Stem Cell Therapy in Neurological Disorders

2nd Edition

Dr. Alok Sharma

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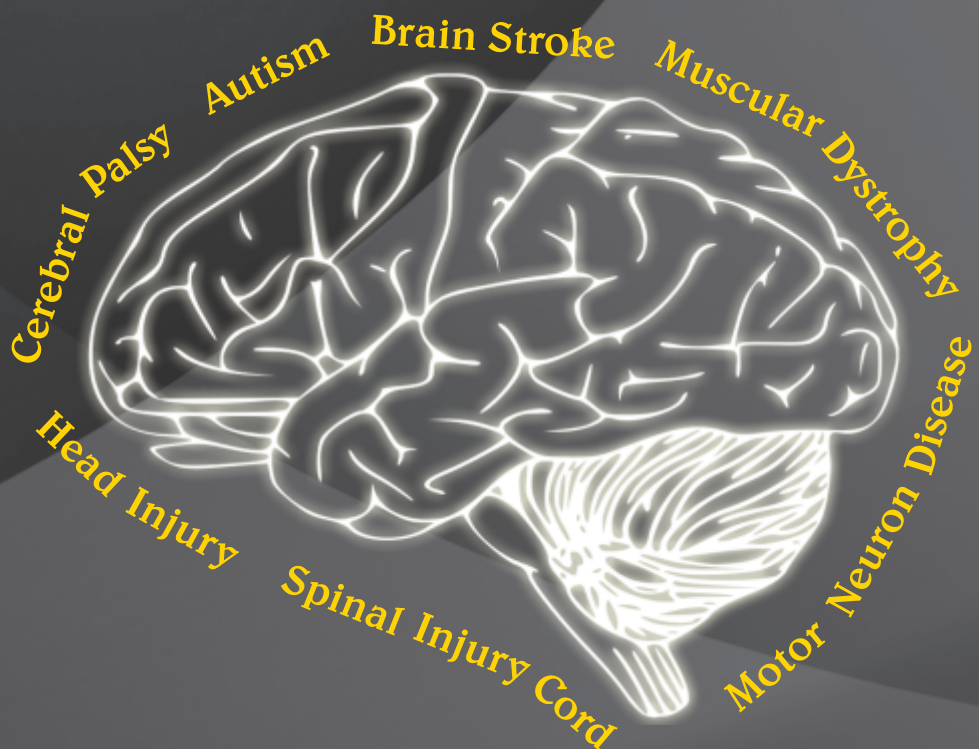
Dr. Nandini Gokulchandran | Dr. Hemangi Sane | Dr. Prerna Badhe



Stem Cell Therapy
In Neurological Disorders

Second Edition

Stem Cell Therapy In Neurological Disorders

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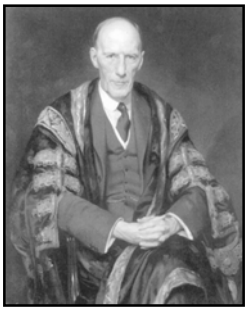
This Book is Dedicated to all our Patients



A Prayer

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

– Sir Robert Hutchison

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

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

– George Bernard Shaw

PREFACE

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

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

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

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

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

Nowhere is this more applicable than in the field of stem cell therapy. Despite the above, caution needs to be exercised in the practise of this therapy since neither the enthusiasm of the medical practitioner, nor the pressure from the patient community and emotional aspects of suffering are enough reasons to overlook the safety aspects of any new medical therapy. However, once safety is established it would further the cause of medicine as a whole, as well as the well being of the patient community, if more practitioners participated in these treatments. This would not only make more data available regarding safety and efficacy, but also by balancing out the supply-demand imbalance, make such treatments more available and affordable. There is a very thin line that separates "helping someone" and "taking advantage of someone's helplessness". It is important that we never cross this line.

There are two sides to the ethical debate on basing our treatment options on evidence based medicine. [1] One side of the debate is " Is it ethical for doctors to offer to patients treatment options that have not become a standard of care as yet?." [2] The other side of the debate is "Is it ethical to deny patients suffering from disabling diseases, treatments options that are safe and available, whilst we wait many years for the results of multicentric international trial to prove that these treatments work ?" Both these questions are answered differently by different people depending on what is at stake for them.

Another question that remains unanswered is when does a treatment that is "unproven or experimental" become a treatment that is "proven or established". How many publications documenting safety and efficacy will it take to make that shift ? Is a single publication enough, or are 10, 50 or 100 ok, or are multicentric international trials the only basis to make any treatment option an excepted form of treatment. Is it necessary to go on reinventing the wheel just to satisfy our intellectual considerations whilst millions of patients continue to suffer? Our own belief is, that based on the already published work and our own clinical experience, this form of treatment is no more experimental since the safety and efficacy of stem cell treatment in many of the neurological disorders has been established and documented in several published articles from several countries. However getting a consensus on these issues is not easy.

The role of regulatory bodies in this field also needs to be relooked. Whereas there is no denying the importance of regulation in all aspects of medical care and research,

it is also important for the regulatory bodies all over the world to ensure that regulations do not hinder or slow down the evolution of newer forms of treatment. They also need to realize that in this field that is evolving at a breathtaking speed, regulations made several years ago may no longer be valid in the present. That the regulations need to be modified as more evidence pours in from all over the world. That the regulations need to adapt and evolve as the research and clinical results are evolving. That individual doctors, medical institutions and medical associations need to trusted and given the responsibility to both develop and implement these newer forms of therapy as well as monitor and prevent its misuse.

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

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

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

Dr. Alok Sharma

Preface to the Second Edition

" Two sides of the Coin"

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

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

To look at the other side we believe that regulatory bodies need to make the following distinctions in creating future guidelines. To explain this we quote from the International Society for Cellular Therapy (ICST) "White paper" published in 2010 in Cytotherapy

[1] Distinction between Experimental therapies and medical innovation:- The White paper states:- "It is important to recognize the difference between clinical trials of experimental treatments and medical invocation. Medical innovation in cellular therapy may be viewed as ethical and legitimate use of non-approved cell therapy by qualified healthcare professionals in their practice of medicine . Patients not eligible for controlled clinical trials should be able to choose unproven but scientifically validated cell therapy medical innovations, if the researchers are competent and those seeking treatment are truthfully and ethically informed. There is a place for both paradigms in the cell therapy

global community." We wish to emphasize this last sentence that - there is place for both paradigms in the cell therapy global community

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

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

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

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

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

Dr. Alok Sharma

Primum non nocere

(First do no harm)

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

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

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

Contents

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

1. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Anjana Nagrajan, Amruta Paranjape, Pooja Kulkarni, Akshata Shetty, Priti Mishra, Mrudula Kali, Hema Biju, Prerna Badhe. Autologous bone marrow mononuclear cell therapy for autism - an open label proof of concept study. Stem cell international. 2013 (2013), Article ID 623875, 13 pages.
2. Alok Sharma, Nandini Gokulchandran, Guneet Chopra, Pooja Kulkarni, Mamta Lohia, Prerna Badhe, V.C.Jacob. Administration of autologous bone marrow derived mononuclear cells in children with incurable neurological disorders and injury is safe and improves their quality of life. Cell Transplantation, 2012; 21 Supp 1: S1-S12.
3. Alok Sharma, Hemangi Sane, Prerna Badhe, Nandini Gokulchandran, Pooja Kulkarni, Mamta Lohiya, Hema Biju, V.C.Jacob. A Clinical Study Shows Safety and Efficacy of Autologous Bone Marrow Mononuclear Cell Therapy to Improve Quality Of Life in Muscular Dystrophy Patients. Cell Transplantation. 2013; Vol. 22, Supplement 1, pp. S139-S146.
4. Sharma A, Gokulchandran N, Sane H, Badhe P, Kulkarni P, Lohia M, Nagrajan A, Thomas N. Detailed analysis of the clinical effects of cell therapy for thoracolumbar spinal cord injury: an original study. Journal of Neurorestoratology. 2013;1:13-22
5. Sharma A, Sane H, Gokulchandran N, Kulkarni P, Thomas N, et al. (2013) Role of Autologous Bone Marrow Mononuclear Cells in Chronic Cervical Spinal Cord Injury-A Longterm Follow Up Study. J Neurol Disord 1: 138.
6. A. Sharma, P. Badhe, N. Gokulchandran, P. Kulkarni, V.C Jacob, M. Lohia, J. George Joseph, H. Biju, G. Chopra. Administration of Autologous bone marrow stem cells intrathecally in Multiple Sclerosis patients is safe and improves their quality of life. Indian Journal of clinical Practice. 2011:21(11):622-625
7. Alok Sharma, Prerna Badhe, Pooja Kulkarni, Nandini Gokulchandran, Guneet Chopra, Mamta Lohia, V.C.Jacob. Autologous Bone marrow Derived mononuclear cells for the treatment of Spinal Cord Injury. The Journal of Orthopaedics. 2011; 1(1): 33-36
8. Alok Sharma, Guneet Chopra, Nandini Gokulchandran, Mamta Lohia, Pooja Kulkarni. Autologous Bone Derived Mononuclear Transplantation in Rett Syndrome. Asian Journal of Paediatric Practice. 2011; 15 (1): 22-24
9. Alok Sharma, Prerna Badhe, Omshree Shetty, Pooja Vijaygopal, Nandini Gokulchandran, V.C. Jacob, Mamta Lohia, Hema Biju, Guneet Chopra. Autologous bone marrow derived stem cells for motor neuron disease with

anterior horn cell involvement. Bombay hospital journal. 2011; 53(1): 71- 75

10. Sharma A, Gokulchandran N, Kulkarni P, Chopra G. Application of autologous bone marrow stem cells in giant axonal neuropathy. Indian J Med Sci 2010; 64:41-4
11. Dr. A. Sharma, Ms. P. Kulkarni, Dr. G. Chopra, Dr. N. Gokulchandran, Dr. M. Lohia, Dr. P. Badhe. Autologous Bone Marrow Derived Mononuclear Cell Transplantation In Duchenne Muscular Dystrophy-A Case Report. Indian journal of Clinical Practice 2012; 23 (3): 169-72
12. Dr. Alok Sharma, Ms. Pooja Kulkarni, Dr. Hemangi Sane, Dr. Nandini Gokulchandran, Dr. Prerna Badhe, Dr. Mamta Lohia, Dr. Priti Mishra. Positron Emission Tomography- Computed Tomography scan captures the effects of cellular therapy in a case of cerebral palsy. Journal of clinical case reports. 2012 J Clin Case Rep 2:195. doi:10.4172/2165-7920.1000195
13. Dr. Suvarna Badhe, Ms. Pooja Kulkarni, Dr Guneet Chopra, Dr Nandini Gokulchandran, Dr Alok Sharma Dystrophin Deletion mutation pattern and Cardiac involvement in 46 cases of Dystrophinopathies. Asian journal of clinical cardiology. Asian Journal of Clinical Cardiology, Vol. 15, No. 6, October 2012: 211-214
14. Dr. Alok Sharma, Dr. Hemangi Sane, Dr. Prerna Badhe, Ms. Pooja Kulkarni, Dr. Guneet Chopra, Dr. Mamta Lohia, Dr. Nandini Gokulchandran. Autologous Bone Marrow Stem Cell Therapy shows functional improvement in hemorrhagic stroke- a case study. Indian Journal of Clinical Practice, 2012:23(2):100-105
15. Alok Sharma, Prerna Badhe, Nandini Gokulchandran, Pooja Kulkarni, Hemangi Sane, Mamta Lohia, Vineet Avhad. Autologous bone marrow derived mononuclear cell therapy for vascular dementia - Case report. Journal of stem cell research and therapy. J Stem Cell Res Ther 2:129. doi:10.4172/2157-7633.1000129
16. Alok Sharma, Hemangi Sane, Amruta Paranjape, Nandini Gokulchandran, Pooja Kulkarni and Anjana Nagrajan, Prerna Badhe. Positron Emission Tomography - Computer Tomography scan used as a monitoring tool following cellular therapy in Cerebral Palsy and Mental Retardation - A Case Report. Case Reports in Neurological Medicine. Volume 2013, Article ID 141983, 6 pages
17. Alok Sharma, Nandini Gokulchandran, Prerna Badhe, Pooja Kulkarni, Priti Mishra, Akshata Shetty and Hemangi Sane. An Improved Case of Autism as Revealed by PET CT Scan in Patient Transplanted with Autologous Bone Marrow Derived Mononuclear Cells. J Stem Cell Res Ther 2013, 3:2
18. Alok Sharma, Nandini Gokulchandran, Akshata Shetty, Hemangi Sane, Pooja Kulkarni and Prerna Badhe. Autologous Bone Marrow Mononuclear

Cells may be Explored as a Novel. Potential Therapeutic Option for Autism. J Clin Case Rep 2013, 3:7

19. Alok Sharma, Amruta Paranjape, Hemangi Sane, Khushboo Bhagawanani, Nandini Gokulchandran, and Prerna Badhe. Cellular Transplantation Alters the Disease Progression in Becker's Muscular Dystrophy. Case Reports in Transplantation. Volume 2013, Article ID 909328, 7 pages
20. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Pooja Kulkarni, Nancy Thomas, Amruta Paranjape, Prerna Badhe. Intrathecal autologous bone marrow mononuclear cell transplantation in a case of adult autism. Autism open access. 2013, 3:2
21. A Sharma, P Kulkarni, N Gokulchandran, P Badhe, VC Jacob, M Lohia, J George Joseph, H Biju, G Chopra. Adult Stem Cells for Spinal Muscular Atrophy. Bangladesh Journal Of Neuroscience. 2009; 25(2): 104-107
22. Alok Sharma, Hemangi Sane, Anjana Nagrajan, et al., "Autologous Bone Marrow Mononuclear Cells in Ischemic Cerebrovascular Accident Paves Way for Neurorestoration: A Case Report," Case Reports in Medicine, vol. 2014, Article ID 530239, 5 pages, 2014. doi:10.1155/2014/530239
23. Sharma A., Sane, H., Paranjape, A., Badhe, P., Gokulchandran, N., & Jacob, V. (2013). Effect of Cellular Therapy seen on Musculoskeletal Magnetic Resonance Imaging in a Case of Becker's Muscular Dystrophy.Journal of Case Reports, 3(2), 440-447.
24. Alok Sharma, Hemangi Sane, Amruta Paranjape, Khushboo Bhagwanani, Nandini Gokulchandran, Prerna Badhe. Autologous bone marrow mononuclear cell transplantation in Duchenne muscular dystrophy - a case report. American journal of case reports (Ahead of Print)
25. Alok Sharma, Hemangi Sane, Dipti Khopkar, Nandini Gokulchandran, Hema Biju, V C Jacob, Prerna Badhe 'Cellular therapy targeting Functional outcome in a case of Cervical Spinal Cord Injury'Advances in Stem Cells 2014 (In Press)
26. Alok Sharma, Hemangi Sane, Dipti Khopkar, Nandini Gokulchandran, V. C. Jacob, Joji Joseph, Prerna Badhe 'Functional recovery in chronic stage of spinal cord injury by Neurorestorative Approach' Case Reports in Surgery 2014 (In Press)
27. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Pradnya Bhovad, Hema Biju,Akshata Shetty, Mrudula Kali and Prerna Badhe, Cell therapy effects portrayed on positron emission tomography computerized tomography scan of the brain serve as a new dimension for autism: A case report. Journal of Paediatric Neurology, (2014) (In Press).

The following case series are under review: Cerebral Palsy, Traumatic Brain Injury, Stroke, Amyotrophic Lateral Sclerosis, Limb Girdle Muscular Dystrophy..

SECTION A

Basics and Technical Aspects

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

– Christopher Reeve

1

Introduction: Neurogeneration and Neurorestoratology

Regenerative medicine is an emerging field of modern medicine, focusing at restoration, repair and replacement of damaged tissues by a safe and effective administration of living cells in solitude or in combination with specially designed materials (1). This has opened up new avenues of therapeutic strategies for multiple disorders with no definitive treatment or cure available, such as neurological disorders (spinal cord injury, autism, cerebral palsy, brain stroke, muscular dystrophy, traumatic brain injury, motor neuron disease, etc.), diabetes, cardiovascular disorders, bone disorders, hematopoietic disorders, cancers, hepatic, renal and dermatological disorders.

One of the building blocks of this field is stem cells. Stem cells have the capability to multiply manifolds and convert or differentiate into any specialized cell types of the body. A variety of stem cells are being used from diverse sources for regeneration. The potency and plasticity of stem cells depends on the source or origin. The embryonic stem cells are the most potent but associated with ethical issues and side effects of teratomas.

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

2006 was a year of breakthrough when Takahashi and Yamanaka demonstrated that it is possible to reprogram embryonic or adult mice skin cells by the use of Yamanakas factors, which can also be performed for human skin cells (2). Currently, efforts are being made towards the attempt of developing patient-specific induced

pluripotent stem cells which will be free from any alterations or genomic instability (3).

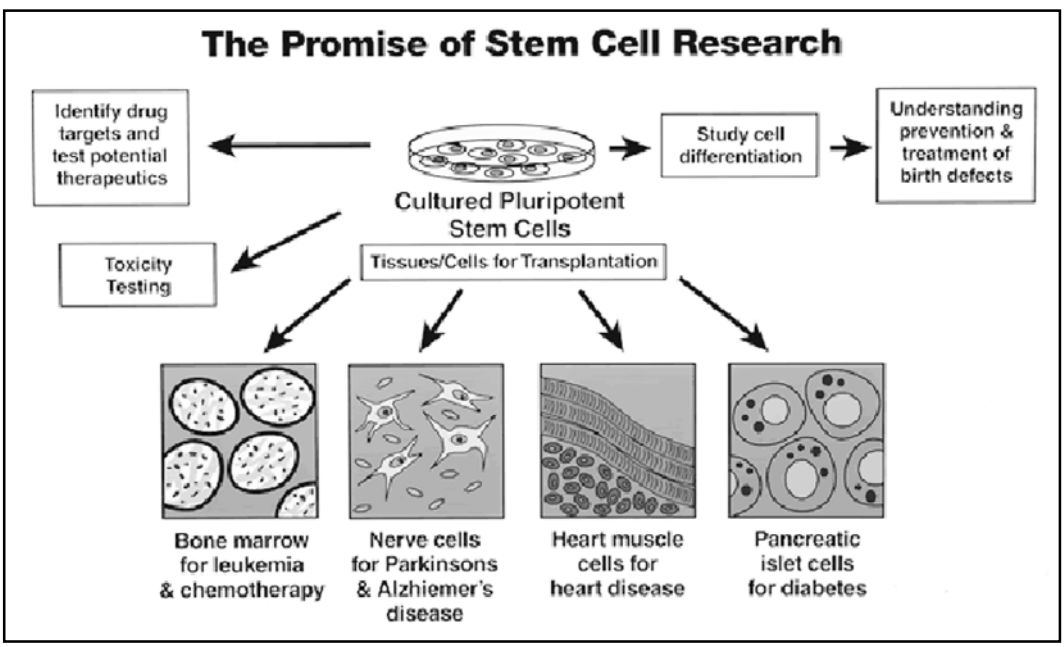
Neurorestoration, as defined by International Association of Neurorestoration, is the concept which forms the basis for increased optimism in the medical community. It is a novel branch of neuroscience which studies and discusses the therapeutic strategies for neural regeneration, repair and replacement of the damaged elements of the central nervous system. The resultant processes like neuroplasticity, neuroprotection, neuromodulation, angiogenesis, immunomodulation are the principal components whose mechanisms are discussed in great depth (4).

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

This book endeavors to assimilate all the current information on understanding stem cells, its potential and more specifically its role in treating incurable neurological and neuromuscular disorders.

