. This means that the preliminary trials that lack statistical rigor but are suggestive of beneficial clinical outcome can be considered as the evidence for efficacy of the treatment. Simply put, it means that it can be reasonably assumed that therapy will be effective in larger population based on a finding with a smaller population.

It was earlier considered unethical to charge for therapies that have shown efficacy in smaller populations. Japan in their recent regulations allowed for marketing of such therapies under a conditional approval and these therapies were also covered under Japan's national health insurance schemes.

Patient's right to seek treatment

Up till the last century availability of the clinical treatments was solely based on decisions of regulatory bodies. If a treatment did not fit the criteria laid out in the regulations then it was not allowed in the market, thereby denied to the patients. Although this was to safeguard patients from adverse effects of under investigated therapies, terminally ill patients were losing out on promising therapies due to strict demands for proving efficacy.

Most of the patients with progressive fatal disorders do not have enough time for an experimental drug which has proven safety and has shown efficacy in smaller trials to be tested in the statistical rigor of bigger trials. These drugs could be potentially lifesaving for these patients. There were many efforts lead by patients and non-profit organizations, which demanded access to such experimental drugs for patients with terminal illnesses.

The origin of compassionate use is in the World Medical Association's Declaration of Helsinki on ethical principles for medical research involving human subjects. The 2013 declaration in their clause on unproven intervention in clinical practice states that, *'In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available'*. (10)

In 2024, the declaration in their clause on unproven intervention in clinical practice states that, *"When an unproven intervention is utilized in an attempt to restore health or alleviate suffering for an individual patient because approved options are inadequate or*

ineffective and enrollment in a clinical trial is not possible, it should subsequently be made the object of research designed to evaluate safety and efficacy. Physicians participating in such interventions must first seek expert advice, weigh possible risks, burdens, and benefits, and obtain informed consent. They must also record and share data when appropriate and avoid compromising clinical trials."

The concept of patient's right to seek treatment is highlighted in the White paper published by the International Society of Cellular Therapy which states that "*Patients seeking medical treatment for cellular therapies have the following rights that must be respected by healthcare providers and all associated with their care. The right to seek treatment: patients and their families/partners have the right to seek treatments for their diseases. No entity should withhold this fundamental right unless there is a high probability of harm to the patients.*"(15)

Efforts made by the patients in accordance with this ethical principle led to changes in the legislation for USA, Europe and several other countries in the world. In USA this was implemented as Treatment/Emergency IND initially and later as Right to try Act in 2015. (5) In Europe this was implemented as Compassionate use Act and recently a program was launched to support to development of priority medicines for unmet medical needs, PRIME. In addition other Acts like Hospital Exemption Act in Europe (10), Special access program in Australia (12) and special access scheme in Canada (13) are based on this principle.

These compassionate use programs highlight patient's right for seeking unproven but safe experimental drugs and allows access to such medicines and therapies at the personal recommendation and responsibility of the treating physician. Such use is deemed ethical and can be charged for after receiving an informed consent from the patient, explaining the possible adverse effects if any and informing the patient about the experimental nature of the therapy.

Unfortunately, in India there are no laws or regulations for compassionate use. Indian regulators and guideline formulators have not taken into consideration the right's of these patients to seek treatments that may potentially save their lives.

Distinction between different types of cellular therapies

In Japan, there is a separate law designed only for the classification of the regenerative medicine products based on their safety profile.(7) These products are divided into 3 separate classes as, class I – High risk, Class II – Medium risk and Class III – Low risk products (Figure).

In European guidelines, the products are divided into minimally and more than manipulated as well. Minimal manipulation is defined as cells procured through simple technologies like cutting, grinding, shaping, centrifugation, soaking in antibiotics or antibiotic solutions, sterilization, irradiation, cell separation, concentra-

tion or purification, filtering, lyophilization, freezing, cryopreservation and vitrification. However, there are no separate guidelines for the use of these products as allowed in Japanese laws.

Distinction between a cellular therapy product and cellular therapy medical service

Advent of cellular therapy has given rise to a huge dilemma for regulators whether to regulate these as a product or a medical service. Every new product is regulated separately and the evidence for one is usually not applicable to the other therefore companies designing different products need to seek different approvals. The guidelines for these are also very strict. However a therapy once proven safe and effective can be used by multiple practitioners and they individually do not need to seek approval for the same.

Japanese guidelines however have been very progressive and they have designed 2 separate laws for products and therapies. These two laws have also been very progressive in their field of application allowing fast track conditional approval for products and mandatory approval from MHLW only for high risk therapies. USA has taken a step ahead in not only allowing a fast track conditional approval for products but also allowing different companies to get faster marketing approval based on exhibited biosimilarity with an already existing approved product.

Current regulatory system in India

The present situation in India with regards to guidelines for stem cell therapy

Distinction between stem cell derived product and stem cell procedures has been made by the Ministry of Health and Family Welfare

1. **Central Drugs Standard Control Organization** is the main regulator for drugs and medical products in India. It is headed by the DCG(I) and they work in accordance with the Drugs and Cosmetics Act, 1940 and the New Drugs and Clinical Trials Rules, 2019. In the New Drugs and Clinical trials Rules, 2019 CDSCO made a very important distinction by defining “stem cell derived product” has been defined as a “New Drug”. Basically, this separates products from procedures. By defining stem cell derived product as a new drug, CDSCO has very clearly stated that stem cell products cannot be manufactured or used without an approval or license from them.
2. **Direction of MoHFW, under section 33P of the Drugs and Cosmetics Act, 1940, issuing clarification on stem cell derived products.**

Department of Health & Family Welfare (Drugs regulation section) MoHFW, under section 33P of the Drugs and Cosmetics Act, 1940, has issued a clarification on stem cell derived products which states that:

Stem cell derived product means a drug which has been derived from processed stem cells and which has been processed by means of substantial or more than minimal manipulation with the objective of propagation and /or differentiation of a cell or tissue, cell activation, and production of a cell-line, which includes pharmaceutical or chemical or enzymatic treatment, altering a biological characteristic, combining with a non-cellular component, manipulation by genetic engineering including gene editing & gene modification.

For the above purpose :

Substantial or more than minimal manipulation means ex-vivo alteration in the cell population (T-Cell depletion, cancer cell depletion), expansion, which is expected to result in alteration of function.

The isolation of tissue, washing, centrifugation, suspension in acceptable medium, cutting, grinding, shaping, disintegration of tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, sterilization by washing or gamma irradiation, freezing, thawing and such similar procedures are regarded as minimal manipulation and are not considered as processing by means of substantial or more than minimal manipulation.

Stem cells removed from an individual for implantation of such cells only into the same individual for use during the same surgical procedure should not undergo processing steps beyond rinsing, cleaning or sizing and these steps shall not be considered as processing.

This clarification further defines what is a stem cell derived product and what is not. Therefore, stem/ cell procedures involving minimal manipulation is not a stem cell derived product and falls outside the purview of the New Drugs and Clinical Trials Rule 2019.

3. Drugs Technical Advisory Board (DTAB) of the Ministry of Health and Family Welfare.

Drugs Technical Advisory Board (DTAB) is the highest statutory decision-making body on technical matters related to drugs in the country. It is part of the Central Drugs Standard Control Organization (CDSCO) in the Ministry of Health and Family Welfare and includes DGHS, DCG(I) other senior government officials.

DTAB has made some very important and landmark distinctions as is evident from the Minutes of the 84th meeting of DTAB clearly states that,

"It is inappropriate to intervene from regulatory angle on routine practices/therapies/surgeries/transplantations undertaken by Registered Medical Practitioners/physicians/doctors in their clinics/hospitals"

Further DTAB also states that ,

"The routine practices/ transplantations/surgeries/therapies undertaken by doctors involving stem cell for treatment of their own patients and not for commercialization of the

same outside their own hospitals/clinics fall outside the purview of Drugs and Cosmetics Act, 1940 and the New Drugs and Clinical Trials Rules, 2019. Therefore, shall be dealt outside the said regulation."

These landmark DTAB decisions very clearly highlight that doctors doing autologous stem cell procedures in their own hospitals not only do not come under the Drugs and Cosmetics Act but also it is inappropriate to intervene from a regulatory angle on such doctors. For doctors practicing autologous cell therapy procedures these DTAB decisions are a major regulatory and legal protection.

Therefore, DTAB by specifying that it is inappropriate to intervene from a regulatory angle on doctors doing stem cell practice in their hospitals has clearly separated the use of stem cell derived products from stem cell procedures.

In January 2022, the DGHS had prepared [1] Standard Operating Procedure (SOP) for control and monitoring of practices and procedures involving cell or stem cell based preparations for therapeutic purposes [2] List of practices and procedures involving Cell or Stem cell based preparations for therapeutic purposes as identified by experts.

To prepare this list, the experts had evaluated and collated worldwide evidence available for use of cell/stem cells in various indications. Use of Cell or Stem cell based preparations for therapeutic purposes for various indications were classified as standard of care or clinical option or under developmental/research.

a. List of indications categorized as Standard of care:

Diabetic Foot/ Wounds, Osteoarthritis, Tendinopathy, Skin rejuvenation, Multiple / Systemic Sclerosis, Wounds, Avascular necrosis of hip (osteonecrosis)

b. List of indications categorized as clinical option :

Autism, Kidney disease, Psoriasis, Spinal cord injury, Cerebral Palsy, Cerebral stroke, COVID 19, Drug resistant Tuberculosis, COPD, Alopecia, Erectile dysfunction, Multiple sclerosis

c. List of indications categorized as under development/research :

Acute respiratory distress syndrome / Acute Lung Injury, Interstitial Lung Disease, Rectifying anaemia at the background of chronic diseases like diabetes, tuberculosis, chronic kidney disease, arthritis including thalassemia, cancer and HIV and its immunotherapeutic potentialities, For treating burn patients, patients with non-healing ulcers due to diabetes, burn and other conditions like gangrene, trauma and accidents and other known or unknown causes and Parkinson Disease, Thymus transplantation at the background of cancer, fetal cortical brain

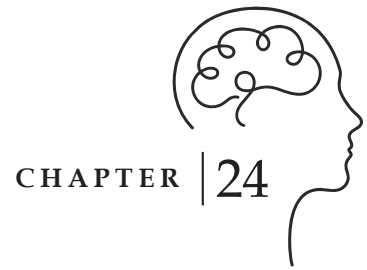
The SOP for control and monitoring of practices and procedures involving cell or

stem cell based preparations for therapeutic purposes mentions responsibility, accountability, procedure for processing, premises requirement, equipment requirement, procedure process information to patient, registration of ethics committee, role of other regulatory bodies, and mechanisms for regulatory actions

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Ethics

Consensus on the potential of stem cell therapy to address various incurable, debilitating disorders is unanimous. Stem Cell research and therapy is the front line of the biomedical field. However, no other area of biomedical research has faced the quantum of ethical, moral and political controversies that surrounds stem cell research. Adult stem cells as an alternative source, other than embryos, have been spared of controversies and have been generally welcomed and encouraged for research and therapy.