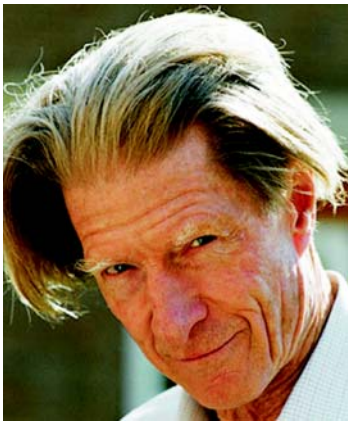
REFERENCES:

1. Langer, R. & Vacanti, J. P. 1993 Tissue engineering. Science 260, 920-926.
2. Takahashi, K. & Yamanaka, S. 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.
3. Park, I.-H., Zhao, R., West, J. A., Yabuuchi, A., Huo, H., Ince, T. A., Lerou, P. H., Lensch, M. W. & Daley, G. Q. 2008 Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451, 141-146.
4. The International Association of Neurorestoratology. Beijing Declaration of International Association of Neurorestoratology (IANR). Cell Transplant 2009;18(4):487.

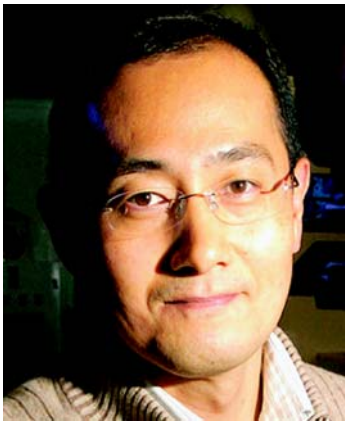


Nobel Prize Winners in Stem Cell Research

2012



John B. Gurdon

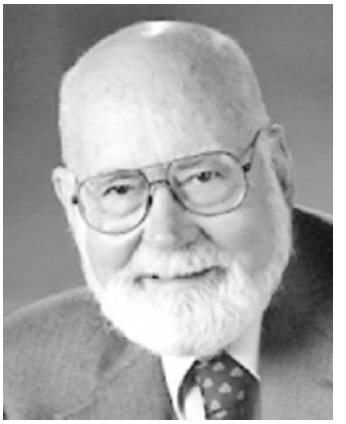


Shinya Yamanaka

2007

Sir Martin Evans

1990



Dr. E. Thomas

2

Historical Review : Evolution of Stem Cell Therapy

For centuries scientists have known that certain animals such as the starfish, newt, earthworm, various reptiles etc can regenerate missing parts of their bodies. Although humans cannot replace a missing finger or limb, we share some of the above abilities since our bodies are constantly regenerating blood, skin and other tissues. The identity of the powerful cells that allowed us to regenerate these tissues was first reveled when experiments with bone marrow in the 1950's established the existence of stem cells in our bodies. This led to the use of bone marrow transplantation as a therapy which is now commonly used in medical practice. This discovery raised the hope in the medical potential of regeneration as a possible treatment for a whole lot of diseases that were considered incurable. Now for the first time in human history it became possible to regenerate damaged tissue with a new supply of healthy cells by drawing upon the unique property of stem cells to create many of the bodies specialized cells. Once the medical potential of regeneration was recognized scientists turned to the embryo to identify similar cells since early human developmental studies had demonstrated that the cells of the embryo were capable of producing all the different types of calls in the body. In the 1980's scientists began to extract embryonic cells from mice however it was in 1998 that scientists first isolated human embryonic cells. The demonstration and use of stem cells in the bone marrow in the 1950's and the isolation of embryonic stem cells in mice could well be considered pivotal moments in medical history and so very appropriately both were recognized with the prestigious Nobel prizes. (Dr. E. Thomas in 1990 and Sir Martin Evans in 2007). In this Chapter we trace the history of stem cells from the early history almost a 100 years ago when the term was first coined to the modern developments 50 years ago with bone marrow transplantation to the recent development in the last 10 years when stem cells are being researched and used for treatment of many other diseases.

Introduction to the Concept of Stem Cells

The origins of stem cell research lie in a desire to understand how tissues are maintained in adult life, rather than how different cell types arise in the embryo. An interest in adult tissues fell, historically, within the realm of pathologists and thus tended to be considered in the context of disease, particularly cancer. It was appreciated long ago that within a given tissue there is cellular heterogeneity: in some tissues, such as the blood, skin and intestinal epithelium, the differentiated cells have a short lifespan and are unable to self-renew. This led to the concept that such tissues are maintained by stem cells, defined as cells with extensive renewal capacity and the ability to generate daughter cells that undergo further differentiation. Such cells generate only the differentiated lineages appropriate for the tissue in which they reside and are thus referred to as multipotent or unipotent.

Stem cells are defined as having the capacity to both self renew and give rise to differentiated cells. Given their proliferation and differentiation capacities, stem cells have great potential for the development of novel cell-based therapies. In addition, recent studies suggest that dysregulation of stem cell properties may be the cause of certain types of cancer. Due to these widespread basic and clinical implications, it is of interest to put modern stem cell research into historical context.

Stem Cell Timeline

1956	First successful bone marrow transplant
1981	Embryonic stem cells are isolated from mouse blastocysts
1988	Hematopoietic stem cells from adult mice are purified and characterized
1992	Stem cells are identified in the adult human brain
1998	The first human embryonic stem cells are isolated

Historical Review And Evolution of Stem Cell Therapy

Early history : Coining of the Term "Stem Cell"

"Stammzelle" and Germline Development

The term stem cell appears in the scientific literature as early as 1868 in the works of the eminent German biologist Ernst Haeckel. Haeckel, a major supporter of Darwin's theory of evolution, drew a number of phylogenetic trees to represent the evolution of organisms by descent from common ancestors and called these trees "Stammbäume" (German for family trees or "stem trees"). In this context, Haeckel used the term "Stammzelle" (German for stem cell) to describe the ancestor unicellular organism from which he presumed, all multicellular organisms evolved and therby, he also proposed

that the fertilized eggalso be called stem cell. Uses of the term stem cell referring to a distinct cell in the embryo capable of giving rise to more specialized cells can be found later in that century. (1)

As embryology evolved in the 19th century along with August Weismann's theory of the continuity of the germplasm (germ cells being different than somatic cells) became the focus of research and debate. Theodor Boveri while tracing the ascaris embryo concluded that the early germline cells maintained the full complement of chromatin so as to transmit the intact genetic material to the next generation, in support of Weissman's theory. In 1892, Boveri explicitly took Haeckel's definition of stem cell as the fertilized egg one step further and proposed that cells along the germline lineage between the fertilized egg and committed germ cells be called stem cells. (2, 3)

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

"Stammzelle" and Hematopoiesis

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

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

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

Modern history:

Hematopoietic Stem Cell Transplantation:

In the early 1900's, the first real stem cells were discovered when it was found that some cells generate blood cells. In the early 1900's physicians administered bone marrow by mouth to patients with anemia and leukemia. Although such therapy was unsuccessful, laboratory experiments eventually demonstrated that mice with defective marrow could be restored to health with infusions into the blood stream of marrow taken from other mice. This caused physicians to speculate whether it was feasible to transplant bone marrow from one human to another (allogenic transplant). Among early attempts to do this, were several transplants carried out in France following a radiation accident in the late 1950's.

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

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

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

Cord blood stem cells have been used in the treatment of blood cancers and/or blood diseases since 1988. That same year, Elaine Gluckman replaced allogenic cord

blood for a bone marrow transplant in order to treat Fanconi Anemia, a rare recessive blood disorder. The child remained completely disease free. In 2001, treatment protocols were developed which permitted the removal of white blood cells from the umbilical cord, making the treatment safe with no risk of Graft-Versus-Host disease.

Recent history

The discovery of embryonic stem cells opened up a new era in the use of stem cells. Basic and experimental work showing that these cells could be useful in the possible treatment of many incurable conditions resulted in researchers and clinicians now looking at stem cells in completely new way. However stem cell research got embroiled in a controversy over the use of human embryonic stem cells for research. This led to scientists and clinicians looking at other sources of stem cells such as from the umbilical cord or from the bone as alternative sources of stem cells.

Embryonic Stem Cells:

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

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

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

McDonald J W et al. in a seminal paper showed that transplanted neural differentiated mouse embryonic stem cells into a injured rat spinal cord after traumatic injury home onto the site and differentiate into astrocytes, oligodendrocytes and

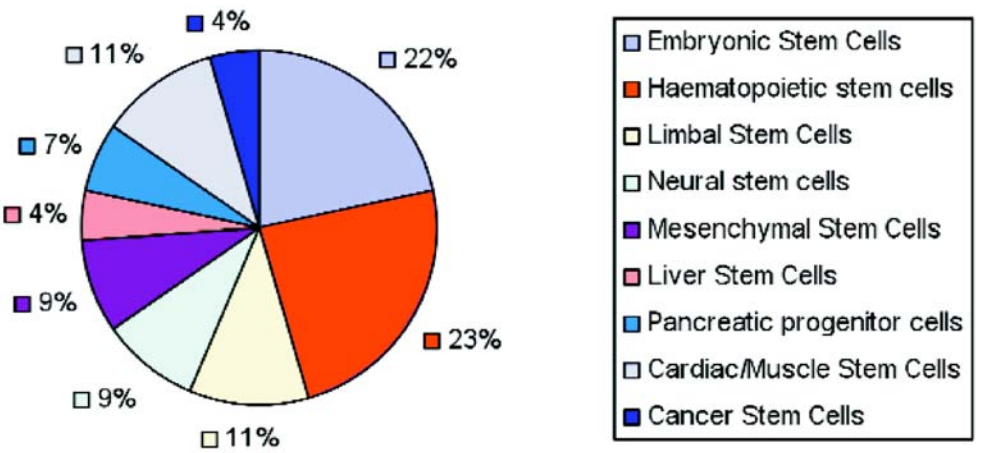
neurons, and migrated as far as 8 mm away from the lesion edge. (13) This lead to an explosion of new thoughts and avenues for research into possible application of this newfound development, especially into treatment of spinal cord injury and other neurological disorders and papers.

However, thereafter, the course of embryonic stem cell research has been greatly influenced by the political decision of President George W. Bush on August 9, 2001. President George W. Bush announced his decision to allow Federal funding of research only on existing human embryonic stem cell lines created prior to his announcement, putting a virtual halt on any further derivation of human stem cell lines and research. This ruling has lead to a setback of almost a decade in the field of stem cell research and therapy. Hence, is construed to be a historical decision in the field of regenerative medicine. Following this landmark, stem cell research in the US and UK slowed down considerably. President B. Obama in 2009 reversed this decision, clearing the way again for the stem cell research to progress again in the US.

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

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

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



Technology and the Ministry of Health of China.

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

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

Major public and private research institution engaged in stem cell research in India

State	City	Institute
Delhi	New Delhi	National Institute of Immunology (NII), All India Institute of Medical Sciences (AIIMS), National Brain Research Centre (NBRC), Institute of Nuclear Medicine and Allied Sciences (INMAS), RR Hospital
Maharashtra	Mumbai, Pune	Tata Institute of Fundamental Research (TIFR), Indian Institute of Technology (IIT), National Institute for Research in Reproduction and Health (NIRRH), King Edward Memorial Hospital (KEM), Lokmanya Tilak Municipal General Hospital (LTMGH), NeuroGen Brain and Spine Institute (NGBSI), National Centre for Cell Sciences (NCCS), Armed Force Medical College (AFMC)
Karnataka	Bengaluru	Indian Institute of Science (IISc), National Centre for Biological Science (NCBS), Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), National Institute of Mental Health & Neurosciences (NIMHANS), Manipal Institute of Regenerative Medicine (MIRM), ANSA Research Foundation (ARF)
Andhra Pradesh	Hyderabad	Centre for Cellular and Molecular Biology (CCMB) LV Prasad Eye Research Institute (LVPERI)
Kerala	Trivandrum	Sree Chitra Tirunal Institute of Medical Science & Technology (SCTIMST), Rajiv Gandhi Centre for Biotechnology (RGCB)
Uttar Pradesh	Lucknow	Central Drug Research Institute (CDRI), Sanjay Gandhi Post Graduate Institute for Medical Education, Indian Toxicology Research Institute
Punjab	Chandigarh	Post Graduate Institute of Medical Education & Research (PGI)
West Bengal	Kolkata	Indian Institute of Chemical Biology (IICB), Bose Institute
Tamil Nadu	Vellore, Chennai	Christian Medical College (CMC), Shankar Netralaya

Major Stem Cell Companies in India

Company	City	Major Focus
NeuroGen Brain and Spine Institute	Mumbai	Hematopoietic stem cells
Advanced Neuroscience Allies Pvt. Ltd. (ANSA)	Bengaluru	Mesenchymal Stem Cells and tumor tissue repository
Stempeutics Research Pvt. Ltd.	Bengaluru	Embryonic and Adult Stem Cell
International Stem Cell Services Ltd.	Bengaluru	Cord Blood
Reliance Life Sciences	Mumbai	Cord blood, Adult and Embryonic stem cells
NCRM	Chennai	Hematopoietic stem cells
Beike Biotech	Delhi	Adult stern cells
Life Cell	Chennai	Cord blood
Stem One	Pune	Cord blood
Cryobank International Incl ia	Kolkata	Cord blood
Stemtherapeutics	Ahmedabad	Cord blood

in the ongoing years.

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

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

To date, more than seventy (70) programs have been identified and supported in various aspects of stem cell research, which broadly encompass basic research on embryonic & adult stem cells as well as translational research and product development for therapeutic use.

There are more than thirty public and private research institutions that are currently engaged in both basic and translational research as well as therapy on stem cells and India. The majority of them are focusing on cord blood stem cell banking. Two companies are involved in embryonic stem cell research and rest are working in adult

stem cell research. A substantial amount of research is being done in the areas of embryonic stem cells (23%) and hematopoietic stem cells (24%). Cardiac/muscle stem cell and limbal stem cell research is about 11%, followed by mesenchymal stem cell and neural stem cell research (9%). The remaining research areas are in liver stem cells, pancreatic progenitor cells and cancer stem cells. Currently in India, five human embryonic stem cell (hESC) lines have been isolated and characterized. All five hESC lines are deposited at the National Centre for Cell Science (NCCS) in Pune, India. Two of these hESC lines are also deposited in the UK Stem Cell Bank.

An increasing numbers of publications on stem cell research and therapy (from 2003 till 2010) along with increasing private companies, non-profit organizations and government funded hospitals and institutes participation in this field (mainly focused on adult stem cells, mesenchymal stem cells and cord blood banking) shows the shifting of the stem cell hub to the Indian subcontinent.(15)

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

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

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

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

As the field evolved, with ethical issues being raised regarding the morality of embryonic stem cells source, researchers began to explore other sources of pluripotent stem cells. The potency of other adult stem cells, especially hematopoietic stem cells began to be understood. In 2002, Catherine Verfaillie at the University of Minnesota proved that CD34+ stem cells from bone marrow could repopulate every single cell in a developing mouse. This study prompted more studies using adult stem cells to generate far more than just blood cells. It was proven that there are great potentials for adult stem cells to treat a wide range of blood diseases, cancers, degenerative diseases, and injuries.

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

stem cells:

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

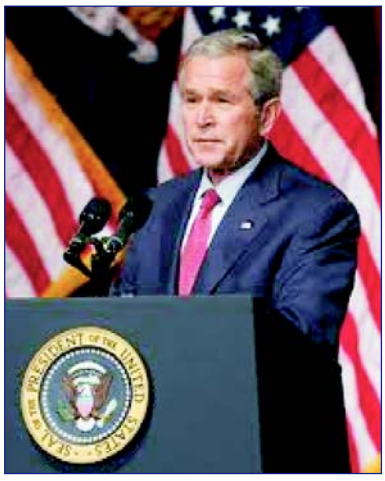
Recently, that is in January 2008 researchers were able to develop the human embryonic stem cells without destroying the embryo.

The field of stem cell research and therapy, thereby, has evolved and come a long way since 1868, when the term "stem cells" was coined. We are now looking toward using various different kinds of stem cells for treating incurable disorders of organs other than hematopoietic, such as, the brain, muscles, liver, heart, etc. Much more can be expected in the years to come by.

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

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

August 09, 2001



(Source: White House Press Office)

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

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

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

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

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

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

One biotech company has already begun producing embryonic human clones for research purposes. Chinese scientists have derived stem cells from cloned embryos created by combining human DNA and rabbit eggs. Others have announced plans to produce cloned children, despite the fact that laboratory cloning of animals has lead to spontaneous abortions and terrible, terrible abnormalities.

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

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

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

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

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

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

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

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

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

I support increasing the research budget of the NIH, and I ask Congress to join me in that support. And at the same time, I strongly support a comprehensive law against all human

cloning. And I endorse the bill -- wholeheartedly endorse the bill -- sponsored by Senator Brownback and Senator Mary Landrieu.

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

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

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

Thank you all for coming. God bless."

President Obama Speech on Stem Cell Policy Change

March 9, 2009

(Source: White House Press Office)

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

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

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

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

and will to pursue this research - and the humanity and conscience to do so responsibly.

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

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

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

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

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

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

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

That is why today, I am also signing a Presidential Memorandum directing the head of the White House Office of Science and Technology Policy to develop a strategy for restoring scientific integrity to government decision making. To ensure that in this new Administration, we base our public policies on the soundest science; that we appoint scientific advisors based on their credentials and experience, not their politics or ideology; and that we are open and honest with the American people about the science behind our decisions. That is how we will harness the power of science to achieve our goals - to preserve our environment and protect our national security; to create the jobs of the future, and live longer, healthier lives.

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

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

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

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

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

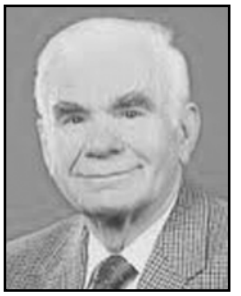
Today, using every resource at our disposal, with renewed determination to lead the world in the discoveries of this new century, we rededicate ourselves to this work.

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

REFERENCES

1. Ramalho-Santos M, Willenbring H. On the origin of the term "stem cell". Cell Stem Cell. 2007; 1(1):35-8.
2. Boveri, T. Befruchtung. In Ergebnisse der Anatomie und Entwicklungsgeschichte, F.S. Merkel and R. Bonnet, eds. (Wiesbaden: Joseph Friedrich Bergmann), 386-485.
3. Boveri, T. Sitzungsber. d. Gesellschaft f. Morphologie und Physiologie 8, 114-225.
4. Wilson, E.B. The Cell in Development and Inheritance (NewYork: Macmillan). 1896.
5. Maienschein, J. Whose View of Life?: Embryos, Cloning, and Stem Cells (Cambridge, MA: Harvard University Press). 2003
6. Becker, A.J., Mc, C.E., and Till, J.E. Nature 1963; 197, 452-454.
7. Till, J.E. and McCulloch, E.A. Radiat. Res. 1961; 14, 213-222.
8. Till, J.E. and McCulloch, E.A. Biochim. Biophys. Acta. 1980; 605, 431-459.
9. Till, J.E., McCulloch, E.A., and Siminovitch, L. Proc. Natl. Acad. Sci. USA 1964; 51, 29-36.
10. Martin GR (1980). "Teratocarcinomas and mammalian embryogenesis". Science 209 (4458): 768-76. Evans M, Kaufman M (1981). "Establishment in culture of pluripotent cells from mouse embryos". Nature 292 (5819): 154-6.
11. Martin G (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells". Proc Natl Acad Sci USA 78 (12): 7634-8.
12. Thomson J, Itskovitz-Eldor J, Shapiro S, Waknitz M, Swiergiel J, Marshall V, Jones J (1998). "Embryonic stem cell lines derived from human blastocysts". Science 282 (5391): 1145-7.
13. John W. McDonald, Xiao-Zhong Liu, Yun Qu et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nature Medicine 5, 1410 - 1412 (1999)
14. Lianming Liao, Lingsong Li and Robert Chunhua Zhao et al. Stem cell research in China, Phil. Trans. R. Soc. B (2007) 362, 1107
15. 2010 World Stem Cell Report.
16. Ema H, Nakauchi H. Bloodlines of haematopoietic stem cell research in Japan. Philos Trans R Soc Lond B Biol Sci, 363(1500), 2089-2097 (2008)
17. David Audley. History of Stem Cells. 2009
18. www.marrow.org
19. http://www.branyonmedicalgroup.com/Stem-Cell-Therapy/stem-cell-therapy-history.html

"Our enduring hope is invested in Biological research"



M. Gazi Yasargil

(Neurosurgeon of The Millenium)

3

Basics of Stem Cells : Types and Sources

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

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

What Are Stem Cells?