Embryonic stem cell, on the other hand, has been hounded by objections and restrictions due to the source of its procurement, by various religious bodies of the world.

Ethical Issues Associated With Embryonic Stem Cell

Research Religion and embryonic stem cell:

The major dictum common to all religions is : 1) Human life is sacred and has to be guarded 2) Alleviation of human suffering should be strived for. Though, there is consensus among all religions regarding the potential of stem cell research being a means towards addressing the second dictum, the opinion on what construes a human life differs vastly.

Should the 5-7 day embryo be given the status of a person and hence have the right to life or is this stage too early to confer this right?

For some religions, ensoulment of the embryo would make it a person. But, then when does ensoulment take place?

These are just a few issues surrounding the embryonic stem cell field. Contrasting opinions among various religions and even within the religion exist. The following is a sample of this diversity among different faiths.

Greek Orthodox and Roman Catholic Churches

The official position of these churches is that a human person begins at conception and the human embryo has the same moral status as human persons. Consequently, research on human embryos, including hES derivation and subsequent use is unethical, and if it involves the willful destruction of embryos, it is homicide. The argument that the cell lines are derived from excess embryos, after the fertility needs are dealt with, is of no consequence, since the production of excess embryos itself is unacceptable to the church. The fact that these embryos and their products would be used for the alleviation of human suffering, does not justify the destruction of the embryos.

Since the underlying belief is in the embryo's right to life, any use of the embryo that is not for its own good is immoral and therefore, impermissible. There is no consequentialist or utilitarian approach that would make this act acceptable. The belief in the personhood of human embryos also means that it is not possible to use hES lines previously derived from human embryos or to use therapies derived from hES research. The idea is that these cell lines and therapies are tainted by the immoral act of killing the embryo. To use them would be to become complicit in the immoral act.

However, this rigid stance, especially of the Roman Church, is somewhat diluted by certain other catholic groups, who do not believe that the embryo is a human person, but believe that its ensoulment is the morally relevant time with regard to personhood.

Protestant Churches

Most protestant churches do not believe that embryos have personhood and are open to embryo research but consider that the goals of the research are of paramount importance. In addition, considerable emphasis is placed on the need for both public discussion and for oversight of the research rather than leaving it as an unregulated private enterprise. They believe that the benefits from this and other medical research be distributed evenly and justly to all those in need, regardless of resources or geography.

Official positions vary from country to country on the moral status of the embryo and therefore, on the morality of embryo research in general. These divisions show just how personal an issue stem cell research can be. For these churches like for the lay public, weighing the moral status of the embryo and the need to help ailing and suffering people is not a simple arithmetic. (1)

Judaism

Orthodox Jews believe that embryos do not have the same moral status as human persons. In fact, gametes and embryos outside a human body do not have any legal status under Jewish law. The result therefore, is that embryos created by IVF have no special moral or legal status. Under Jewish law (Halcha) the fetus does not become a person (nefesh) until the head emerges from the womb.

They believe that when the embryo is implanted it is "as water" up to the fortieth day. After that time and before the fetus emerges from the woman's body it is a potential life and has great value. Ensoulment is generally thought to occur sometime after the fortieth day. It gains full human status, however, only once it emerges from the woman's body. Since embryos used in hES research are outside the body, according to the Jewish faith it is possible to use excess IVF embryos in research.

In addition to the Jewish views on the moral status of the human embryo, this religion places emphasis on preventing and alleviating suffering. This leads to a deep belief in the morality of and value in pursuing medical research. The commitment to preserving one's body and health is joined by a commitment to helping others and alleviating suffering. So there is a moral imperative to help those who are suffering from diseases and to explore the potential of all types of stem cell research. This belief leads Jews to have a generally favorable view of stem cell research including hES research. (2)

Islam

In Iran, Turkey, Singapore (with a majority of Muslims) and other Islamic countries, embryo research policies are influenced by the religious belief that full human life with its attendant rights begins only after the ensoulment of the fetus. This is generally believed by Muslim scholars to take place at 120 days after conception (although a minority belief indicates ensoulment takes place 40 days after conception). This fact, in conjunction with the importance articulated in the Qur'an of preventing human suffering and illness, means that the use of surplus IVF embryos for stem cell research is relatively uncontroversial. What remains controversial in the Muslim world is creating embryos for the purpose of research. As with other religions, Islam and its followers have differing points of view 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 (karuna) - that divide Buddhists. Some Buddhists argue that embryonic stem cell research is in accordance with the Buddhist tenet of seeking knowledge and ending human suffering, while others argue that it is a violation of the notion of not harming others.