A stem cell is defined by two distinct properties of self renewal and differentiation into various cell types. These cells can divide indefinitely, producing a population of identical cells. Stem cells can, on cue, undergo differentiation by asymmetric division to produce two different cell lines. One is identical to the parent and continues to contribute to the original stem cell line. The other cell contains a different set of genetic instructions (resulting in an alternative pattern of gene expression) and is characterized by a reduced proliferative capacity and more restricted developmental potential than

its parent. Eventually a stem cell becomes known as a "progenitor" or "precursor" cell, committed to producing one or a few terminally differentiated cells such as neurons, muscle cells etc. (1)

Potency of Stem Cells:

There exists a hierarchy in the stem cell compartment, depending on their 'potency' or fate restriction.1) Totipotent stem cells give rise to embryonic as well as the extra embryonic tissue. This means, it has the capacity to form the whole of the embryo, including the placenta. The physiological totipotent stem cell is a fertilized oocyte (zygote) or first blastomere which comprises of the 8 cell stage. The artificial counterpart is a clonote obtained by somatic cell nuclear transfer (SCNT) to an enucleated oocyte.2) Pluripotent stem cells in turn have the capacity to give rise to cells of all the three germ layers of the embryo, i.e., endoderm, mesoderm and the ectoderm. Pluripotent stem cells are cells from the inner cell mass of the blastocyst (ICM), epiblast (EPSC) and SC obtained as immortalized cell lines - blastocyst derived embryonic stem cells (ES) and Primordial Germ Cell-derived embryonic germ cells (EG). 3) Multipotent stem cells give rise to cells of one of the germ cell layers only, either ecto-, meso- or endoderm. Sources range from 8 day old embryo to adult bone marrow. 4) Monopotent/Unipotent stem cells are tissue-committed stem cells that give rise to cells of one lineage, e.g., hematopoietic stem cells, epidermal stem cells, intestinal epithelium stem cells, neural stem cells, liver stem cells or skeletal muscle stem cells. (2)

Though the above classification has evolved over decades, understanding of the potency of these cells are everchanging. Many of these cells, which were earlier considered to be multipotent, have shown limited pluripotent properties Also, transdifferentation of monopotent/unipotent cells by external stimulation or manipulation have shown that these classifications, based on fate restriction or potency, are fast becoming redundant.

Classification of Stem Cells

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

1. Embryonic Stem Cells
2. Fetal Stem Cells
3. Umbilical Cord Stem Cells
4. Adult Stem Cells
5. Adult Somatic Stem Cells
6. Induced Pluripotent Stem Cells

1. Embryonic Stem cells:

Embryonic stem cells are pluripotent in nature which are derived from the inner cell mass (ICM) of 5 to 7 day blastocyst, obtained from IVF clinics. (3) Developmental

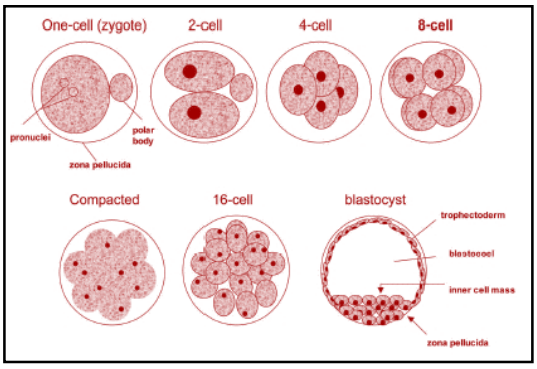


Figure 1: Development of a zygote to a blastocyst (from where embryonic stem cells are derived)

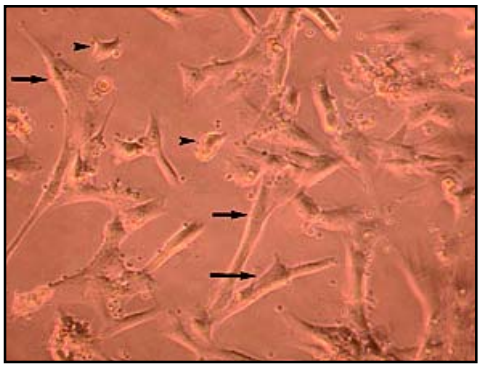


Figure 2 : Mesenchymal stem cells

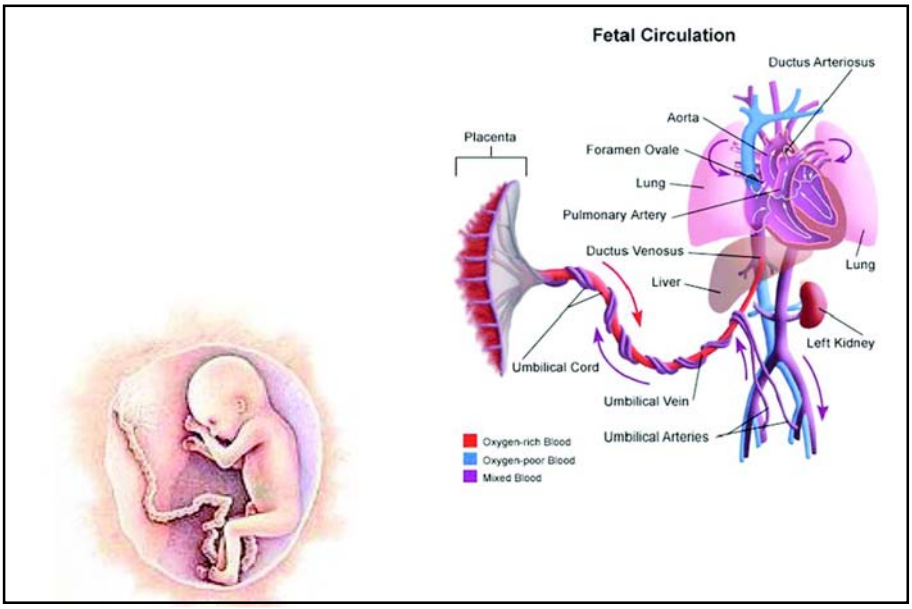


Figure 3 : The umbilical cord and placenta : a rich source of stem cells.

studies in mouse revealed that the fertilized oocyte, the zygote, has the capacity to form the whole embryo. It further divides progressively to give rise to an 8 cell staged, 16 celled, 32 celled blastomere and then finally the blastocyst. The blastocyst is demarcated into the outer transparent trophoblast (which forms the extra embryonic tissue/the placenta) and the Inner cell mass (ICM) which is a 30-34 celled clump. (Figure 1) The ICM ultimately gives rise to the three germ layers and subsequently the whole embryo. Hence, the inner cell mass is the source for the derivation of the embryonic stem cells, which has lost the "totipotency" of the zygote, but is now "pluripotent".

The potential of the embryonic stem cell to form the "germ layers" & its capacity to self renew indefinitely as well as its ability to form any cell type of the body, has led to opening up of this field widely, not only with respects to its use in regeneration, but has thrown up debates regarding ethics and legalities.

However, even before the first embryonic stem cell line was derived in 1981, embryonal carcinoma cells derived from germline tumors called "teratocarcinomas" were widely studied. After transplantation to extra-uterine sites of appropriate mouse strains, these produced benign teratomas or malignant teratocarcinomas. (4)

Embryonic Stem cell lines could be maintained in vitro without any apparent loss of differentiation potential. The "pluripotency" of these cells was demonstrated in vivo by the introduction of ES cells into blastocysts. The resulting mouse chimeras demonstrated that ES cells could contribute to all cell lineages including the germ line. In vitro, mouse ES cells showing the capacity to reproduce the various somatic cell types were found to develop into cells of the germ line

Human (h) ES cell lines are generated from preimplantation embryos produced by in vitro fertilization and after in vitro culture of blastocysts. The resulting hES cells share some fundamental characteristics of murine lines, such as Oct-3/4 expression, telomerase activity, and the formation of teratomas containing derivatives of all three primary germ layers in immunodeficient mice. (5, 6) hES cells maintain proliferative potential for prolonged periods of culture and retained a normal karyotype even in clonal derivatives . In contrast to mES, hES forms cystic embryoid bodies and express SSEA 3 and 4. (7) Several potentially important differences exist between mouse and human ES cells. hES cells show a longer average population doubling time than mES cells [30-35 h vs. 12-15 h]. Also, LIF alone is insufficient to maintain hES in their undifferentiated state (as compared to mES cells which can be maintained feeder free on LIF alone).

At the end of 2001, 70 hES lines had been established using feeder layers of mouse embryonic fibroblasts. This panel of cells, however, suffers from significant limitations, including possible murine retrovirus infections (from the feeder cells) that have rendered them inappropriate for therapeutic applications.

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

Genetic Manipulation of Embryonic Stem Cells

It is well known that embryonic stem cells can differentiate into any cell type of the body. However, channelizing of this potential appropriately requires proper differentiation protocols to delineate cells to specific cell types. Two impediments initially prevented the full potential of the in vitro ES cell model from being realized, 1) Very little was known about the differentiation pathways in culture & 2) Differentiation protocols resulted in the simultaneous production of heterogeneous cell populations, thus constraining studies on selected subsets of cells. To overcome these limitations, genetic tools have proven indispensable to the study of ES cells and their progeny, both in vitro and in vivo. The capacity of ES cells to be clonally expanded permits the identification of independent and stable integration events, and a number of technologies have been developed to rapidly generate stably transfected ES cell clones and transgenic mouse models. Recent advances have shown that hES cells are also amenable to genetic manipulation, thus opening the door to genetic analysis of human development and disease in vitro. (10)

Uses of Embryonic Stem Cells:

1. Embryonic stem cells as cellular models

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

2. Embryonic stem cells in pharmacology

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

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

3. In stem cell based therapies:

The in vitro developmental potential and the success of ES cells in animal models demonstrate the principle of using hES-derived cells as a regenerative source for transplantation therapies of human diseases. Before transfer of ES-derived cells to humans can proceed, a number of experimental obstacles must be overcome. These include efficient derivation of human ES cells in the absence of mouse feeder cells, and an understanding of genetic and epigenetic changes that occur with in vitro cultivation. It will be necessary to purify defined cell lineages, perhaps following genetic manipulation, that are suitable for cell-based therapies. If manipulated, then it will be important to guard against karyotypic changes during passaging and preparation of genetically modified ES-derived cells. Once introduced into the tissue, the cells must

function in a normal physiological way. Finally, assurances against the formation of ES cell-derived tumors and donor/recipient immunocompatibility are additional requirements of stem cell-based therapies. As pointed out, significant progress has been made in the isolation of defined cell lineages in mouse, and important advances have already been seen with hES cells. Before therapeutic application, any ES-based treatment must overcome obstacles of toxicity, immunological rejection, or tumor formation. (13, 14)

2. Fetal Stem Cells:

Fetal Stem Cells (FSCs) are relatively a new addition into the community of different sources of stem cells, exhibiting unique and fascinating features (15). FSCs can not only be isolated from the fetal blood and hemopoietic organs in early pregnancy, but also from a variety of somatic organs as well as amniotic fluid and placenta throughout gestation (16). They can also be extracted from extra-embryonic sources (17). Fetal blood is a rich source of hemopoetic stem cells (HSCs). These cells exhibit rapid proliferative rate than those present in cord blood or adult bone marrow. As these cells share similar growth kinetics and expressing pluripotency markers, it provides us with a strong notion that these cells may be biologically closer to embryonic stem cells. These cells represent as intermediates between embryonic stem cells and adult stem cells, with respect to proliferation rates and plasticity features. Populations of non-hematopoetic stem cells (MSCs), present in the first trimester fetal blood, support hemopoesis and possess the ability to differentiate along multiple lineages. In terms of clinical application both fetal HSCs and MSCs have advantages over their adult counterparts. They possess the properties of better homing and engraftment, with greater multipotentiality and better immunologic compliance. Fetal stem cells are less ethically litigious than embryonic stem cells, as it can be argued that FSCs are currently been obtained from terminated fetuses, thus using the tissue that would be discarded otherwise. The various types of fetal stem cells include hemopoetic stem cells, mesenchymal stem cells, endothelial stem cells, epithelial stem cells and neural stem cells (18).

3. Umbilical Cord Stem Cells

Umbilical cord blood stem cells can be obtained from the umbilical cord immediately after birth. Like bone marrow, umbilical cord blood is another rich source of hematopoietic stem cells, since 1988. The blood remaining in the umbilical vein following birth contains a rich source of hematopoietic stem and progenitor cells, has been used successfully as an alternative allogeneic donor source to treat a variety of pediatric genetic, hematologic, immunologic, and oncologic disorders. Fresh cord blood is also a promising source of non-hematopoietic stem cells. Among others, it contains endothelial cells, MSCs and unrestricted somatic stem cells (USSC). These hematopoietic stem cells are less mature than those stem cells found in the bone marrow of adults or children.

Umbilical cord blood contains circulating stem cells and the cellular contents of umbilical cord blood appear to be quite distinct from those of bone marrow and adult peripheral blood. The frequency of umbilical cord blood hematopoietic stem cells equals

or exceeds that of bone marrow and they are known to produce large colonies in vitro, have different growth factor requirements, have long telomeres and can be expanded in long term culture. Cord blood shows decreased graft versus host reaction compared with bone marrow, possibly due to high interleukin-10 levels produced by the cells and/or decreased expression of the beta-2-microglobulin. Cord blood stem cells have been shown to be multipotent by being able to differentiate into neurons and liver cells.

While most of the attention has been on cord blood stem cells and more specifically their storage for later use, there have also been reports that matrix cells (wharton's jelly) from the umbilical cord contain potentially useful stem cells. Wharton's jelly has been a source for isolation of mesenchymal stem cells. These cells express typical stem cell markers, such as c-kit and high telomerase activity; have been propagated for long population doubling times; and can be induced to differentiate in vitro into neurons.

Sarugaser et al. postulated that the MSC population of the Wharton's Jelly matrix is located close to the vasculature of the cord and specifically isolated these cells, which they called human umbilical cord perivascular cells (HUCPVCs). Their work provided an initial characterization of HUCPVCs with respect to their nonhematopoietic phenotypic profile and capacity to generate colonies of fibroblastic and osteogenic cells.

HUCPVCs were found to have a colony forming unit-fibroblast (CFU-F) frequency of about 1:300 and a population doubling time of 20 hours by passage 2, resulting in significant cell expansion and producing over 1010 HUCPVCs from 2-5 x 106 cells after 30 days of culture. These cells, which are major histocompatibility complex (MHC) class II negative, not only express both an immunoprivileged and immunomodulatory phenotype, but their HC class I expression levels can also be manipulated, making them a potential cell source for SC-based therapies. In addition, HUCPVCs represent a noncontroversial source of primitive mesenchymal progenitor cells that can be harvested after birth, cryogenically stored, thawed, and expanded for therapeutic use. (19)
The advantages of using cord blood as a source of stem cells are:

1. It is a non-invasive source and can be obtained from the umbilical cord immediately after birth.
2. Available in vast abundance; thousands of babies are born each day and the umbilical cord and placenta are discarded as waste.
3. Despite its high content of immune cells, it does not produce strong graft-versus-host disease
4. Therefore, cord blood grafts do not need to be as rigorously matched to a recipient as bone marrow grafts. A 4 out of 6 match is sufficient for clinical use.
5. Higher proliferative capacity

However, there are a few disadvantages (20):

1. Slow engraftment
2. Limited cell dose- small volume of unit, additional cell dose unavailable
3. Autologous donation- limited benefit owing to hereditary disorders

4. Storage issues - unknown length of long term storage, Cost related to long term storage,
5. Quality control

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

All over the world, innumerable cord blood banks have cropped up for storage of umbilical cord stem cells. These are generally either pure public banks or private banks. There are certain banks which offer both types of banking (mixed type). Umbilical cord stem cells banks also differ in the type of biological material that they store. Some banks only store the cord blood (from the umbilical vein) which predominantly carries the haematopoietic stem cells. Increasingly, banks have started storing pieces of the placenta and cord, which are a rich source of mesenchymal stem cells.

4. Adult Stem Cells

Adult stem cells are pluripotent, self renewing and have the ability to differentiate into the mature cell of it resident environment and also, may have transdifferentiating abilities.

Adult stem cell niches have been found in most organs of the human body, eg. bone marrow, adipose tissue, heart, liver, brain, muscles etc. The primary role of these adult stem cells is initiation of repair process in the organ following an injury. There is practical difficulty to obtain these cells due to the following reasons:

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

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

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

Bone Marrow Derived Cells

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

Initially, the bone marrow was thought to contain only haematopoietic stem cells. The excitement regarding HSCs diminished after it was found to have limited potency. However, increasingly, evidence is pouring in regarding the heterogenous population of cells having varying plasticity.

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

1) Mononuclear Cells:

Bone marrow mononuclear cells are a heterogeneous population that includes hematopoietic lineage cells such as lymphocytes, monocytes, stem cells and progenitor cells as well as mesenchymal stromal cells, along with endothelial progenitor cells (EPCs) and very small embryonic like (VSELs) stem cells. Mononuclear cells are isolated from human adult bone marrow, peripheral blood and umbilical cord. This mixture of cells has shown promising therapeutic potential in various neurological conditions (53).

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

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

3) Multipotent Adult Progenitor Cells (MAPC):

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

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

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

5) Multipotent Adult Stem Cells (MACS):

These cells express pluripotent-state-specific transcription factors (Oct-4, Nanog and Rex1) and were cloned from human liver, heart and BM-isolated mononuclear

cells. MACS display a high telomerase activity and exhibit a wide range of differentiation potential. Again the potential relationship of these cells to MSC,MAPC and MIAMI described above is not clear, although it is possible that these are overlapping populations of cells identified by slightly different isolation/expansion strategies.

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

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

Other Organs Where Potential Stem Cell Population Exists:

1) Bone and cartilage stem cells:

Mesenchymal Stem Cells in bone marrow can differentiate into bone and cartilage under appropriate conditions. If, bone or cartilage is injured, there are stem cells inherent in bone or cartilage to participate in the repair process. Bone itself has been found to have both uncommitted stem cells as well as committed osteoprogenitor cells. In addition, when bone is fractured, there is exposed marrow and abundant bleeding with hematoma formation in the marrow space, which results in good repair potential. In vivo, articular cartilage has a very limited capacity for repair if injured. It is currently not clear whether there is a committed chondrocyte progenitor cell located within cartilage. In the presence of injury to cartilage, stem cells do participate in the repair process. The numbers, however, are small and the regulatory factors are limited. It is postulated that these cells may be derived from surrounding tissues such as muscle, bone or other non-cartilaginous tissues. (26, 27)

2) Epidermal stem cells (skin and hair):

The human skin comprises the outer epidermis and underlying dermis. Hair and sebaceous glands also make up the epidermis. The most important cell type in the epidermis is the keratinocyte which is an epithelial cell that divides and is housed in the basal layer of the epidermis. Once these cells leave the basal layer they undergo terminal differentiation resulting in a highly specialized cell called a squame which eventually forms either the hair shaft or the lipid-filled sebocyte that form an outer skin layer between the harsh environment and underlying living skin cells. The epidermis houses stem cells at the base of the hair follicle and their self-renewing properties allow for the re-growth of hair and skin cells that occurs continuously. New keratinocytes are produced continuously during adult life to replace the squames shed from the outer skin layers and the hairs that are lost. Stem cells differentiate into an

intermediate cell called the "transient amplifying cell" which gives rise to the more differentiated cell types inclusive of the keratinocytes and sebocytes. (28)

3) Neural stem cells:

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

Site/origin of neural stem cells:

In the mammalian adult brain, the genesis of new neurons continues throughout life within two 3-layered cortical regions, the hippocampus and olfactory bulb (OB), where it is sustained by endogenous stem cells. Stem cell niches have now been identified in adult mammalian forebrain, a) in the subventricular zone (SVZ), subgranular zone (SGZ) and b) dental gyrus of the hippocampus. The most active NSC compartment is found in SVZ which represents a remnant of the embryonic germinal neuroepithelium, and persists throughout life as an active mitotic layer in the wall of the telencephalic lateral ventricles and along its rostral extension toward the olfactory bulb.(30) A complete turnover of the resident proliferating cell population occurs every 12 to 28 days in the SVZ; about 30,000 new neuronal precursors (neuroblasts) being produced every day and migrating to the OB. Two main cell types are found in the SVZ: migratory, proliferating neuroblasts and astrocytes. These cells reach the more superficial OB layers and terminally differentiate into granule and periglomerular neurons. Glial tubes are composed of a special type of astroglia that expresses the marker of mature CNS astrocytes [glial fibrillary acidic protein (GFAP)] and also contain the cytoskeletal proteins vimentin and nestin. (31)

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

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

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

axonal regeneration by producing insulating myelin sheaths around growing and damaged axons, secreting growth factors, and generating structural and matrix macromolecules that lay the tracks for axonal elongation. (33, 34)

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

(iii) **Adipose tissue :** The adipose tissue is a highly complex tissue and consists of mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages and lymphocytes. Hence, this tissue compartment provides a rich source of pluripotent adipose tissue-derived stromal cells. It has been demonstrated that AT contains stem cells similar to BM-MSCs, which are termed processed lipoaspirate (PLA) cells. Exhibiting a neuronal-like morphology and expressing several proteins consistent with the neuronal phenotype.(36, 37)

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

5) Eye stem cells

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

6) Dental Stem Cells:

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

7) Gut stem cells:

The gastrointestinal epithelial lining undergoes continuous and rapid renewal throughout life. Differentiation programs thus exist in specific regions of the tract.

Epithelial cell renewal in the intestine is sustained by multipotent stem cells located in the crypts of Lieberhahn. In the small intestine, epithelial cells of enterocytic, goblet and enteroendocrine origin differentiate as they migrate from a crypt up an adjacent villus and leave the intestine once they reach the villus tip. In the colon, it is different. Epithelial cells migrate from the crypt to a flat surface cuff that surrounds its opening.

The stem cell hierarchy in the gut and the fact that stem cells and their progeny are located in well defined anatomic units make the gut an ideal in vivo model for stem cell research.(25)

There are other sources from where stem cells can be derived. These origin include vascular, endometrial and skeletal muscle.

5. Adult Somatic Cells:

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

6. Induced Pluripotent Stem Cells:

One of the emerging areas in laboratory investigations of stem cells is the attempt to induce differentiated somatic stem cells into pluripotent stem cells by inducing certain factors which will initiate cellular reprogramming (48, 49). The induced pluripotent human stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers (50). These IPSCs sidesteps the ethical issues that have limited the use of embryonic stem cells, as they can be generated without the use of oocytes or cell from the preimplantation embryo (51). These cells can be autologous, thereby surmounting the problem of immune reaction. Thus, development of IPS cell technology can add to the sources of autologous cells for transplantation therapy (52).

REFERENCE:

1. Gerald D. Fischbach and Ruth L. Fischbach. Stem cells: science, policy, and ethics. J Clin Invest.2004; 114(10): 1364-1370.
2. Mariusz Z. Ratajczak, Ewa K. Zuba-Surma, Marcin Wysoczynski, Wu Wan, Janina, Ratajczak, and Magda Kucia . Hunt for Pluripotent Stem Cell - Regenerative Medicine Search for Almighty Cell. J Autoimmun. 2008 ; 30(3): 151-162.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. Embryonic stem cell line from

human blastocysts. Science 1998; 282: 1145-1147.

4. Evans MJ. The isolation and properties of a clonal tissue culture strain of pluripotent mouse teratoma cells. J Embryol Exp Morphol 1972;28: 163-176.
5. Richards M, Fong CY, Chan WK, Wong PC, and Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. Nat Biotechnol 2002;20: 933-936
6. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, and Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998;282: 1145-1147
7. Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, and Benvenisty N. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 2000;6: 88-95.
8. Richards M, Fong CY, Chan WK, Wong PC, and Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. Nat Biotechnol 2002;20: 933-936
9. Lee JB, Lee JE, Park JH, Kim SJ, Kim MK, Roh SI, and Yoon HS. Establishment and maintenance of human embryonic stem cell lines on human feeder cells derived from uterine endometrium under serum-free condition. Lee JB, Lee JE, Park JH, Kim SJ, Kim MK, Roh SI, Yoon HS. Biol Reprod. 2005;72(1):42-9
10. Eiges R, Schuldiner M, Drukker M, Yanuka O, Itskovitz-Eldor J, and Benvenisty N. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. Curr Biol 2001;11: 514-518
11. Durick K, Mendlein J, and Xanthopoulos KG. Hunting with traps: genome-wide strategies for gene discovery and functional analysis. Genome Res 1999; 9: 1019-1025.
12. Davila JC, Cezar GG, Thiede M, Strom S, Miki T, and Trosko J. Use and application of stem cells in toxicology. Toxicol Sci 2004; 79: 214-223.
13. Hochedlinger K and Jaenisch R. Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. N Engl J Med 2003; 349: 275-286.
14. Brustle O, Spiro AC, Karram K, Choudhary K, Okabe S, and McKay RD. In vitro-generated neural precursors participate in mammalian brain development. Proc Natl Acad Sci USA 1997; 94: 14809-14814.
15. Kalliopi I Pappa and Nicholas P Anagnou 'Novel sources of fetal stem cells: where do they fit on the development continuum?' Regen. Med. (2009) 4(3) 423-433
16. Pascale V. Guillot, Keelin O'Donoghue, Hitoshi Kurata, Nicholas M. Fisk' Fetal Stem Cells: Betwixt and Between Semin Reprod Med 2006; 24(5): 340-347
17. Akiva J. Marcus, Dale Woodbury 'Fetal stem cells from extra-embryonic tissues: do not discard' J. Cell. Mol. Med. Vol 12, No 3, 2008 pp. 730-742
18. Keelin O'Donoghue, Nicholas M. Fisk 'Fetal stem cells' Best Practice & Research Clinical Obstetrics and GynaecologyVol. 18, No. 6, pp. 853-875, 2004

19. Alison MR, Vig P, Russo F, et al. Hepatic stem cells: From inside and outside the liver? Cell Prolif 2004; 37: 1-21.
20. Kenneth J. Moise Jr Umbilical Cord Stem Cells (Obstet Gynecol 2005;106:1393-1407
21. Sabine Hombach-Klonisch, Soumya Panigrahi, Iran Rashedi et al. Adult stem cells and their trans-differentiation potential-perspectives and therapeutic applications. J Mol Med. 2008 ; 86(12): 1301-1314
22. Cosimo De Bari, Francesco Dell'Accio, Przemyslaw Tylzanowski, and Frank P. Luyten. Multipotent Mesenchymal Stem Cells From Adult Human Synovial Membrane. Arthritis & rheumatism. 2001 : 44(8), 2001, 1928-1942
23. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-9.
24. Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, et al. A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 2006;20:857-69.
25. Alison MR, Poulsom R, Forbes S, et al. An introduction to stem cells. J Path 2002; 197: 419-423.
26. Metsaranta M, Kujala UM, Pelliniemi L, et al. Evidence for insufficient chondrocytic differentiation during repair of full thickness defects of cartilage.Matrix Biol 1996; 15: 39-47.
27. Nakajima H, Goto T,Horikawa O, et al. Characterization of cells in the repair tissue of full thickness articular cartilage defects. Histochem Cell Biol 1998; 109: 331-338.
28. Blanpain C, Lowry WE, Geohegan A, et al. Self-renewal,multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell 2004; 118: 530-532.
29. Graziadei PP, Monti Graziadei GA. Neurogenesis and neuron regeneration in the olfactory system of mammals. III. Differentiation and reinnervation of the olfactory bulb following section of the fila olfactoria in rat. J Neurocytol 1980; 9 : 145-62.
30. Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc NatlAcad Sci USA 1993; 90: 2074-2077.
31. Syed Ameer Basha Paspala, Avvari Bhaskara Balaji, Parveen Nyamath,et al. Neural stem cells & supporting cells - The new therapeutic tools for the treatment of spinal cord injury. Indian J Med Res 2009; 130, 379-391.
32. Ramón-Cueto A, Nieto-Sampedro M. Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. Exp Neurol 1994; 127 : 232-44.
33. Ramer LM, Au E, Richter MW, Liu J, Tetzlaff W, Roskams, AJ. Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. J Comp Neurol 2004; 473 : 1-15.

34. Saporta S, Kim JJ, Willing AE, Fu ES, Davis CD, Sanberg PR. Human umbilical cord blood stem cells infusion in spinal cord injury: engraftment and beneficial influence on behavior. J Hematother Stem Cell Res 2003; 12 : 271-8.

35. Zhao ZM, Li HJ, Liu HY, Lu SH, Yang RC, Zhang QJ, et al. Intraspinal transplantation of CD34+ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats. Cell Transplant 2004; 13 :113-22.

36. Nurse CA , Macintyre L, Diamond J. Reinnervation of the rat touch dome restores the Merkel cell population reduced afterdenervation. Neuroscience 1984; 13 : 563-71.

37. Caspar-Bauguil S, Cousin B, Galinier A, Segafredo C, Nibbelink M, André M, et al. Adipose tissues as an ancestral immune organ: site-specific change in obesity. FEBS Lett 2005; 579 : 3487-92.

38. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13 : 4279-95.

39. Pennon A, Calancie B, Oudega M, Noga BR. Conduction of impulses by axons regenerated in a Schwann cell graft in the transected adult rat thoracic spinal cord. J Neurosci Res 2001; 64 : 533-41.

40. Zulewski H, Abraham EJ, Gerlach MJ, et al. Multipotential nestin positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine and hepatic phenotypes.Diabetes 2001; 50: 521-533

41. Schwarz SC, Schwarz J. Translation of stem cell therapy for neurological diseases. Transl Res 2010; 156(3): 155-160.

42. Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, Lipton SA, Ding S. Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell 2011; 9(2): 113-118.

43. Ieda M. Direct reprogramming into desired cell types by defined factors. Keio J Med 2013; 62(3): 74-82.

44. Kim J, Efe JA, Zhu S, Talantova M, Yuan X, Wang S, Lipton SA, Zhang K, Ding S. Direct reprogramming of mouse fibroblasts to neural progenitors. Proc Natl Acad Sci U S A 2011; 108(19): 7838-7843.

45. Ring KL, Tong LM, Balestra ME, Javier R, Andrews-Zwilling Y, Li G, Walker D, Zhang WR, Kreitzer AC, Huang Y. Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell 2012; 11(1): 100-109.

46. Kim HS, Kim J, Jo Y, Jeon D, Cho YS. Direct lineage reprogramming of mouse fibroblasts to functional midbrain dopaminergic neuronal progenitors. Stem Cell Res 2014; 12(1): 60-68.

47. Son EY, Ichida JK, Wainger BJ, Toma JS, Rafuse VF, Woolf CJ, Eggan K. Conversion of mouse and human fibroblasts into functional spinal motor neurons. Cell Stem

Cell 2011; 9(3): 205-218

48. Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Prolif 2008; 41 Suppl 1: 51-56.
49. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4): 663-676.
50. Junying Yu et al. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells Science (2007) 318, 1917
51. Hanley J, Rastegarlari G, Nathwani AC. An introduction to induced pluripotent stem cells. Br J Haematol 2010; 151(1): 16-24.
52. Robbins RD, Prasain N, Maier BF, Yoder MC, Mirmira RG. Inducible pluripotent stem cells: not quite ready for prime time? Curr Opin Organ Transplant 2010; 15(1): 61-67.
53. Glover, L., Tajiri, N., Ishikawa, H., Shinozuka, L., Kaneko, Y. et al. (2012) A Step-up Approach for Cell Therapy in Stroke: Translational Hurdles of Bone Marrow - Derived Stem Cells. Translational Stroke Research. 3(1), 90-98.
54. Bojic, S., Volarevic, V., Ljujuic, B., Stojkovic, M. Dental Stem Cells- characteristics and potential. Histol. Histopathol. 2014, Jan21

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

– Ivan Pavlov

4

Mechanism Of Action

Stem cells are instrumental in the formation of new tissues and thereby promoting repair and regeneration. Their role, in the normal wear and tear of the body, appears to be assistance of repair and maintenance of normal tissue structure and function. Recreation of this ability in vitro as well in animal models of various diseases is the basis of devising therapeutic modalities for degenerative disorders through remodeling of the injured tissues. Cell-based therapy, could therefore potentially be used to treat a wide array of clinical conditions where cellular damage is the underlying pathology.

More importantly, the use of adult stem cells as opposed to human embryonic stem cells for therapy avoids ethical problems and has two additional advantages:1) Adult stem cells can be isolated from patients, and this overcomes the problem of immunological rejection and 2) The risk of tumor formation is greatly reduced as compared to the use of embryonic stem cells.(1)

Plasticity, Pluripotency And Production

While pluripotency and plasticity are considered properties of early ESC, adult stem cells are traditionally thought to be restricted in their differentiation potential to the progeny of the tissue in which they reside. However, a remarkable plasticity in differentiation potential of stem cells derived from adult tissues has been seen. (2).

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

cells. (11-13)In most of these plasticity studies, genetically marked cells from one organ of an adult mouse apparently gave rise to cell type characteristics of other organs following transplantation, suggesting that even cell types once thought to be terminally differentiated are far more plastic in their developmental potential than previously thought. A critical aspect of the observation of adult stem cell plasticity is that in order for plasticity to occur, cell injury is necessary. (14)This suggests that micro-environmental exposure to the products of injured cells may play a key role in determining the differentiated expression of marrow stem cells. (15)

The events underlying stem cells plasticity could relate to a variety of mechanisms such as dedifferentiation, trans-differentiation, epigenetic changes, and/or cell fusion. Rerouting of cell fate may result from the multistep process known as **dedifferentiation** where cells revert to an earlier, more primitive phenotype characterized by alterations in gene expression pattern which confer an extended differentiation potential. In urodele amphibians, cell dedifferentiation is a common mechanism resulting in the functional regeneration of complex body structures throughout life, including limbs, tail, and even spinal cord .Recent studies on the plasticity of murine myotubes and other cells derived from adult tissues suggest that dedifferentiation may also be possible in mammals. (16-17) Molecular and epigenetic changes have shown to be involved in the process of dedifferentiated, possibly mediated by signals released after cellular injury.

Another mechanism put forward to explain stem cell switch to a novel phenotype is a process known as **trans-differentiation**. Cells may differentiate from one cell type into another within the same tissue or develop into a completely different tissue without acquiring an intermediate recognizable, undifferentiated progenitor state. (18)

Recent studies show clearly that bone-marrow-derived cells can colonize a wide variety of tissues in the body of a host. (19, 20) Although derived from the embryonic mesoderm, the developmental potential of bone marrow cells is not restricted to this germ layer, but these cells have also been shown to populate tissues of ectodermal and endodermal origin .(21) Both mesenchymal stem cells and bone marrow- derived cells can give rise to a wide array of non-hematopoietic cell types such as astrocytes and neurons in the brain, cardiac myocytes in models of infarction, skeletal muscle, and hepatocytes. However, the reported frequencies of colonization are low, and it is unlikely that there is much repair of organ damage by bone marrow in the normal individual. Despite examples of trans-differentiation events of adult stem cells being reported, these findings are still controversial. (22) Most of the reports could not be confirmed in subsequent investigations, and to date, trans-differentiation has never been conclusively demonstrated in any experimental setting. In every case, differentiation from a rare population of stem cells has never been excluded, or "trans-differentiation" events turned out to be misinterpretations caused by **cell fusion** resulting in nuclear reprogramming and changes in cell fate. (23-24)

It is now recognized that adult stem cells from bone marrow may fuse with cells of the target organ. So far, bone-marrow-derived cells were shown to form fusion heterokaryons with liver, skeletal muscle, cardiac muscle, and neurons .There is evidence that such fused cells become mono-nucleated again, either by nuclear fusion or by

elimination of supernumerary nuclei.(25) Fusion and nuclear transfer experiments demonstrated that genes previously silenced during development could be reactivated by cytoplasmic factors modulating the epigenetic mechanisms responsible for the maintenance of a specific state of cell differentiation. . Despite the limitation of the low frequency of this event and its dependence of the developmental stage of donor nuclei, cell fusion may be considered as a potential avenue for tissue repair. The physiological purpose of adult cell fusion is speculative. As outlined by Helen Blau , fusion could be a means by which cells 1) Deliver healthy genetic material to dying cells (rescue function), 2) Supply cells with new genes (repair function), or 3) Correct genetically defective cells such as in muscular dystrophy (gene replacement).

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

In addition to the aforementioned phenomena of cell fate switching, the presence of a rare population of pluripotent primitive stem cells may also explain the acquisition of an unexpected phenotype. Non-hematopoietic cell populations from bone marrow and umbilical cord blood were enriched by in vitro culture and demonstrated to have the potential to differentiate into derivatives of all three germline layers with meso-, endo-, and ectodermal characteristics. (26,27) Known as multipotent adult progenitor cells (MAPC), these cells contribute to most, if not all, somatic cell lineages, including brain, when injected into a mouse blastocyst . (28) Interestingly, while MAPC express Oct4, a transcription factor required for undifferentiated embryonic stem cells maintenance at levels approaching those of ESC, MAPC do not express two other transcription factors known to play a major role in ESC pluripotency, Nanog and Sox2. (29) This particular expression profile may contribute to the fact that the use of ESC, but not MAPC, carries the risk of generating tumors. Thus, MAPC are a promising source of autologous stem cells in regenerative medicine. Their low tumorigenicity, high regenerative plasticity, and optimal immunological compatibility are essential assets for the successful transplantation of MAPC-derived tissue-committed cells without immune-mediated rejection. (30)

The Paracrine Effect

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

part, by paracrine mechanisms. The reasons for the above postulations are as follows: (31)

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

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

Third, and perhaps most importantly, in vitro and in vivo animal studies have revealed that much of the functional improvement and attenuation of injury afforded by stem cells can be replicated by cell free, conditioned media derived from stem cells. Taken together, these indirect and direct data suggest that stem cells may improve injured organ performance and limit injury not via differentiation but rather via complex paracrine actions rather than an organogenetic role.

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

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

Increased Angiogenesis

Stem cells produce local signaling molecules that may improve perfusion and enhance angiogenesis to chronically ischemic tissue. Although the particular growth factors contributing to this neovascular effect remain to be defined, the list includes vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (FGF2). (32, 33)

VEGF is a strong promoter of angiogenesis. Chen et al. have recently shown that treatment with bone marrow stromal cells enhances angiogenesis by increasing endogenous levels of VEGF and VEGFR2. They previously demonstrated that administration of recombinant human VEGF165 to rats 48 h after stroke significantly increased angiogenesis in the penumbra and improved functional recovery.

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

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

Thus, VEGF, HGF, and FGF2 may be important paracrine signaling molecules in

stem cell-mediated angiogenesis, protection, and survival.

Decreased Inflammation

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

Anti-Apoptotic and Chemotactic Signaling

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

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

Beneficial Remodeling of the Extracellular Matrix

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

Activation of Neighboring Resident Stem Cells

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

activate subsequently the resident cardiac stem cells. (35)

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

Remyelination

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

Recent studies have shown that remyelination can be accomplished by supplying demyelinated regions with cells like Schwann cells, oligodendrocyte lineage cells lines, Olfactory ensheathing cells (OECs), embryonic stem cells and neural stem cells , Adult bone marrow derived stem cells. The remyelinating effect of these cells may be via one or more mechanisms, including: the stem cells act as an immunomodulator by producing soluble factors; they carry out direct cell replacement by differentiating into neural and glial cells in the lesion; and they indirectly promote neural and glial differentiation of endogenous cells. Interactions with viable axons and supportive astrocytic responses are required for endogenous immature cells to fulfill their potential remyelinating capacity.(36,37)

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

REFERENCE

1. Smith AG. Embryo-derived stem cells: of mice and men. Annu Rev Cell Dev Biol 2001;17:435-462
2. Amy J Wagers and Irving L Weissman. Plasticity of Adult Stem Cells. Cell; 2004, 116(5): 639-648.
3. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998;279:1528-1530.
4. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168-1170.

5. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest 2000;105:71-77.
6. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 2001;98:10344-10349.
7. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. Science 2000;290:1775-1779.
8. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107:1395-1402.
9. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M,Weissman IL, Grompe M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6:1229-1234.
10. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105:369-377.
11. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999;96:10711-10716.
12. Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 1999;401:390-394.
13. Pang W. Role of muscle-derived cells in hematopoietic reconstitution of irradiated mice. Blood 2000;95:1106-1108.
14. Abedi M, Greer DA, Colvin GA, Demers DA, Dooner MS, Harpel JA, Pimentel J, Menon MK, Quesenberry PJ. Tissue injury in marrow transdifferentiation. Blood Cells Mol Diseases 2004;32:42-46.
15. Quesenberry PJ, Colvin G, Dooner G, Dooner M, Aliotta JM, Johnson K. The stem cell continuum: cell cycle, injury, and phenotype lability. Ann N Y Acad Sci 2007;1106:20-29.
16. Odelberg SJ, Kollhoff A, Keating MT. Dedifferentiation of mammalian myotubes induced by msx1. Cell 2000;103:1099-1109.
17. Tsai RY, Kittappa R, McKay RD. Plasticity, niches, and the use of stem cells. Dev Cell 2002;2: 707-712.
18. Sabine Hombach-Klonisch, Soumya Panigrahi, Iran Rashedi. Adult stem cells and their trans-differentiation potential- perspectives and therapeutic applications. J Mol Med. 2008 ; 86(12): 1301-1314.
19. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell

fusion. J Clin Invest 2003;111:843 850.

20. Jang YY, Sharkis SJ. Metamorphosis from bone marrow derived primitive stem cells to functional liver cells. Cell Cycle 2004;3:980-982.
21. Direkze NC, Forbes SJ, Brittan M, Hunt T, Jeffery R, Preston SL, Poulsom R, Hodivala-Dilke K, Alison MR, Wright NA. Multiple organ engraftment by bone-marrow-derived myofibroblasts and fibroblasts in bone-marrow-transplanted mice. Stem Cells 2003;21:514-520.
22. Morshead CM, Benveniste P, Iscove NN, van der Kooy D. Hematopoietic competence is a rare property of neural stem cells that may depend on genetic and epigenetic alterations. Nat Med 2002;8:268-273.
23. Vassilopoulos G, Russell DW. Cell fusion: an alternative to stem cell plasticity and its therapeutic implications. Curr Opin Genet Dev 2003;13:480-485.
24. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422:897-901.
25. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425:968-973.
26. D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC. Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci 2004;117:2971-2981.
27. Kogler G, Sensken S, Airey JA, et al. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. J Exp Med 2004;200:123-135.
28. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-49.
29. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 2005;122:947-956.
30. Shizuru JA, Negrin RS, Weissman IL. Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. Annu Rev Med 2005;56:509-538.
31. Paul R. Crisostomo, Troy A. Markel, Yue Wang, and Daniel R. Meldrum. Surgically relevant aspects of stem cell paracrine effects. Surgery. 2008 May; 143(5): 577-581.
32. Crisostomo PR, Wang M, Herring CM, Markel TA, Meldrum KK, Lillemoe KD, et al. Gender differences in injury induced mesenchymal stem cell apoptosis and VEGF, TNF, IL-6 expression: Role of the 55 kDa TNF receptor (TNFR1) J Mol Cell Cardiol. 2007;42(1):142-149.
33. Vandervelde S, van Luyn MJ, Tio RA, Harmsen MC. Signaling factors in stem cell

mediated repair of infarcted myocardium. J Mol Cell Cardiol. 2005;39(2):363-376.

34. Markel TA, Crisostomo PR, Wang M, Herring CM, Meldrum DR. Activation of Individual Tumor Necrosis Factor Receptors Differentially Affects Stem Cell Growth Factor and Cytokine Production. Am J Physiol Gastrointest Liver Physiol. 2007; 293(4):G657-62.
35. Wang M, Crisostomo PR, Herring C, Meldrum KK, Meldrum DR. Human progenitor cells from bone marrow or adipose tissue produce VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism. Am J Physiol Regul Integr Comp Physiol. 2006;291(4):R880-884.
36. Jingxian Yang, Abdolmohamad Rostami, Guang-Xian Zhang et al. Cellular remyelinating therapy in multiple sclerosis. Journal of the Neurological Sciences.2009 ;Vol 276(1), 1-5
37. Louis N. Manganas and Mirjana Maletic-Savatic . Stem cell therapy for central nervous system demyelinating disease. Current Neurology and Neuroscience Reports . 2005; 5 (3), 225-231

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

–Aristotle

5

Laboratory Aspects Of Stem Cell Therapy

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

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

Basic methodology

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

Bone marrow harvesting

Open Method

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

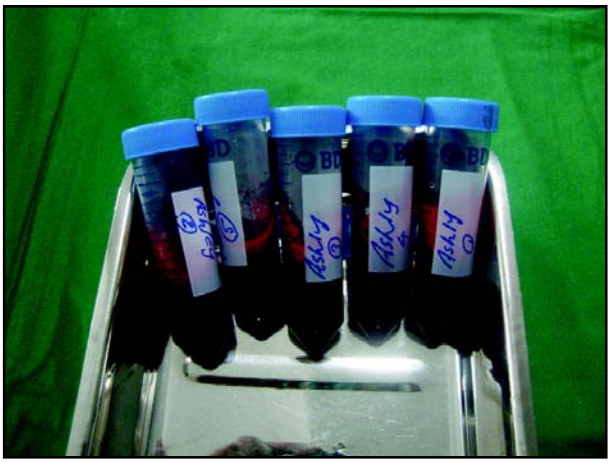


Figure 1: Aspirated bone marrow in tubes. Each tube contains about 20 ml bone marrow mixed with heparin.

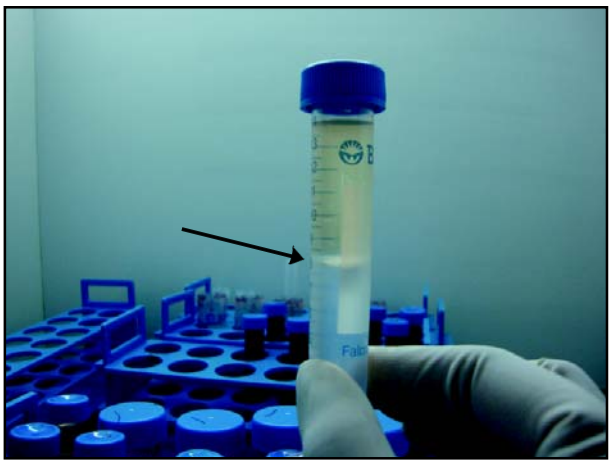


Figure 2: Buffy coat containing separated fraction of mononuclear concentrate (arrow indicating)

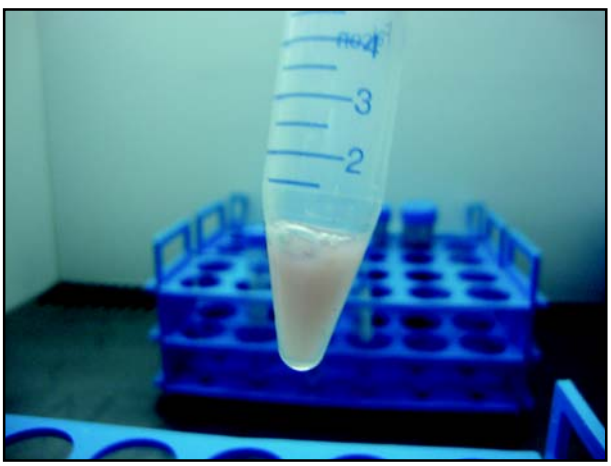


Figure 3 : Purified concentrate of mononuclear cells in solution (heterogenous mixture of stem cells - mainly hematopoietic)

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

Closed Method

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

A total of 240 mL of marrow aspirate was processed using the point of care SmartPReP System (Harvest Technologies, Plymouth, MA) to yield 40 mL of treating volume. (5)

Peripheral blood

A short prototype is as follows:

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

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

Ex vivo hSC selection

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

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

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

Cord blood processing

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

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

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

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

Endometrial cell processing and expansion

Harvesting

Before the collection procedure a "collection tube" is prepared in a class 100 Biological Safety Cabinet located in a Class 10,000 Clean Room. To prepare the collection tube, 0.2 ml amphotericin B (Sigma-Aldrich, St Louis,MO), 0.2 ml penicillin/ streptomycin (Sigma) and 0.1 ml EDTA-Na2 (Sigma) are added to a 50 ml conical tube containing 30 ml of GMP-grade phosphate buffered saline (PBS). Collection of 5 ml of menstrual blood is performed according to a modification of the published procedure. Collection is performed by the donor. A sterile Diva cup inserted into the vagina and left in place for 30-60 minutes. After removal, the contents of the Diva cup are to be decanted into the collection tube. The collection tube is then taken to the clean room where it is centrifuged at 600 g for 10 minutes. The collection tube is then transported to the Biological Safety Cabinet where the supernatant is removed, and the tube is topped up to 50 ml with PBS in the Biological Safety Cabinet and cells are washed by centrifugation at 600 g for 10 minutes at room temperature. The cell pellet is to be

washed 3 times with 50 ml of PBS, and mononuclear cells are collected by Ficoll-Paque (Fisher Scientific, Portsmouth NH) density gradient. Mononuclear cells are washed 3 times in PBS and resuspended in 5 ml complete DMEM-low glucose medium (GibcoBRL, Grand Island, NY) supplemented with 10% Fetal Bovine Serum selected lots having endotoxin level < = 10 EU/ml, and hemoglobin level < = 25 mg/dl clinical grade ciprofloxacin (5 mg/mL, Bayer A.G., Germany) and 4 mM L-glutamine (cDMEM).

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

Expansion

Cells are plated in a T-75 flask containing 15 ml of cDMEM, cultured for 24 hours at 37°C at 5% CO2 in a fully humidified atmosphere. This allows the ERC precursors to adhere. Non-adherent cells are washed off using cDMEM by gentle rinsing of the flask. Adherent cells are subsequently detached by washing the cells with PBS and addition of 0.05% trypsin containing EDTA (Gibco, Grand Island, NY, USA) for 2 minutes at 37°C at 5% CO2 in a fully humidified atmosphere. Cells are centrifuged, washed and plated in T-175 flask in 30 ml of cDMEM. This results in approximately 10,000 ERC per initiating T-175 flask. The flask is then cultured for 5 days which yields approximately 1 million cells in the T-175 flask (Passage 1). Subsequently cells are passaged at approximately 200,000 cells in a T-175 flask. At passage 3-4, approximately 100-200 million cells are harvested. (7)

Induced pluripotent cell processing

Induced pluripotent cells (iPSCs) are generated by reprogramming somatic cells to embryonic-like state cells. The somatic cells are introduced with a defined and limited set of factors and are cultured under embryonic stem cell like conditions. (8) For the first time, Yamanaka et al carried out a retroviral mediated introduction of four transcription factors - octamer-binding transcription factor-3/4 (OCT3/4), SRY-related high-mobility-group (HMG)-box protein-2 (SOX2), MYC and Kruppel-like factor-4 (KLF4) in mouse fibroblast to produce iPSCs. (8,9) Since then, the same protocol has been used for other types of mouse cells and human somatic cells. Once, the factors are introduced, cells are cultured where they form colonies resembling pluripotent cells. These cells are then isolated based on the morphology, surface markers , etc. Generation of iPSCs takes around 1-2 weeks for mouse cells and 3-4 weeks for human cells. Recently, the iPSCs are being generated virus and vector free to avoid viral induced tumor formation.

The growth factors and cytokines used for differentiation of iPSCs should be extensively tested to ensure high biological activity, high purity, freeze-thaw stability, and structural homogeneity.(10) They should also allow optimal growth, expansion, and storage of differentiated cells. The major steps in obtaining iPSCs are

reprogramming, culturing, engineering, differentiation and cell analysis. It is essential to validate their pluripotency and differentiation capacity into the desired cell lineage. (11)

References

1. Hyung Chun Park, Yoo Shik Shim,Yoon Ha Seung Hwan Yoon, Et Al. Treatment of Complete Spinal Cord Injury Patients by Autologous Bone Marrow Cell Transplantation and Administration of Granulocyte-Macrophage Colony Stimulating Factor. Tissue Engineering 2005;11(5-6):913-922
2. https://www.miltenyibiotec.com/~/media/Files/Navigation/Research/Stem%20Cell/SP_MC_BM_density_gradient.ashx
3. http://www.translationalresearch.ca/documents/SOP%20VI%20MONONUCLEAR%20CELL%20ISOLATION.pdf
4. http://www.springerprotocols.com/Abstract/doi/10.1007/978-1-60327-169-1_1)
5. www.harvesttech.com
6. http://www.neocells.com/html/processing.html
7. Zhaohui Zhon, Amit N Patel, Thomas E Ichi et al. Feasibility investigation of allogeneic endometrial regenerative cells. J Transl Med 2009; 7(1):15.
8. Ye L, Swingen C, Zhang J. Induced pluripotent stem cells and their potential for basic and clinical sciences. Curr Cardiol Rev. 2013 Feb 1;9(1):63-72.
9. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76.
10. http://www.lifetechnologies.com/in/en/home/references/protocols/cell-culture/stem-cell-protocols/ipsc-protocols.html
11. http://www.lifetechnologies.com/in/en/home/life-science/stem-cell-research/induced-pluripotent-stem-cells.html

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

Prof. Harvey Cushing

Neurosurgeon of the Millenium

6

Surgical Aspects of Stem Cells Therapy: Routes of Administration

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

Procurement of Stem cells - Bone marrow aspiration

The choice of site may be dependent on various factors such as age, weight marrow distribution, physical status of the patient, physicians experience etc. However the most common site is the pelvis. The aspiration is easily done from either of the iliac crests (posterior or anterior). The posterior superior iliac spine is easily accessible and identifiable, however to access this, the patient has to be turned in the lateral or prone position which can be troublesome and cumbersome. The anterior superior iliac spine can be accessed with the patient lying comfortably in the supine position. In obese patient, the landmarks may be obliterated due to fat distribution. Sampling is not normally discordant between the anterior or posterior iliac spines.

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

needle. A 1 cm area is anesthetized.

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

The stylet is now removed and a 10 ml or 20 ml syringe, with some heparin in it, is attached and the aspiration is done. A total of 100-120 ml is aspirated in adults and 80-100 ml in children. This is collected in heparinized tubes which need to be appropriately labeled. The bone marrow collected is transported to the laboratory in a special transporter under sterile conditions.(1)

Transplantation of Stem Cells in neurological disorders

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

Intrathecal delivery

The patient is positioned in the lateral decubitus position, in the curled up "foetal ball" position. Occasionally, the patient is made to sit, leaning over a table- top. Both these maneuvers help open up the spinous processes. The back is painted and draped and local anaesthetic is injected into the L4-5 or L3-4 space. An 18G Touhy needle is inserted into the sub-arachnoid space. After ascertaining free flow of CSF, an epidural catheter is inserted into the space, far enough to keep 8-10 of the catheter in the space. The stem cells are then injected slowly through the catheter, keeping a close watch on the hemodynamics of the patient. The cells are flushed in with CSF. The catheter is removed and a benzoin seal followed by a tight compressive dressing is given. This procedure is usually done under local anesthesia. General anesthesia is given to children.

A spinal needle instead of a catheter is preferred in patients with cardiac problems, where excessive intravenous infusion is to be avoided, in patients on anti-coagulant or

Figure 1: Bone marrow J needle

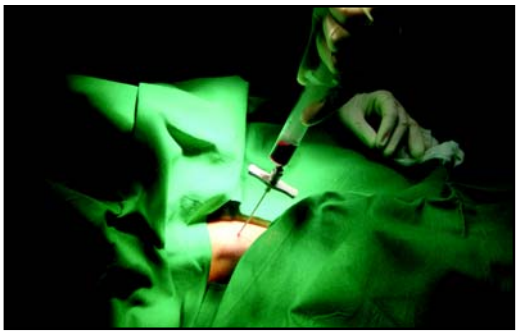


Figure 2: Bone marrow aspiration

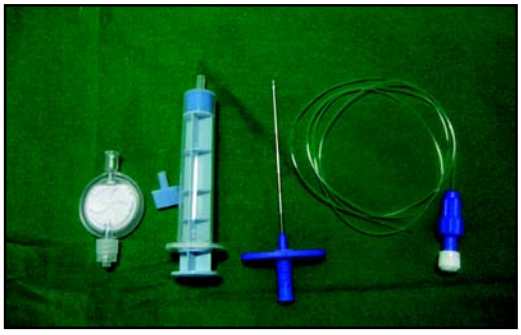


Figure 3: Epidural set (18 G) for intrathecal Inj.

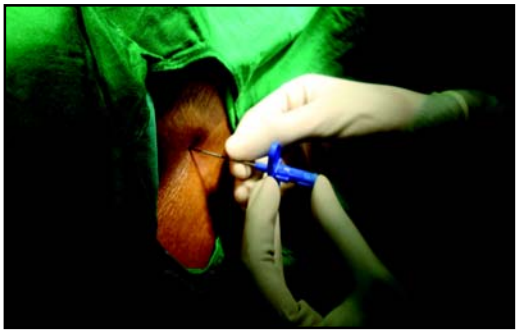


Figure 4: Intrathecal Injection step 1

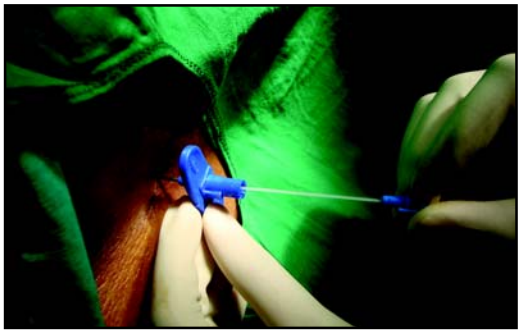


Figure 5: Intrathecal Injection step 2

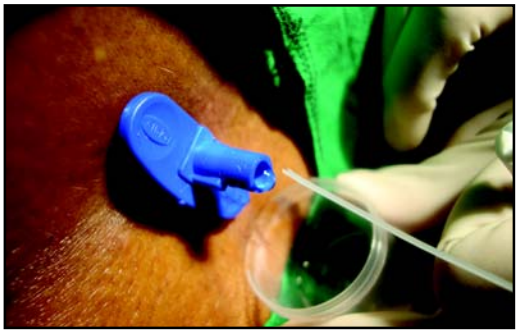


Figure 6 : Intrathecal Injection step 3

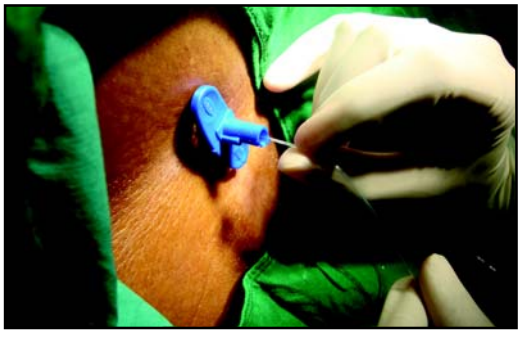


Figure 7: Intrathecal Injection step 4

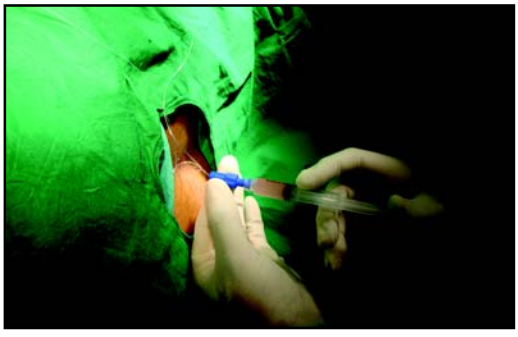
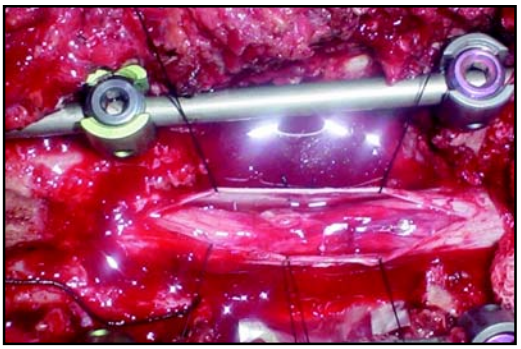


Figure 8: Intrathecal Injection - delivery of stem cells



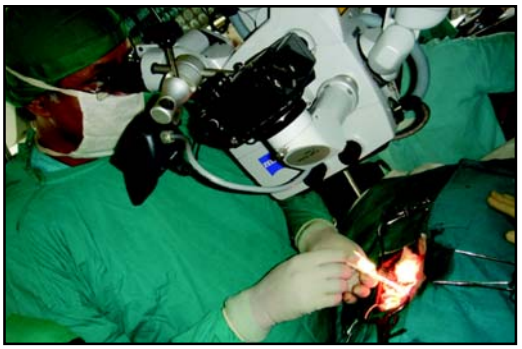


Figure 9 & 10 : Intraspinal transplantation of stem cells in a case of thoracic spinal cord injury.