A central belief of Hinduism and Buddhism is that an individual's soul or self is eternal. In Hinduism the soul is believed to be passed from one living being to another in a process called reincarnation. In Buddhism reincarnation is described differently as the rebirth of the self. These beliefs, that the soul or the self are reborn lead to a greater acceptance of cloning technology. Although the use of embryos in stem cell research remains a divisive issue in these religions, the use of cloning technology in stem cell research is less controversial.(3-5)

Medical And Other Ethical Issues And ES Cell Research:

Proponents of embryonic stem cell research advocate that obtaining human ES-cells from the embryos left over after successful pregnancy in the course of IVF-treatment for the goal of treating diseases and saving lives justifies the symbolic loss that arises from destroying embryos in the process. They emphasize on the significance of saving life of many patients who need cell replacement therapy, as an essential reason for permission of research on embryos and obtaining ES-cells from them. A different set of ethical issues arises once researchers have learnt safe and effective ways to direct human ES-cell to differentiate into specified cell or tissue types, and to transplant them for therapeutic effects in patients. An important clinical issue at this point will be whether ES-cell not derived from the patient, will be rejected by the patient's immune system. The strategy for dealing with this problem, would then be to use a patient's nuclear DNA to create an embryo from which ES-cells compatible with that patient could then be derived. This process, known as somatic cell nuclear transfer could prove to be a safe and effective use of ES-cell derived replacement therapies. However, this would raise more ethical issues beyond the destruction of leftover embryos to obtain human ES-cells. One issue would be ethical concerns about creating human embryos for the sole purpose of destroying them to obtain replacement cells for the patient who provided

ed 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 EScell research has been that of the United States. This has been mainly attributed to be-influenced by the ethical, moral & religious stand of the catholic church. In 1973 a moratorium was placed on government funding for human embryo research. In 1988 a NIH panel voted 19 to 2 in favor of government funding. In 1990, Congress voted to override the moratorium on government funding of embryonic stem cell research, which was vetoed by President George Bush. President Clinton lifted the ban, but changed his mind the following year after public outcry. Congress banned federal funding in 1995. In 1998 DHHS Secretary Sullivan extended the moratorium. In 2000, President Bill Clinton allowed funding of research on cells-derived from aborted human fetuses, but not from embryonic cells. On August 9, 2001, President George W. Bush announced his decision to allow Federal funding of research only on existing human embryonic stem cell lines created prior to his announcement. His concern was to not foster the continued destruction of living human embryos. In 2004, both houses of Congress asked President George W. Bush to review his policy on embryonic stem cell research. President George W. Bush released a statement reiterating his moral qualms about creating human embryos to destroy them, and refused to reverse the federal policy banning government funding of ESC research (other than for ESC lines established before the funding ban). In the November 2004 election, California had a Stem Cell Research Funding authorization initiative on the ballot that won by a 60% to 40% margin. It established the "California Institute for Regenerative Medicine" to regulate stem cell research and research facilities. It authorizes issuance of general obligation bonds to finance institute activities up to \$3 billion dollars subject to an annual limit of \$350 million. Under President Obama, it is expected that federal funding will be made available to carry out research with hESC lines not on the NIH list and to derive new hESC lines from frozen embryos donated for research after a woman or couple using invitro fertilization (IVF) has determined they are no longer needed for reproductive purposes. However, federal funding may not be permitted for creation of embryo expressly for research or for derivation of stem cell lines using somatic cell nuclear transfer (SCNT)

The Korean Stem Cell Controversy

The meteoric rise and equally sudden fall of Korean scientist Woo-Suk Hwang depicts all that can possibly go awry, ethically and scientifically, in the world of stem cell research. What would have been regarded as a seminal paper in SCNT technology and human ES therapeutics turned out to complete fraud and hogwash. Not only were the results fabricated, but also, unethical practices were employed to procure oocytes for the research.

At the end of 2005, the scientific community was shocked by one of the greatest

cases of misconduct in the history of science. Two breakthrough articles about stem cell technology from a Korean laboratory headed by Woo-Suk Hwang, published in *Science*, appeared to be almost completely fabricated and were therefore retracted. The two fraudulent papers concentrated on the concept of therapeutic cloning in humans. In this somatic cell nuclear transfer (SCNT) technology, a nucleus from a patient's somatic cell is transplanted into an enucleated donor oocyte. The resulting blastocyst embryo is used for the isolation of embryonic stem cell (ESC) lines that possess virtually all the patient's characteristics and thus will minimize immune rejection upon transplantation. Until the publication of the fraudulent papers, therapeutic cloning was a cumbersome and inefficient technique and successful therapeutic cloning in humans had not been reported before. In their 2004 paper, Hwang and his associates claimed to have isolated the first human ESC line derived from SCNT and in their second paper they reported to have improved the efficiency to such an extent that clinical application became within reach. Two months following the first paper, criticism arose on the ethics of obtaining the human oocytes used in the study. After initial denial it became clear that egg donors had been paid and two lab members had provided oocytes. This forced Hwang to admit these unethical practices. Subsequently, the scientific content itself raised questions. Duplications of microscopic photographs in different panels, and designated as different ESC lines, in the publication of 2005 were uncovered, but these were parried as an accidental mistake by Hwang and the *Science* editorial board. Furthermore, DNA fingerprint comparison of presumed donor and derived ESC lines showed no inter-experimental variety and were in fact performed on the same fingerprint profile. Hwang agreed to an independent investigation by Seoul National University. His three most important recent works were investigated: the retracted 2004 and 2005 *Science* papers and a publication in *Nature* about a cloned dog. The conclusions were clear. The claim of being the first laboratory to create a pluripotent human ESC line through SCNT was reported to be false. Verification of the DNA fingerprints of cell lines, teratomas and donors showed that the NT-1 cell line was not derived from the designated donor. Second, no evidence was found to verify the conclusions of 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 typing 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 follow-