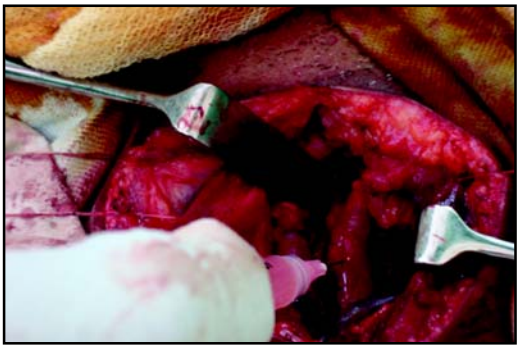


Figure 11: Intra-arterial direct injection of stem cells into the carotid artery following carotid endartrectomy

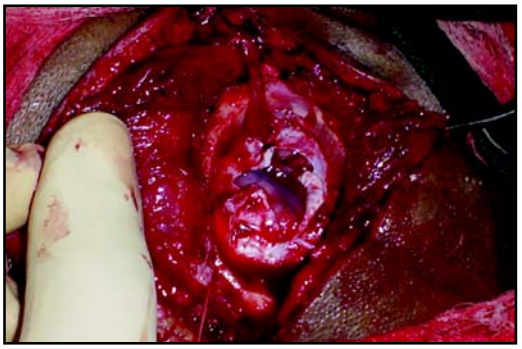


Figure 12: STA-MCA bypass

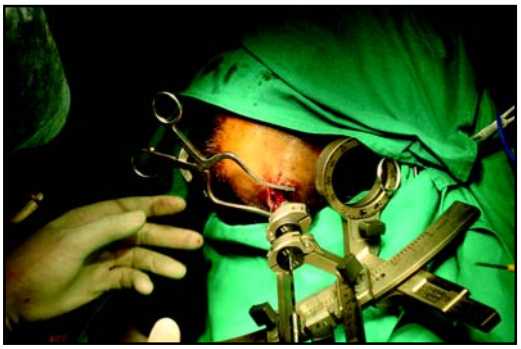


Figure 13: Leksell Stereotactic Frame for direct stem cell implantation into the brain.

anti-platelet drugs so as to avoid bleeding into the sub-arachnoid space, in case where the spine is scoliotic which happens often in patients with muscular dystrophy and in some previously operated cases of lumbar spine surgery.

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

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

Intraspinal transplantation

Direct implantation into the spinal cord may be done in one of many ways :-

a) Through a complete laminectomy from one level above to one level below the injury site so that there is sufficient access to the transplantation site. The dura is incised, sparing the arachnoid, which is subsequently opened separately with a microscissors. The dorsal surface of the contusion site is located under high-power microscopic magnification. After exposure of sufficient surface in the contusion site, 300µL aliquots of cell paste (total volume, 1.8 mL) are injected into six separate points surrounding the margin of the contusion site. To avoid direct cord injury, 2×10^8 cells are delivered at a rate of 30 µL/min, using a 27-gauge needle attached to a 1-mL syringe. The depth of the injection site is 5 mm from the dorsal surface. To prevent cell leakage through the injection track, the injection needle is left in position for 5 min after completing the injection, after which the dura and arachnoid are closed. The muscle and skin are closed in layers. (3)

b) Though a minilaminectomy and exposure of the spinal cord. The dura is opened and a 27 gauge scalp vein is used by cutting one of the wings. The other wing is held by a hemostat and inserted at a 45 degree angle into the Dorsal root entry zone. It is inserted 3mm deep into the spinal cord. Two injections are made on either side above the injury site and two injections are made below the injury site. In China, surgeons are injecting 35 µL of stem cells. In his planned trials, Wise Young is intending to inject an escalating dose of 4 µL, 8 µL and 16 µL.

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

Intra-arterial injection

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

Stereotactic implantation into the brain

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

Intramuscular injection

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

Intravenous injection

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

Anaesthesia considerations

Muscular Dystrophy

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

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

Multiple Sclerosis

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

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

Cerebral Palsy

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

Anaesthesia Considerations: Increased chances of GE reflux. Increased chances of aspiration, both from the regurgitant contents and pooled salivary secretions. Skeletal and muscle spasticity resulting in contractures and joint deformities, which can hamper positioning. increased sensitivity to anaesthetic drugs, resulting in slow emergence.

Spinal Cord Injury

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

REFERENCES

1. Bernadette F. Rodak, George A. Fritsma, Kathryn Doig. Hematology: clinical principles and applications.
2. Callera et al. Magnetic resonance tracking of magnetically labelled autologous bone marrow CD 34+ cells transplanted into the spinal cord via lumbar puncture technique in patients with chronic spinal cord injury: CD 34+ cells' migration into the injured site. Stem Cells Dev. 2007; 16(3): 461-6.
3. Treatment of Complete Spinal Cord Injury Patients by Autologous Bone Marrow Cell Transplantation and Administration of Granulocyte-Macrophage Colony Stimulating Factor,hyung chun park1 yoo shik shim,yoon ha seung hwan yoon, so ra park,byung hyune choi, hyun seon park,TISSUE ENGINEERING Volume 11, Number 5/6, 2005
4. Bjorklund A., Dunnett S.B., Brundin P., Stoessl A.J., Freed C.R., Breeze R.E., Levivier M., Peschanski M., Studder L., Barker R. Neural transplantation for the treatment of Parkinson's disease. Lancet Neurology 2: 437-45, 2003.
5. Freed, C.R., Greene, P.E., Breeze, R.E. Tsai, W.-Y., DuMouchel, W., Ko, R., Dillon, S., Winfield, H., Culver, S.,Trojanowski, J.Q., Eidelberg, D., and Fahn, S.: Embryonic dopamine cell transplantation for severe Parkinson's disease. New England Journal of Medicine 344(10):710-719, 2001.
6. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA, Cox CS Jr. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev. 2009 Jun;18(5):683-92.

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

– Goethe

7

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

Motor point is the point at which the main nerve enters the muscle or, in case of deeply placed muscle, at the point where the muscle emerges from under covers of the more superficial ones.

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

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

Concept of motor point stimulation

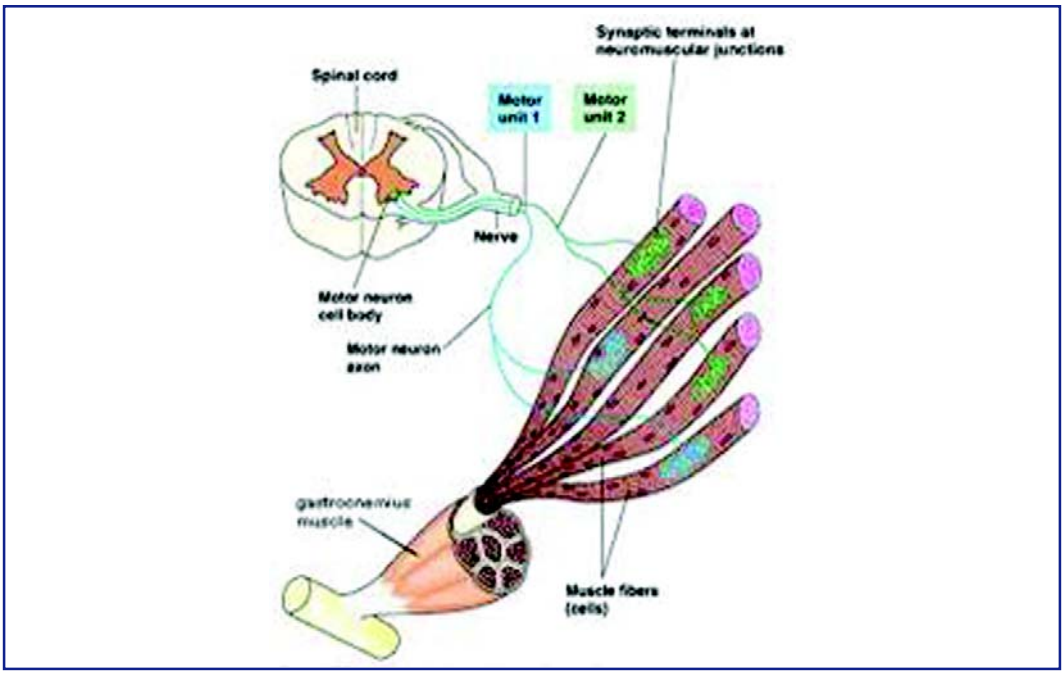


Figure 1: A Neuromuscular Junction

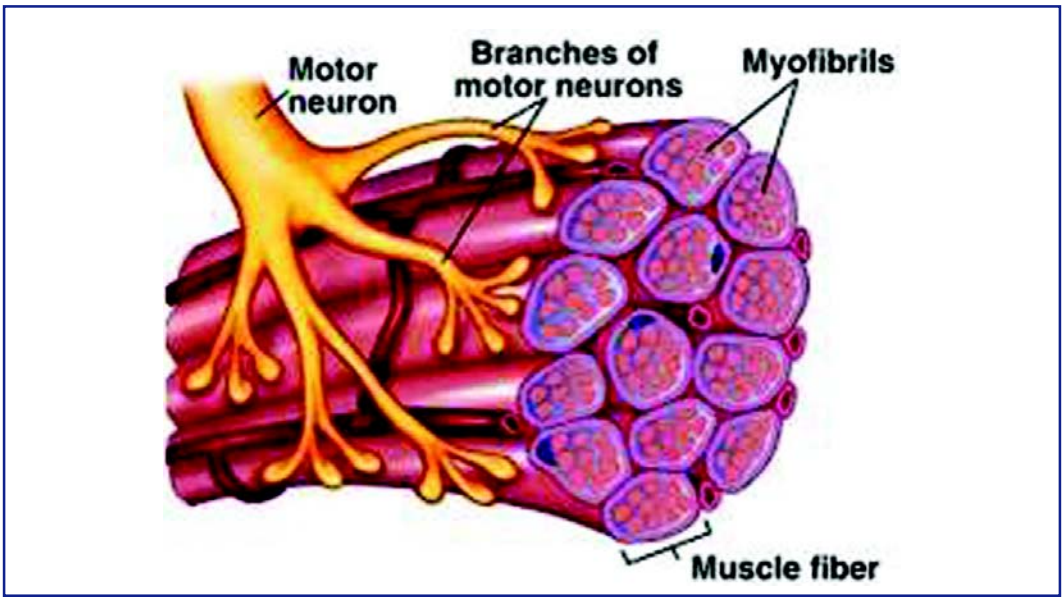


Figure 2 : The Motor Unit

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

When a sensory nerve is stimulated the downward travelling impulse has no effect, but the upward travelling impulse is appreciated when it reaches conscious levels of the brain. If impulses of different durations are applied, using the same current for each, it is found that the sensory stimulation experienced varies with the duration of the impulse. Impulses of long duration produce an uncomfortable stabbing sensation, but this becomes less as the duration of the impulse is reduced until with impulses of 1 ms & less only a mild prickling sensation is experienced.

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

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

Preparation of apparatus

Faradic type of current

A low frequency electronic stimulator with automatic surger is commonly used. A faradic current is a short -duration interrupted direct current with a pulse duration of 0.1 - 1 ms & a frequency of 50 - 100 Hz. Strength of contraction depends on the number of motor units activated which in turn depends on the intensity of the current applied & the rate of change of current. To delay fatigue of muscle due to repeated contractions, current is commonly surged to allow for muscle relaxation.

Stimulation of Motor points

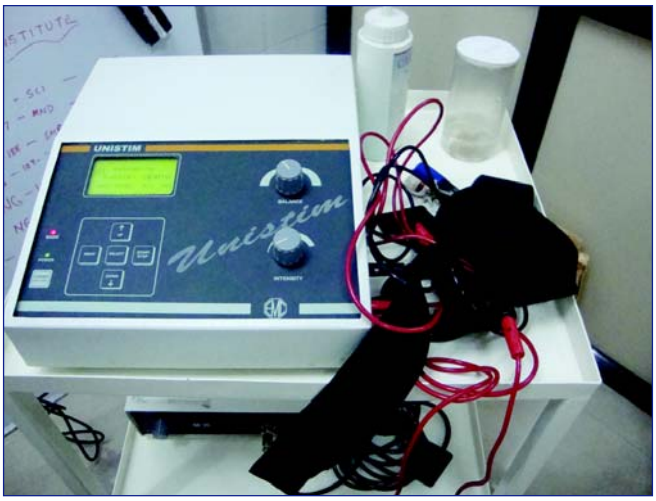


Figure 3 : Electrical stimulator used for stimulation and plotting of motor points.

Figure 4 : Preparation of the patient for motor point plotting

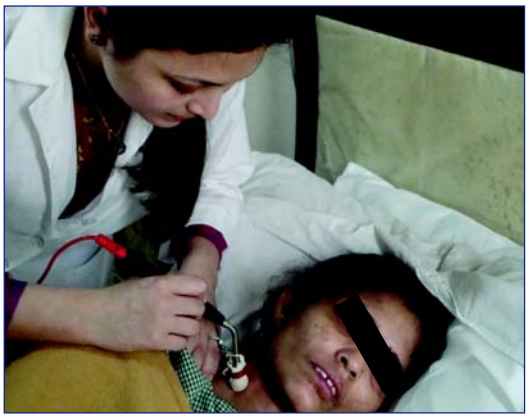


Figure 5 : Plotting of motor point (strenomastoid muscle)

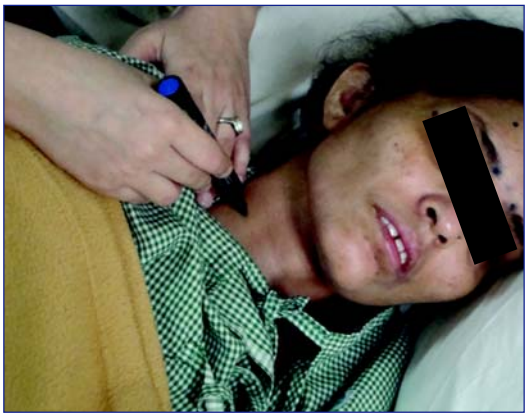


Figure 6 : Marking of sternomastoid muscle motor point.

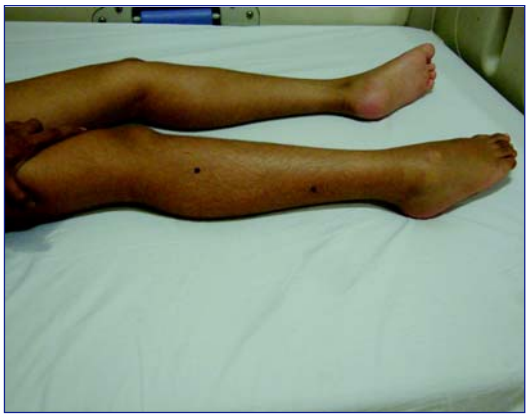


Figure 7 : Plotted motor points of tibialis anterior and peronei muscle

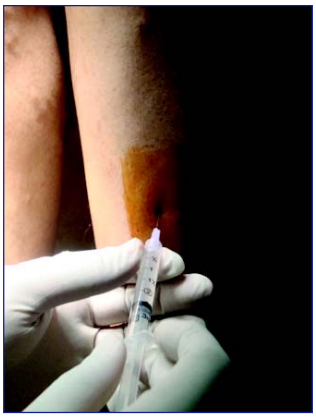


Figure 8 : Injection of stem cells in tibialis anterior muscle motor point.

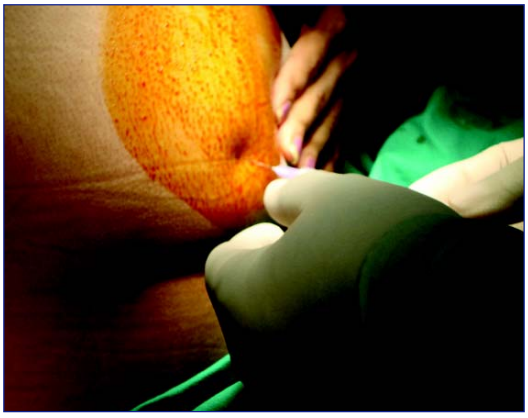


Figure 9 : Injection of stem cells in the glutei muscle motor point.

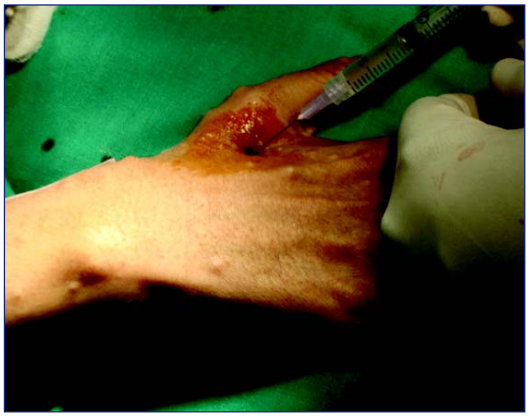


Figure 10 : Injection of stem cell injection in the adductor pollicis muscle motor point.

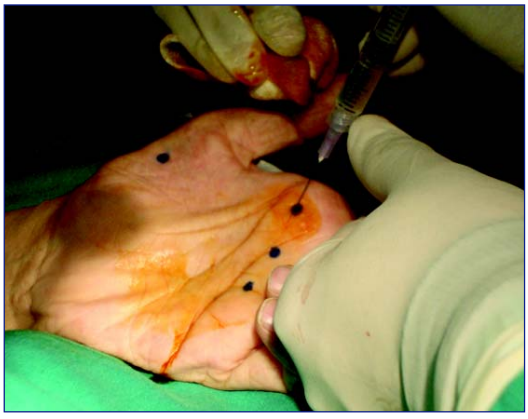


Figure 11 : Injection of stem cells in the lumbrical muscle motor points

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 point. The indifferent electrode is applied & secured in a suitable area. The indifferent electrode is placed over the motor point of the muscle to be stimulated. Firm contact ensures a minimum of discomfort, & where possible the whole of operators hand should be in contact with the patient's tissues so that she /he can feel the contractions produced.