ing: body integrity, respect of privacy and confidentiality of data, promotion of solidarity, fairness of access to healthcare and information and consent of the donors. Umbilical cord blood banking process should comprise of a detailed consent explained clearly to the woman or to the couple of the prospective new treatments, but stress that they are still very much at the experimental stage. Principally, tissue bank activities should be reserved to public health institutions or non-profit making organizations. All public and private banks tissue banks should be monitored for quality measures and standards. These guidelines are based on the principle of respect for human dignity and integrity which asserts the principle of non commercialization of the human body; principle of autonomy or the right to self-determination on the basis of full and correct information; principles of justice and solidarity, as regards to fair access to healthcare services; principle of beneficence, or the obligation to do good, especially in the area of health care; principle of non-maleficence, or the obligation not to harm, including the obligation to protect vulnerable groups and individuals, to respect privacy and confidentiality; and principle of proportionality which implies a balance between means and objectives. (11)

There are also some value conflicts regarding the Umbilical cord blood banking. The values of freedom and free enterprise can conflict with the principles of solidarity and justice, according to which access to healthcare should be on an equitable basis and based on realistic needs, as well as with the principle of protection of vulnerable groups.

Informed Consent:

Informed consent is a vital step to any research project. It is the process in which a patient/participant consents to participate in a research project after being informed of its procedures, risks, and benefits (12) After fully comprehending the information about the project, the patient/participant gives full and conscious consent for the physician/scientist to continue with the procedure. The consent is obtained after giving all the information to the patient in comprehensible non-medical terms, preferably in the local language about the diagnosis; nature of treatment; risks involved, prospectus of success, prognosis if the procedure is not performed and alternative treatment. The three main aspects of the informed consent are information, voluntariness and capacity. In keeping the observations of the Supreme Court, the National Commission of India stated that all information would imply adequate information to enable the patient to make a balanced judgement to whether or not to be a part of the trial or treatment.

Ethics in medical practice

Greek word Ethos, meaning character, is the origin of the word ethics. Ethics is part of philosophy in modern world and explores the rights, wrongs and morality of human behavior. Ethical principles related to medical practices form bioethics.

Bioethical principles guide the current regulatory systems and are the foundation of modern research practices [13].

Four core principles of bioethics are Autonomy, Nonmaleficence, Beneficence and Justice.

Autonomy:

Principle of autonomy states that the patient should be considered capable of taking an informed decision about the treatment after understanding the benefits and adverse events of the treatment should be given the right to exercise his/her choice of the treatment without any external influence preventing voluntary action.

Nonmaleficence:

The second principle of Nonmaleficence suggests that there should be no harm caused to the patient by providing or denying a treatment.

Beneficence:

The third principle of Beneficence is self explanatory and suggests that the physician has a duty to benefit the patients and also to prevent them from any harm that may be caused by a medical treatment.

Justice:

The fourth and final of the basic principles, is the principle of Justice; meaning a physician should be fair in offering his services and there should not be any preferential attribution of services.

The principles have been intentionally or unintentionally violated in past while offering medical treatments and performing medical research. Medical experiments conducted on the prisoners of war in Germany were intentional examples and occurrence of phocomelic infants after thalidomide consumption was an unintentional example of this violation. Because of these the need to enforce strict adherence to the biomedical principle in medical research and practice was necessitated. In modern world the ethical principles are implemented through various regulatory bodies to protect the patients from being taken advantage of and to protect them from any harm caused by the treatment or medical incompetence. The guidelines formed by these regulatory bodies are based on Evidence Based Medicine (EBM).

Principles of Evidence based medicine

Evidence based medicine (EBM) is defined as, “The conscientious and judicious use of current best evidence from clinical care research in the management of individual patients.” [14] To cope with the growing medical information and to de-

termine the true efficacy of medical treatments, EBM was used. EBM helps to form most informed conclusions about the efficacy of existing treatment options [14]. Various steps involved in coming to this conclusion are forming a relevant clinical question, searching for the evidence to answer that question, appraising the evidence, integrating that evidence into the clinical practice keeping in mind patient preferences, evaluating the outcome of such integration using standardized scientific tools and then documenting and disseminating these findings for others to appraise [15]. This makes the whole process combusive.

Information gathered clinical experience, expert opinions, individual cases or series of cases and research trials using cohort studies or randomized can be considered as evidence but there is a hierarchy. While the clinical experience and collective expert opinions are considered to have least generalizability, randomized controlled trials and systematic reviews of such trials are considered to be the most applicable evidence. The quality of the evidence is based mainly on the epidemiological principles [16].

The primary aim of using EBM model is to provide synthesis of the available information to empower the doctors to take most informed clinical decisions. Using this system has prevented the consumption of unsafe drugs, serious adverse effects and use of ineffective medical practices. However, most of the revolutionary concepts of modern medicine were developed in the late 19th and early and mid 20th century when the notion of EBM was not there. This period has witnessed some of the most extra-ordinary medical inventions and they happened in the absence of regulatory bodies.

Although EBM is the most scientific approach towards concluding the information gathered, the process of EBM is very time consuming and has its limitations in assessing the effectiveness. It is difficult to apply the evidence gathered collectively from patients to an individual patient with dissimilar characteristics [16]. EMB relies on the empirical evidence for informed conclusions and is unable to formulate clinically relevant conclusions in absence of such evidence. Therefore most common fallacy of EBM is that lack of evidence of efficacy is considered as lack of efficacy itself. [16]. Empiricism of EBM undermines the philosophical origin of the medical innovations that are based on the clinical expertise and pathophysiological knowledge of the disease [16].

With the advent of evidence based medical practices, the development of new therapies and drugs has become very time consuming and the burden of evidence also increases the economical burden. Current process of developing new drugs includes preclinical laboratory & animal testing as well as multiple phases of human testing to first establish its safety and then efficacy first in a small group and then in larger groups [17]. It takes approximately 6 to 8 years and \$5,000,000,000 to develop a new marketable drug [18]. It is almost impossible for a single medical

practitioner to spend the time and money required for this process. It can only be done collectively or by corporate. It is based on commercial interest and demand from the consumers rather than medical expertise. Therefore the rare disease get neglected and development of drugs for rare diseases is limited.

So the debate is:

1. Are current medical practices for developing new treatments adhering to the principle of Justice?
2. Are we then really observing the principle of beneficence?[c] Is the system of EBM which was developed for making efficient, scientific and evidence informed clinical decisions, now perhaps slowing the pace of medical evolution. Therefore although very essential, practice of EBM needs to be relooked especially with reference to cell therapy.

Ethical Dilemmas of cellular therapy

The last decade has seen the evolution of cellular therapy. This is the field of regenerative medicine where healthy tissues could be used to replace or repair damaged tissues. Cell therapy holds a special place in the development of Neurorestorative treatments for otherwise incurable neurological conditions. Development of cellular therapy has also sprouted debates on various ethical grounds based on religious, social, political and capitalistic beliefs. It has been unanimously accepted that the science of regenerative medicine is vast and holds a tremendous potential of finding cure to various untreatable diseases. But in the past there has been a strong opposition to research associated with embryonic stem cell therapy. The genesis of this was the ban put by President George W Bush on the federal funding of embryology stem cell lines developed after 2001. This ban still remains in public memory with the result that subsequent clinical developments in the field have not received the recognition these should have. In any case, this ban was subsequently lifted by President Barak Obama. Cellular therapy consists of various other types of cells which are not of embryonic origin. The ethical dilemmas associated with embryonic stem cells need not be applied to the cells of non embryonic origin like umbilical cord stromal cells and adult stromal cells. The medical community, patients and regulatory authorities need to make this distinction and the objections for the use of one type of cells should not be applied to the other types.

Another facet of the ethical considerations is using cellular therapy as a form of treatment. Clinical practitioners in the field of cellular therapy face a moral and an ethical dilemma every single day of whether it is ethical to offer a treatment that has not yet been approved as a standard of care for that disease? But if they deny a safe and available treatment to the patients who have incurable diseases, just because their efficacy has not been established by the modern medical standards

and wait for it to be established while the patients wither away, then Is that ethical? The basis of this dilemma lies in the conflict of the two fundamental principles of bio ethics, Beneficence and Non-maleficence. On one hand one is expected to always benefit the patient on the other one may not offer the treatments to the patients unless those are approved as per the modern medical standards. By not offering such treatments one is actually violating the principle of non-maleficence through omission. EBM in modern medicine has become synonymous with ethical medical practice. But the unique challenges put forth by evolution of cellular therapies demands us to rethink about this. Cellular therapies are far more dynamic and diverse than drug therapies and therefore it may take decades or more to generate empirical evidence validating use of cellular therapy as a treatment form. The generation of such evidence will also be dependent on various socio-politico-economic variables and not on the need for medical innovation alone. Is it fair then, that on one hand we claim to protect patients from adverse effects and ineffectiveness of the therapy using EBM whereas on the other hand we let them die or suffer waiting for the treatment? It is time to revisit the concept of 'Practice Based Evidence' (PBE) and not rely solely on 'Evidence Based Medicine'. Whereas EBM may perhaps jeopardize the autonomy of the patients in choosing an unproven treatment for the lack of any other proven treatment, PBE protects such autonomy. PBE respects the evidence generated from a single practitioner whereas EBM will disregard it as the lowest form of the evidence. But this lowest form of the evidence is what has given medicine its most brilliant inventions. Medical field progressed when the individual clinical practitioners pioneered newer forms of therapy based on their clinical experience and expertise. Most of the surgical practice comes from individual surgical expertise and cannot be tested using multicenter randomized controlled trials. Day to day decisions made in an intensive care setups and operating rooms are primarily influenced by the clinical circumstances at hand and the clinicians own experiences in dealing with such situations. Not everything in the medicine can be measured on the yardsticks of EBM; neither can it all be tested using EBM. Further the question remains when can a particular form of treatment be considered to have conclusive evidence? How many clinical trials will it take for us to get that evidence? The more daunting question is whether we have the funds, the resources and the time to conduct multitude of trials. Should we accept to slow the pace of medical advancements for lack of funds and resources for formal clinical trials? PBE could therefore be a key to faster progress in medicine. Both the paradigms of evidence based medicine and practice based evidence need to co- exist. The science of cellular therapies provides a unique opportunity for this co- existence. This does not mean that the treatments should not be tested scientifically. Novel treatments must be very stringently monitored for its safety. However once the safety has been established, the use of such treatments may be permitted in case of the diseases where there are no other treatments available. Not only these treatments should be permitted but also more and more number