Selection of the Individual muscles for Stem cell transplant

Depending on the manual muscle testing & patient's complain of weakness & difficulty in ADL, physiotherapist decides which muscles need to be injected with stem cells. Post stem cell injection these muscles need specific training & individual muscle strengthening program so that they can help the patient in gaining efficiency & independency in ADL. Apart from injecting stem cells intrathecally, injecting them in the motorpoints of the muscles facilitates further specific implantation of the stem cells in isolated individual muscles.

A) Major muscles of UL that are generally considered:
   a) Deltoid: Anterior, middle & posterior fibres.
   b) Biceps brachialis.
   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) Dorsilflexors: Tibialis anterior, Peronei longus & brevis, EHL.

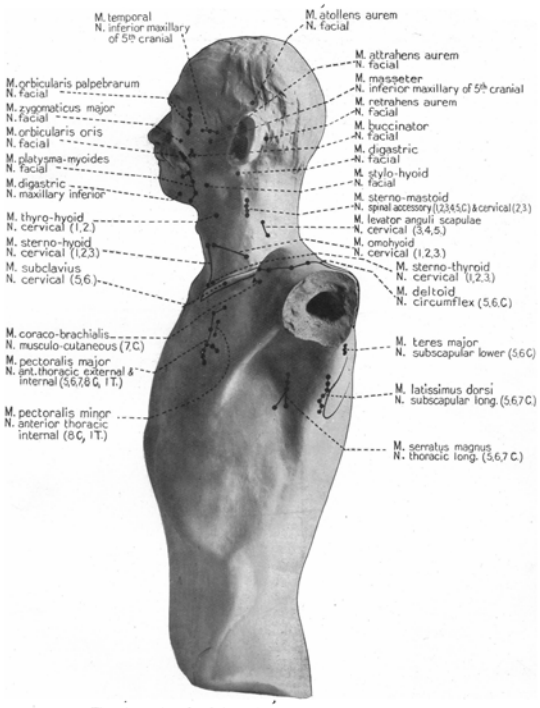
C) In trunk:

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

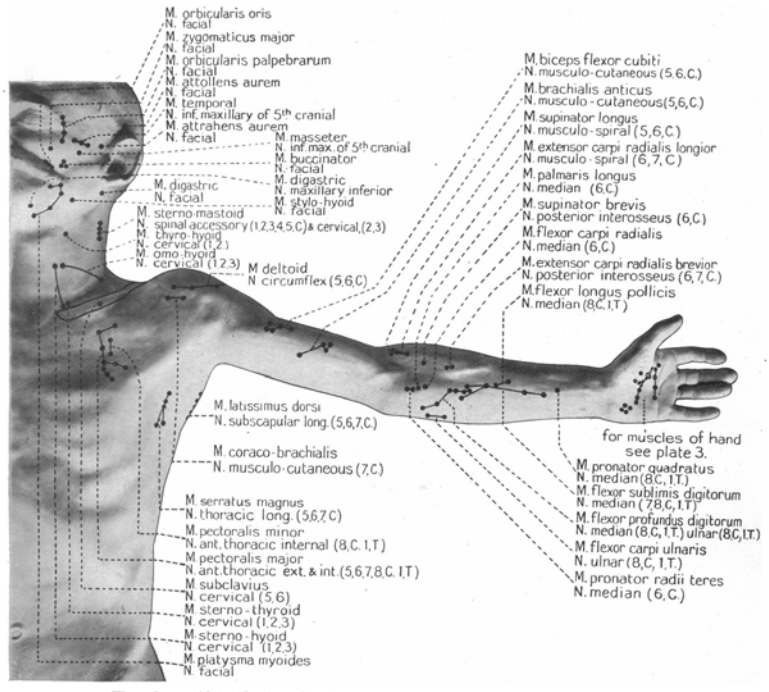
D) Facial Muscles:

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

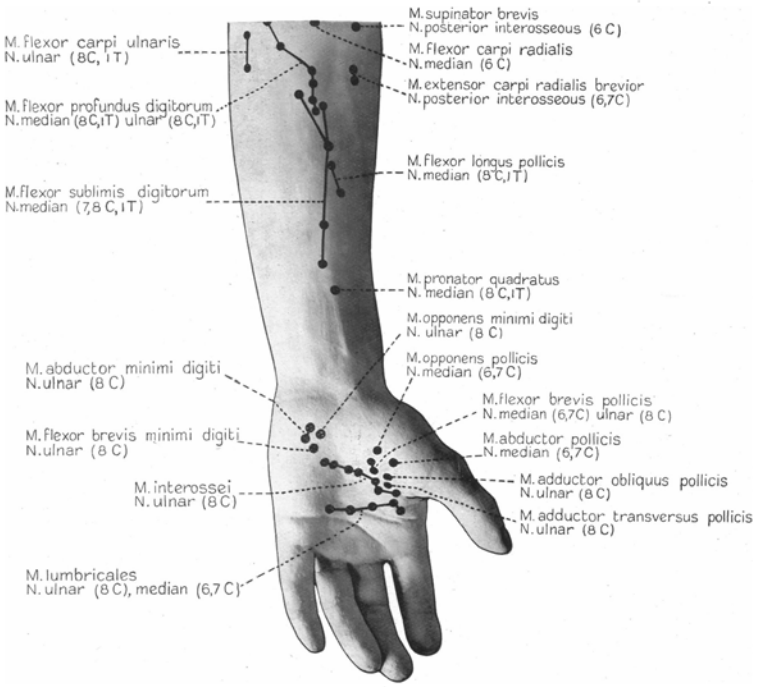
Intramuscular stem cells injection in motor points within the muscle, ie the area with high concentration of motor end plates is very specific transplantation. Also multiple motor points in choosen muscle group allows for a graded response, thus allowing increment in muscle strength clinically depending on, further specific training & strengthening of individual injected muscles. An injection of stem cell in the motor end plate potential, can be identified in the neuromuscular system within few hours,



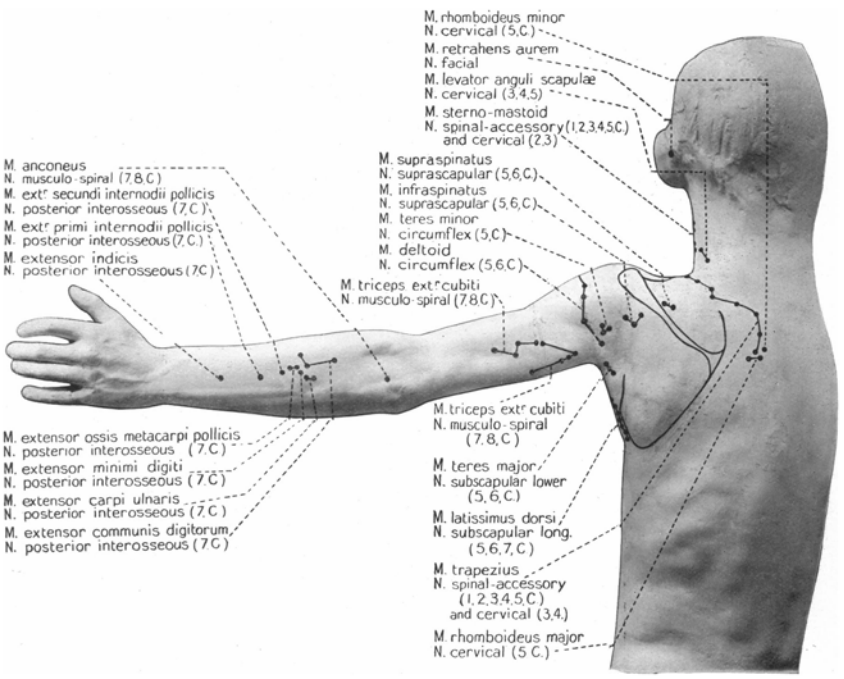
View of cast of trunk of living male from left side showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



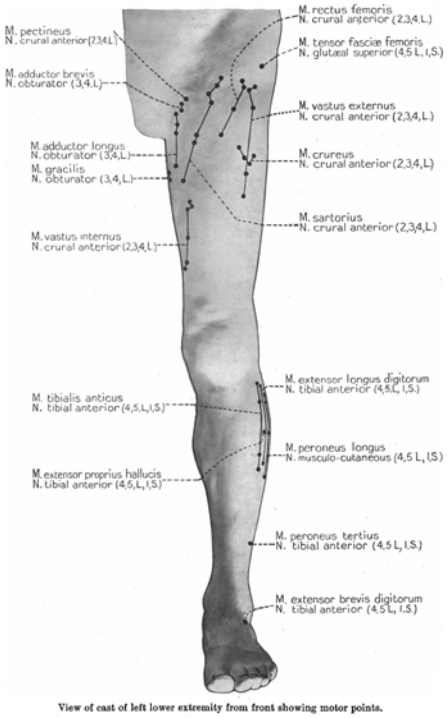
View of cast of left side of trunk and upper extremity from front showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



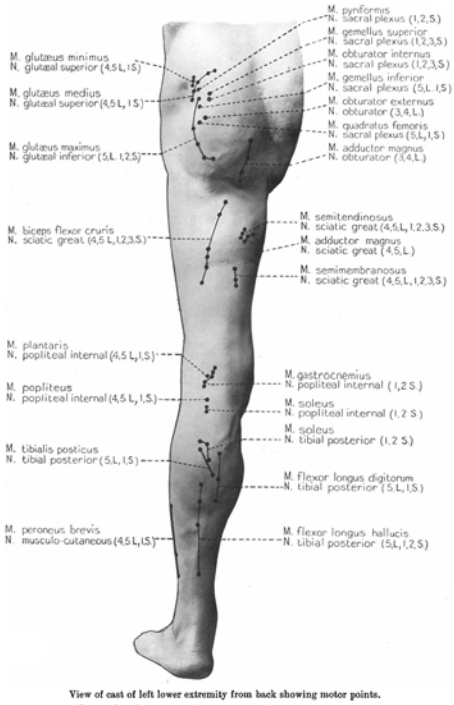
View of cast of left fore-arm and hand from front showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



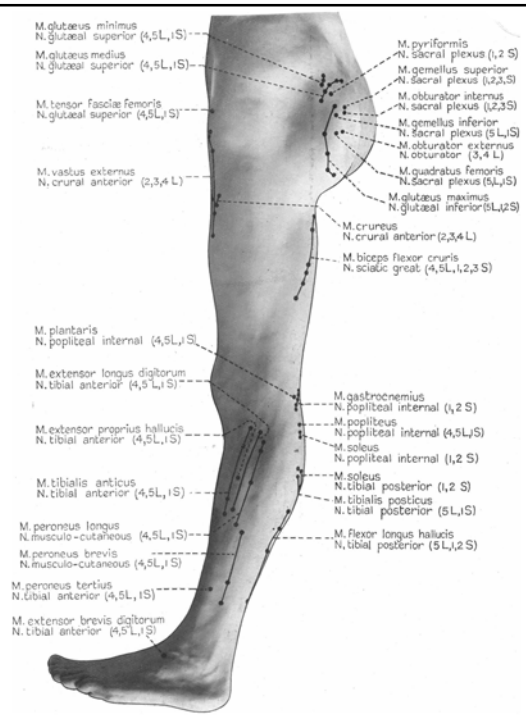
View of cast of left side of trunk and upper extremity from behind showing motor points.
(Prepared in the Anatomy Department of University of Aberdeen.)



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



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



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

although the onset of clinical effects is noticed as early as 72 hours post transplant, which varies from patient to patient.

REFERENCE

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

SECTION B

Clinical Application of Stem Cells

"Things don't change.
You change your way of looking at them"

– Carlos Castaneda

8

Role of Stem Cells In Autism

Autism spectrum disorders (ASD) are pervasive developmental disorders characterized by impairments or delays in social interaction, communication and language along with repetitive behavior. A report by the Surgeon General, states that autism has roots in both structural brain abnormalities and genetic predispositions. (1) They are called spectrum disorders because of the wide range of severity of symptoms. The prevalence of autism has increased radically over the few decades for reasons not yet known. It is seen three to four times more in boys than girls. (2)

ASD is associated with known genetic causes in 10-15% of cases. (3)The exact etiology and pathophysiology of autism remains poorly understood. The numerous biochemical abnormalities detected in autism are oxidative stress; endoplasmic reticulum stress; mitochondrial dysfunction; decreased methylation; underproduction of glutathione; intestinal dysbiosis and toxic metal burden. (4) The environmental factors like organophosphates and heavy metals are also attributed to the origin of the disease. [5] Brain hypoperfusion and immune dysfunctions have been postulated as the two main underlying pathologies in autism. (6,7) The degree of hypoperfusion is proportional to the severity of the symptoms of autism. The extent of hypoxia was shown to be inversely correlated to Intelligent Quotient (IQ). [8]

A range of findings have suggested autism as a disorder of growth of the neural systems and connections, likely to be responsible for the under development of functions such as communication, behavior and socialization. [9] Utah and Chris Frith (2010) proposed a social brain hypothesis to explain theory of mind deficits in ASD. [10] The social brain concept tries to localize the complex social perception to superior temporal sulcus (STS), amygdala, orbital frontal cortex (OFC), and fusiform gyrus (FFG). [11] The key roles implicated are STS region in analysis of perception, FFG in face detection and recognition, OFC in social reinforcement and reward processes, the amygdala in

analysis and regulation of emotions. [12] These areas form neural interconnections to establish a pathway from perception to action. [13]

Autism, similar to other neurodevelopmental disorders, is incurable and requires chronic management. Currently, the only treatment options available for autism are behavioral, nutritional and medical intervention. These interventions facilitate development and learning, promoting socialization, self awareness, reducing maladaptive behaviors and educating and supporting families. (14).

Unmet medical needs

Like other complex neurodevelopmental disorders, ASD is thought to be the final common pathway of multiple etiological and neuropathological mechanisms (15), thus, complicating the search for autism-specific biological markers. As there are no definitive biological markers, diagnosis relies on the recognition of an array of behavioral symptoms that vary from case to case, heterogeneous and overlap with other childhood neuropsychiatric disorders. The treatment available does not address the core pathology of autism but only manages the symptoms and associated medical conditions.

Stem cell therapy in autism

Stem cell therapy is emerging as one of the treatment strategies for autism. It has the therapeutic potential to repair the damaged neural tissue at molecular, structural and functional level. They are known to address the underlying core neuropathology of autism viz. Hypoperfusion and immune dysregulation via neuroprotection, neuromodulation and neurorestoration. (16) Hypoperfusion results in hypoxia. Reversal of hypoxia may lead to self repair and neural proliferation, which is observed in many animal models of cerebral ischemia. In cerebral ischemia animal models, bone marrow stem cells have shown to repair the ischemia-damaged neural networks and restore the lost neuronal connections. (17) Hence, stem cells may be used to stimulate angiogenesis and lead to reperfusion. 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. (18) Thus, stem cells are capable of suppressing the pathological immune responses as well as stimulating neovascularisation. Cell therapy may also prove useful for the treatment of T cell defect associated with autism. (19)

Not many preclinical and clinical trials have been conducted till date to study the benefits of stem cell therapy in autism. It is very challenging to study the effect of any intervention on animal models of autism due to lack of characteristic social interaction and language deficits found in autism. There are some case reports (20,21) and case series which are recently published and have shown beneficial effects of cellular therapy.

Sharma et al published the first clinical study which was an open label proof of concept study in 32 patients of autism. The results of their trial demonstrated the safety and efficacy of stem cell therapy for autism. (22) They administered autologous bone marrow mononuclear cells intrathecally.. These patients showed improved

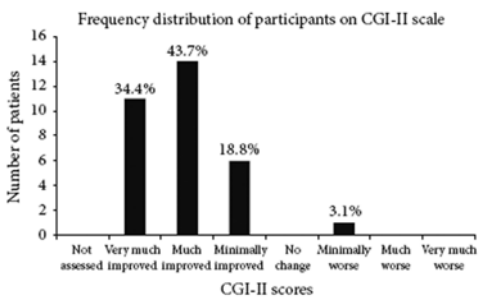
neurological functions which were recorded on objective scales such as ISAA and CGI and also the improvement in brain metabolism was observed in PET CT scans of few patients.

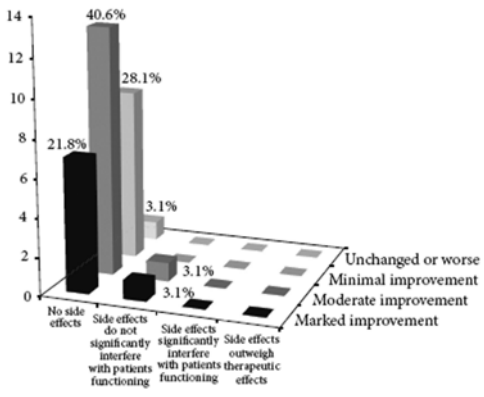
The next clinical study was published by Yong-Tao Lv et al where they studied use of human cord blood MNCs and MSCs. This study also showed a positive outcome. (23)

Our results

Published data

An open label proof of concept study of autologous bone marrow mononuclear cells (BMMNCs) intrathecal transplantation in 32 patients with autism followed by multidisciplinary therapies was performed. All patients were followed up for 26 months (Mean 12.7) Outcome measures used were ISAA, CGI and FIM/ Wee-FIM scales. Positron Emission Tomography computed Tomography (PET-CT) scan recorded objective changes. Out of 32 patients, a total of 29 (91%) patients improved on total ISAA scores and 20 patients (62%) showed decreased severity on CGI-I. In the domain of Social relationships and reciprocity 29 out of 32 (90.6%) patients showed improvement. Improved emotional responsiveness was observed in 18 out of 32 (56%) patients. Under the Speech-language and communication domain there was an improvement observed in 25 patients out of 32 (78%). Behavior patterns of 21 out of 32 patients (66%) improved. Hyperactivity or restlessness (71%) and engaging in stereotype and repetitive motor mannerisms (65%) decreased significantly. Sensory aspects improved in 14 out of 32 patients (44%). Cognitively they showed improved consistency in attention and concentration and response time. 71% patients showed better attention and concentration, 45% patients showed reduction in the delay in responding. The difference between pre and post scores was statistically significant ($p < 0.001$) on Wilcoxon Matched-Pairs Signed Rank Test. On CGI-II 96% of patients showed global improvement. The efficacy was measured on CGI-III efficacy index. Functional neuroimaging in the form of PET - CT scan in eight patients, documented changes in