of practitioners should be involved in providing these treatments so that a large body of evidence can be generated. Greater availability would also make such treatments easily available and less expensive. Rather than relying on the corporates to generate evidence, individual practitioners and institutions should also be empowered and entrusted.

An ethical dilemma that often confronts practitioners of cellular therapy is whether it is appropriate to charge for treatments that are yet unproven. The answers to questions like this are complex however in general it could be said that what is important is whether a treatment method should or should not be offered to a patient. If it is ethical to offer a treatment to a patient that will benefit the patient then it there should not be any ethical issues about charging for the same.

Ethical Basis Of Stem Cell Therapy:

Ethical principles like autonomy, beneficence, non-maleficence and justice are expected to be adhered to in the medical research and treatment according to the modern regulatory practices. The primary intention of these is to safeguard the patients who are the consumers of medical services from any risk and exploitation during research and therapy. The evolution of cellular therapy as a new form of treatment has opened up a new debate about ethics of the stem cell practice.

American society of gene and cell therapy defines cell therapy as the, '*Administration of live whole cells or maturation of a specific cell population in a patient for the treatment of a disease by American society of gene and cell therapy.*' The primary aim of cellular therapy is to repair the damaged tissue by replacement or regeneration of new cells. Therefore cellular therapy is different from the drug therapies where a single molecule is investigated for a putative beneficial effect.

When considering whether stem cells can be offered as a form of treatment or not, there are two extreme views. There are those that strongly oppose the offering of cellular therapy as a treatment form till there is definitive evidence of its effectiveness. On the other hand there are the practitioners of these treatments who believe that patients suffering from many of the untreatable conditions should not be denied these treatments just because they are still unproven. Both sides are correct in their own ways and both these views are like two sides of a coin. The existence of one does not negate the other. Together they make a whole. It is therefore time to relook at the ethics, regulations and principles of evidence based medicine in a new light in connection with cellular therapy.

The ethical basis of offering stem cell therapy as a treatment option is based on the Paragraph no. 32, World Medical Association Declaration of Helsinki- Ethical Principles for Medical Research Involving Human Subject. It states that "In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent

from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing 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 patient's point of view respecting the fact that even small functional improvements can mean a lot to a particular patient.
3. That there is an ethical ground for offering stem cell therapy as a treatment option based on the Helsinki declaration.
4. That there is enough published clinical evidence about the safety and efficacy of adult stem cells in neurological disorders and based on this evidence there is no need to keep on doing trials.

To elaborate on the above points:

That there is a need to make a clear cut distinction between embryonic stem cells and adult stem cells whilst strict regulations for embryonic stem cell work are completely justified the same are not needed for adult stem cell work:-

It is clear from all the above that the entire ethical debate regarding stem cell therapy revolves around the use of embryonic stem cell and cloning. There are no ethical issues with the use of autologous stem cells derived from bone marrow. Yet there are various restrictions in place for the use of any types of stem cells in different countries. Until everyone concerned starts looking at stem cells of non-embryonic origin differently from embryonic stem cells we will continue to

involved in debating the issue and the price for these delays are paid for by the patients for no fault of theirs. Herein lies the tragedy. There is available a form of cellular replacement therapy that can give relief to millions of patients, for which there is enough published clinical evidence of safety and a satisfactory published evidence of efficacy yet this treatment cannot be freely used by one and all. It is

our belief that by letting patient suffer and at time side when there are treatment option with stem cells that could possibly benefit them is unethical. That there is a need to look at the whole issue from the patients point of view respecting the

fact that even small functional improvements can mean a lot to a particular patient: We tend to judge improvements from normal peoples point of view. We don't realize that even small improvements, seemingly unimportant to us, can make a quantum difference in the lives of patients paralyzed with neurological problems. The Beijing Declaration of the International Association of Neurorestoratology (IANR) says it "recognizes the importance of small functional gains that have significant effects on quality of life". We need to stop being arm chair professors and talking only about evidence based medicine. We have to look at this from the point of view of the patients. To highlight this we highlight a case which shows us how improvements that may mean nothing to us can mean the world to suffering patients. This was one of the first cases of multiple sclerosis treated with stem cells. Patient had a lot of improvements including significant improvements in her speech, ability to use her hand to hold a cup and her mobility, ability to sit without support, ability to stand with support. All of these were not possible before the stem cell therapy treatment. Yet the improvement that mattered to her more than all of these was something very small. Earlier when lying in the prone position she could not turn in bed by herself. After the stem cell therapy she could do so. Prior to the treatment every night she would have to wake up her grandmother 3-4 times a night to help her turn her position in bed. This used to upset the patient since it used to emotionally hurt and pain her that she had to wake up her grandmother multiple times in the night just to turn her. And she needed to turn since sleeping in one position would make her very uncomfortable. So despite all her other improvements with her speech and hands what made her most happy and the improvements that mattered to her the most was after the treatment she could turn in bed by herself and did not have to wake up her grandmother every night. This has been highlighted just to make one very simple point. That we must look at this entire issue from the patients point of view. We must recognize that small improvements that do not mean anything to us can mean a lot to a patient with severe physical limitations. That at the end of the day all ethics, moral, values principles, laws and regulations have just one purpose. The well being of the common man.

What has unfortunately happened in the field of stem cell therapy is that the regulations we have made to protect ourselves are now limiting us and tying us up. These regulatory chains need to be unshackled. Physicians need to be free to use whatever modality of treatment they believe is in the patients best interests. However the other side of the argument is that these are helpless patients and they are likely to be exploited by physicians offering stem cell therapy. We must however note that there are black sheep in every profession. That those who don't have values and principles are doing all manner of unprincipled and unethical practices.

es with conventional treatments also. On the other hand there are researchers who have been working in this field for many years both in the laboratory as well as clinically. They should be permitted to offer treatments they believe are safe and will benefit patients. Unless more physicians offer

these treatments there will always be a supply demand gap with the result that fly by night operators will enter the field to make money. Therefore freeing up the field will bring more transparency and accountability to this aspect of medical treatment.

That there is an ethical ground for offering stem cell therapy as a treatment option based on the Helsinki declaration:-

The Helsinki Declaration that has been discussed earlier in this chapter makes one thing very clear that for diseases for which there are no cures or the cures have been ineffective the physician is justified in using an unproven treatment if the physician believes that it will benefit the patient. This is the ethical bedrock on which we offer stem cell therapy as a form of treatment for neurological disorders for which there are no other treatments.

That there is enough published clinical evidence about the safety and efficacy of adult stem cells in neurological disorders and based on this evidence there is no need to keep on doing trials. In the section on clinical aspects we have mentioned in this book numerous studies that have clearly shown the safety and efficacy of adult stem cells in various neurological disorders. A question that remains unanswered is when does a treatment that is "unproven or experimental" become a treatment that is "proven or established". How many publications documenting safety and efficacy will it take to make that shift? Is a single publication enough, or are 10, 50 or 1000, or are multicentric international trials the only basis to make any treatment option an accepted form of treatment. Is it necessary to go on reinventing the wheel just to satisfy our intellectual considerations whilst millions of patients continue to suffer?

Ethical issues surrounding the stem cell research and practice are complicated. There is growing published evidence about safety and efficacy of stem cell therapy explains the ethics of offering it to patients with incurable disorders who otherwise have no other option than to endure the suffering.

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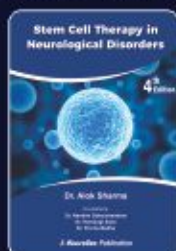


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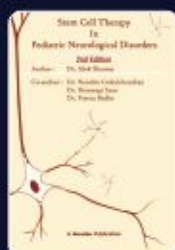
A separate pediatric neurorehabilitation facility and other play areas makes it very child friendly. The institute is very scientific and academic in its approach and to date has published 112+ scientific papers in international and national journals. 18+ books have also been published and chapters contributed to several international textbooks. NeuroGen also has many international tie ups with leading organizations from America and other countries for research and treatment collaborations. The institute is very quality conscious and has several certifications (1. ISO 9001:2015, 2. GLP & 3. GMP certification). Despite all the international partnerships and treatments offered to patients from all over the world the institute is very socially conscious and through the Stemcare foundation financially supports patients from the lower socioeconomic strata to be able to avail of the treatments that are needed. It's a policy of the institute that no patient should be deprived of any treatment due to financial reasons. NeuroGen doctors conduct free medical camps all over the country. Conferences, workshops and CME's are regularly conducted to impart knowledge to doctors, therapists as well as patient families. Cutting edge research, pioneering new treatments, the best medical professionals, comprehensive treatment facilities all under one roof and a caring holistic approach and make the NeuroGen Brain and Spine institute a unique and special facility for patients with Neurological problems.

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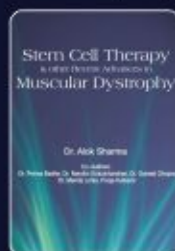
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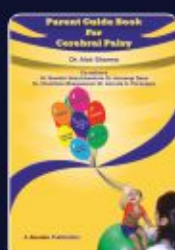
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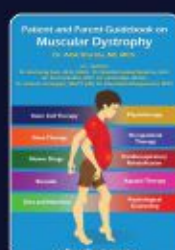
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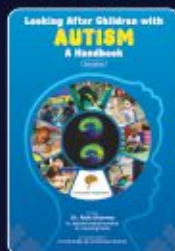
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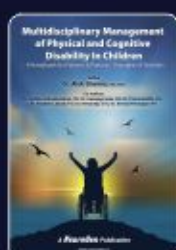
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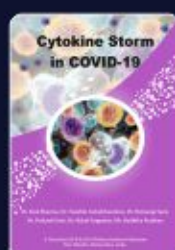
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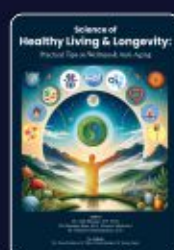
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