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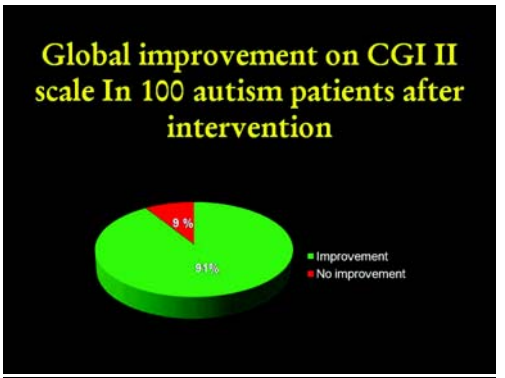
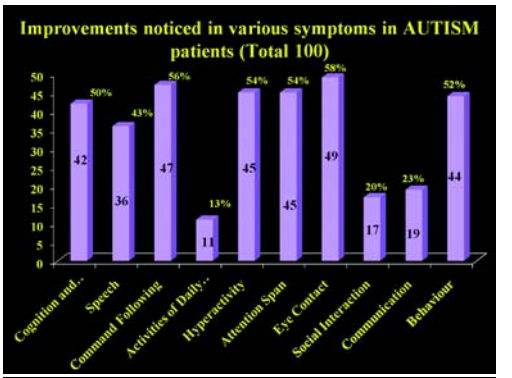
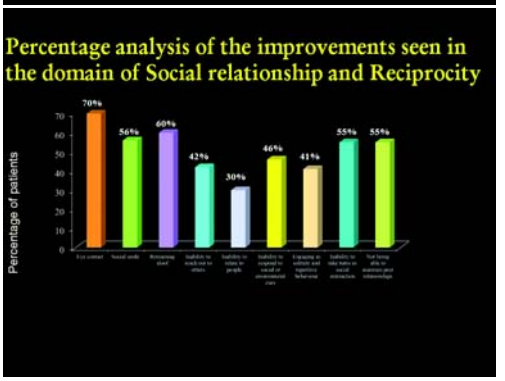
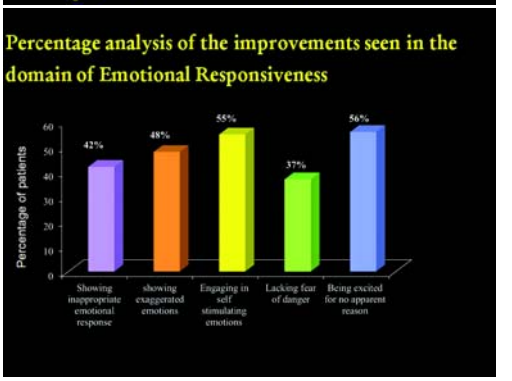
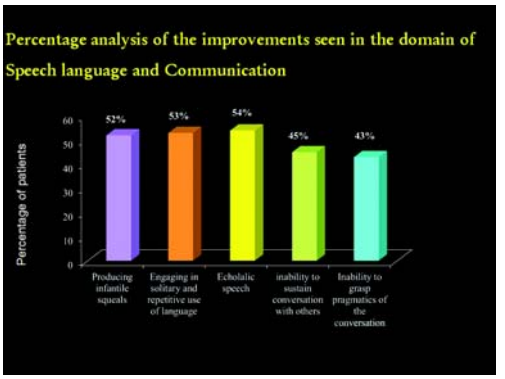
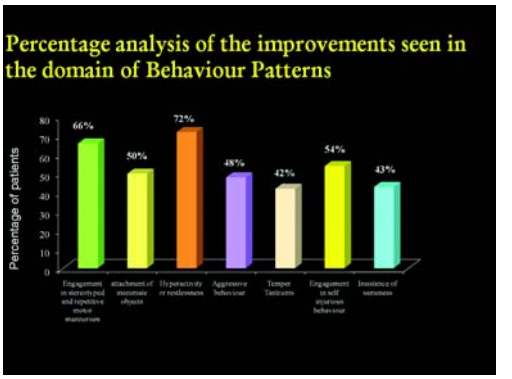


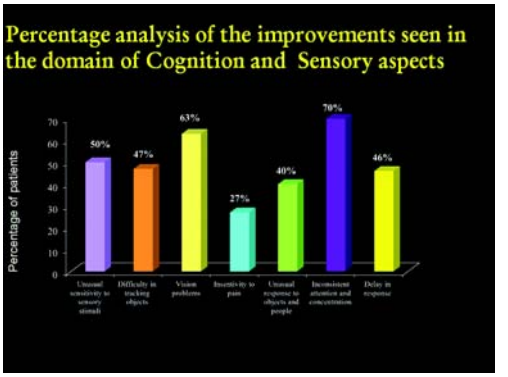












brain metabolism which correlated with clinical improvements. Few adverse events including seizures in three patients were controlled with medications. The encouraging results of this leading clinical study provide future directions for application of cellular therapy in autism.

Unpublished

100 patients with diagnosis Autism were included in the analysis. All the patients were assessed on Clinical Global Impression II (CGI-II) scale. 91% patients showed global improvement. 9% did not show any improvement. 70% eye contact, 60% initiated socializing, 56% initiated social smile, 55% started maintaining peer relationships, self stimulating behavior decreased in 55%, 54% reduced echolalic speech,72% showed reduced hyperactivity, 66% shoed reduced repetitive motor mannerisms and 48% showed reduced aggressive behavior.

Future Directions

Several clinical trials using adipose tissue cells, bone marrow cells, umbilical cord blood cells are being carried out all over the world including countries like Mexico, India, USA, Italy, etc. For stem cell therapy to translate as a standard treatment for autism extensive trials are required. Types of cells, route of administration, quantity of cells to be injected, frequency of injections are few factors which need to be tested to optimize the intervention. .

Functional neuroimaging is thought to give more lucid information about neural connectivity. [24] PET - CT scan and Functional MRI (FMRI) scan are most widely used functional neuroimaging techniques. But these techniques need to be studied in detail and standardized.

We hypothesize that cellular transplantation cause functional restoration of specialized neural systems by neuroprotection, neural circuit reconstruction, neural plasticity, neurogenesis and immunomodulation. Individual therapies like occupational therapy, psychological intervention and speech therapy employ the principles of learning to facilitate neural plasticity. In addition, they also provide the opportunity and tools for social engagement. Enhancement of the neural and functional restoration can be optimized by combining these therapies with cellular transplantation.

REFERENCES

1. Mental Health: A Report of the Surgeon General", The Surgeon General of the Public Health Service, 1999, pp. 6-7
2. Ritvo ER, Freeman BJ, Pingree C, Mason-Brothers A, Jorde L, Jenson WR, McMahon WM, Petersen PB, Mo A, Ritvo A. The UCLA-University of Utah epidemiologic survey of autism: prevalence. Am J Psychiatry. 1989 Feb;146(2):194-9.
3. Kumar R, Christian S. Genetics of autism spectrum disorders. Curr Neurol Neurosci Rep.2009;9:188-197

4. Bradstreet, J., Smith, S., Baral, M., Rossignol, D. (2010) Biomarker-guided interventions of clinically relevant conditions associated with autism spectrum disorders and attention deficit hyperactivity disorder, Alternative Medicine Review,15, 1, 15-32.
5. Herbert,M. (2010) Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders, Current Opinion in Neurology, 23, 2,103-110.
6. Yang WH, Jing J, Xiu LJ. Regional cerebral blood flow in children with autism spectrum disorders: a quantitative 99mTc-ECD brain SPECT study with statistical parametric mapping evaluation. Chin Med J. 2011;124(9):1362-1366.
7. Molloy CA, Morrow AL, Meinzen-Derr J, et al. Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunol. 2006;172:198-205.
8. Hashimoto, T., Sasaki, M., Fukumizu, M., Hanaoka, S., Sugai, K., Matsuda, H. (2000) Pediatric neurology, 23(5), 416-20
9. Johnson, C. & Meyers, S. (2007) Council on Children with Disabilities, Identification and Evaluation of Children with Autism Spectrum Disorders, Pediatrics, 120(5),1183-1215.
10. Frith U, Frith C. The social brain: allowing humans to boldly go where no other species has been. Philos Trans R Soc Lond B Biol Sci. 2010 Jan 12;365(1537):165-76.
11. Brothers, L., Ring, B., Kling, A. (1990) Response of neurons in the macaque amygdala to complex social stimuli. Behaviour Brain Research, 41(3), 199-213.
12. Pelphrey, K., Shultz, S., Hudac, C., Vander Wyk, B. (2011) Research review: Constraining heterogeneity: the social brain and its development in autism spectrum disorder. Journal of child psychology and psychiatry, 52(6), 631-44
13. Allison, T., Puce, A. & McCarthy, G. (2000) Social perception from visual cues: Role of the STS region, Trends in Cognitive Sciences,4,267-278.
14. Scott M. Myers, Chris Plauché Johnson. Management of Children With Autism Spectrum Disorders. Pediatrics Vol. 120 No. 5 November 1, 2007 pp. 1162 -1182
15. Bailey A, Phillips W, Rutter M. Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. J Child Psychol Psychiatry. 1996 Jan;37(1):89-126
16. Ichim TE, Solano F, Glenn E, Morales F, Smith L, et al. (2007) Stem cell therapy for autism. J Transl Med. 5 (1):30.
17. Song M, Mohamad O, Gu X, Wei L, Yu SP. Restoration of Intracortical and Thalamocortical Circuits after Transplantation of Bone Marrow Mesenchymal Stem Cells into the Ischemic Brain of Mice. Cell Transplant. 2013;22(11):2001-15
18. Siniscalco D. (2012) Stem Cell Research: An Opportunity for Autism Spectrum Disorders Treatment. Autism. 2:2
19. Siniscalco D, Sapone A, Cirillo A, Giordano C, Maione S, et al. (2012) Autism spectrum disorders: is mesenchymal stem cell personalized therapy the future? J

Biomed Biotechnol. 2012: 480289.

20. Alok Sharma, Nandini Gokulchandran, Prerna Badhe, Pooja Kulkarni, Priti Mishra, Akshata Shetty and Hemangi Sane. An Improved Case of Autism as Revealed by PET CT Scan in Patient Transplanted with Autologous Bone Marrow Derived Mononuclear Cells. J Stem Cell Res Ther 2013, 3:2
21. Alok Sharma, Nandini Gokulchandran, Akshata Shetty, Hemangi Sane, Pooja Kulkarni and Prerna Badhe. Autologous Bone Marrow Mononuclear Cells may be Explored as a Novel. Potential Therapeutic Option for Autism. J Clin Case Rep 2013, 3:7
22. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Anjana Nagrajan, Amruta Paranjape, Pooja Kulkarni, Akshata Shetty, Priti Mishra, Mrudula Kali, Hema Biju, Prerna Badhe. Autologous bone marrow mononuclear cell therapy for autism - an open label proof of concept study. Stem cell international. 2013 Volume 2013 (2013), Article ID 623875, 13 page
23. Lv YT, Zhang Y, Liu M, Qiuwaxi JN, Ashwood P, Cho SC, Huan Y, Ge RC, Chen XW, Wang ZJ, Kim BJ, Hu X. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. J Transl Med. 2013 Aug 27;11(1):196.
24. Schifter T., Hoffman, J., Hatten, H. Jr., Hanson, M., Coleman, R., DeLong G. (1994) Neuroimaging in infantile autism, Journal of child neurology, 9(2), 155-61.

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

– Hellen Keller

9

Stem Cell Transplantation for Cerebral Palsy

Cerebral palsy (CP) is a non progressive encephalopathy with clinical syndrome of restricted movement and posture with diverse etiologies. (1) Its development could be attributed to prenatal, perinatal or post natal factors. Evidence suggests that prenatal factors result in 70-80% of cases of cerebral palsy. (2) Cerebral palsy (CP) is known to affect 2/1000 live-born children. The symptoms of CP vary in terms of severity. The main symptoms include muscle spasticity, muscle weakness, uncontrolled movements, impaired mobility, speech impairment and/or challenges in eating, dressing, bathing, etc depending on the area of the brain affected. Movement dysfunction is often accompanied by visual impairment, hearing loss, osteoporosis, learning disabilities, cognition impairment, behavioral issues and seizures. Risk factors for cerebral palsy include prenatal anemia, improper nutrition, infections, premature delivery, etc. Hypoxia and ischemia are the major risk factors prenatally and during delivery.

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.

Unmet medical needs

The prevalence of CP is increasing due to decrease in mortality of low birth weight infants and increase in the rate of CP in these children. Hence, establishing a standard therapeutic approach is the focus of researchers and clinicians all over the world. Although the available treatment options are helpful in managing the symptoms to some extent, none of them repair the underlying damaged brain. There are no definitive treatment options to accelerate the development of cerebral palsy patients.

Role of stem cell therapy in Cerebral palsy

One of the common causes of cerebral palsy is hypoxic ischemia. The underlying neuropathology of CP mainly includes periventricular leukomalcia (PVL). It consists of diffuse cerebral white matter injury with or without focal necrosis. (7) In PVL, there is also loss of pre-myelinating oligodendrocytes (pre-OLs) along with astrogliosis and microglial infiltration. (8) The loss of pre-OLs lead to disruption in the production of mature OLs which further leads to disturbance in myelination followed by neuronal dysfunctions. (9,10) In CP, another contributing factor is the microglial activation which instigates the secretion of tumor necrosis factor alpha (TNF-?), interferon gamma (INF-?), Interleukin -1 beta (IL-1?), superoxide radicals, nitrogen species, glutamates, adenosine exerting a toxic effect on neurons and oligodendrocytes.(11) Stem cell therapy regulates these cellular mechanisms. Stem cells migrate and home onto the damaged areas and initiate repair process. They exert an anti-inflammatory effect by reducing the levels of TNF-?,IL-1?, IL-1?, IL-6 and increasing levels of IL-10 (12); therefore, enhancing the endogenous brain repair. Stem cells also restore the damaged myelin by replacing lost OLs and pre-OLs.

Animal studies

Various preclinical studies have demonstrated the potential of stem cell therapy in cerebral palsy. Administration of these cells in animal models have led to survival, homing and differentiation into neurons, oligodendrocytes, astrocytes, etc. (13,14) The homing property of these cells was confirmed by Chen et al, who transplanted magnetically labeled mesenchymal stem cells in a model of perinatal brain injury and found that these cells migrate to lesion sites and proliferate.(15)

Woodbury et al. have demonstrated the differentiation of bone marrow cells into neurons in adult rats. (16) Similarly, studies have shown that umbilical cord blood stem cells proliferate into neural cells via Sonic hedgehog (Shh) signaling pathway. (17) Park et al reported differentiation of clonal neural stem cells (NSCs) into neurons and oligodendrocytes. (18) Titomanlio et al implanted neurosphere-derived precursors in neonatal mouse models which migrated to the lesion site and differentiated into oligodendrocyte and neurons and triggered reduction in lesion size alongwith improvement in memory performance. (19) Transplantation of umbilical cord blood cells in rat models have shown to improve sensorimotor deficits along with other neurological functions. (20-25) Other cells such as multipotent progenitor cells (MPCs) and oligodendrocyte precursor cells were also found to be efficacious in rat models. (26, 27)

Human studies

Not many human clinical studies have been performed till date in CP. Few researchers have reported a positive outcome of intravenous and intrathecal administration of cord blood (CB) cells (28-34) Li et al reported a case of intravenous autologous BMSCs transplantation, wherein improvements were observed after 6 months. (35-37) Similarly, other studies involving intrathecal autologous BMSCs have

also demonstrated motor and functional improvements. (38) 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. (39) Chen et al and Luan et al administered neural stem cell like cells and neural progenitor cells respectively. They reported these cells to be safe and efficacious. (40,41)

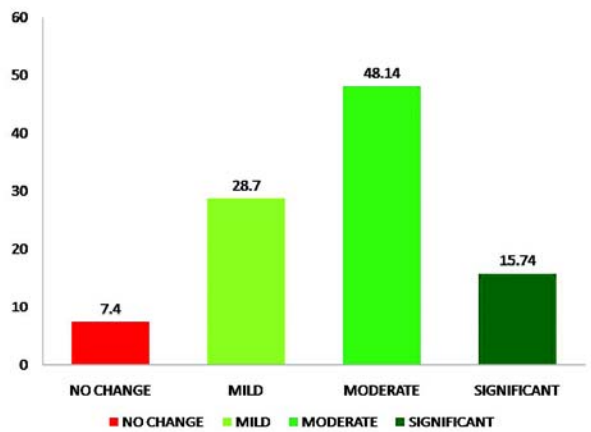
Our results

Published data

Two case reports have been published demonstrating the beneficial effects of autologous bone marrow mononuclear cell intrathecal transplantation. The results were supported by PET CT scans of brain. (42-45)

Unpublished data

108 patients with diagnosis of cerebral palsy were included in the analysis. These patients had attained a plateau stage with respect to symptomatic and functional improvements. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant improvements. The symptoms included were oromotor/speech, balance, trunk activity, upper limb activity, lower limb activity, muscle tone, ambulation and Activities of Daily Living. Mild improvement was defined as improvements till 3 of the symptoms mentioned. Moderate was considered when 4 to 6 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 7 to 8 of the symptoms. Analysis revealed that out of 108 patients, 7.4% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 28.7% of patients, moderate in 48.14% of patients, whereas, 15.74% of patients showed significant improvements. Most of the patients contributed to the moderate improvements. The possible factors associated with this could be the time of intervention, presence of seizures along with cerebral palsy, which is quite common.



Future directions

Various countries all over the world are performing clinical trials in this field (42) A double-blinded, placebo-controlled study is being conducted by Charles S. Cox, Jr., M.D., the Children's Fund, Inc, USA, comparing the safety and effectiveness of banked cord blood to bone marrow stem cells. (43)

The future research should focus on studying the effect of intervention in chronic models of CP as almost all the preclinical studies have been carried out in acute CP. It should also focus on the types, sources and number of cells to be administered, time and frequency of transplantation. Appropriate outcome measures and monitoring tools need to be standardized to study the effect of intervention.

REFERENCES

1. Richer LP, Dower NA, Leonard N, Chan AKJ and Robertson CMT. Familial Recurrence of Cerebral Palsy with Multiple Risk Factors. Case Rep Pediatr. 2011; 2011: 307857.
2. Jacobsson B, Hagberg G. Antenatal risk factors for cerebral palsy. Best Pract Res Clin Obstet Gynaecol. Jun 2004;18(3):425-36
3. Venter A, Leary M, Schoeman J, et al. Hyperbaric oxygen treatment for children with cerebral palsy. S Afr Med J. 998;88:1362-1363
4. Cronje F. Hyperbaric oxygen therapy for children with cerebral palsy. S fr Med J. 1999;89:359-361
5. Montgomery D, Goldberg J, Amar M, et al. Effects of hyperbaric oxygentherapy on children with spastic diplegic cerebral palsy: a pilot project.Undersea Hyperb Med. 1999;26:235-242
6. Graham HK, Aoki KR, Auii-Rämö I, Boyd RN, Delgado MR, Gaebler- Spira DJ, Gormley ME, Guyer BM, Heinen F, Holton AF, Matthews D, Molnaers G, Motta F, Garcia Ruiz PJ, Wissel J. (2000) Recommendations for the use of botulinum toxin type A in the management of cerebral palsy. Gait Posture 11: 67-79.
7. Folkerth RD. Neuropathologic substrate of cerebral palsy. J Child Neurol. 2005;20(12):940-9.
8. Volpe JJ. Cerebral white matter injury of the premature infant-more common than you think. Pediatrics. 2003;112:176-9.
9. Miron VE, Kuhlmann T, Antel JP. Cells of the oligodendroglial lineage, myelination, and remyelination. Biochim Biophys Acta. 2011; 812(2):184-93.
10. Susuki K. Myelin: A Specialized Membrane for Cell Communication. Nature Education 2010; 3(9):59
11. Hansson E, Ronnback L. Glial neuronal signaling in the central nervous system. FASEB J 2003; 17:341-348
12. Brenneman M, Sharma S, Harting M, Strong R, Cox CS Jr, Aronowski J, GrottaJC,Savitz SI. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow

Metab. 2010;30(1):140-9.

13. Qu SQ, Luan Z, Yin GC, Guo WL, Hu XH, Wu NH, Yan FQ, Qian YM. Transplantation of human fetal neural stem cells into cerebral ventricle of the neonatal rat following hypoxic-ischemic injury: survival, migration and differentiation. Zhonghua Er Ke Za Zhi. 2005;43(8):576-9.
14. Zheng T, Rossignol C, Leibovici A, Anderson KJ, Steindler DA, Weiss MD.Transplantation of multipotent astrocytic stem cells into a rat model of neonatal hypoxic-ischemic encephalopathy. Brain Res. 2006;1112(1):99-105.
15. Chen G, Wang Y, Xu Z, Fang F, Xu R, Wang Y, Hu X, Fan L, Liu H. Neural stem cell-like cells derived from autologous bone mesenchymal stem cells for the treatment of patients with cerebral palsy. J Transl Med. 2013;11:21.
16. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons.Journal of Neuroscience Research. 2000;61:364-370.
17. Wang XL, Zhao YS, Hu MY, Sun YQ, Chen YX, Bi XH. Umbilical cord blood cells regulate endogenous neural stem cell proliferation via hedgehog signaling in hypoxic ischemic neonatal rats. Brain Res. 2013;1518:26-35
18. Park KI, Himes BT, Stieg PE, Tessler A, Fischer I, Snyder EY. Neural stem cells may be uniquely suited for combined gene therapy and cell replacement: Evidence from engraftment of Neurotrophin-3-expressing stem cells in hypoxic-ischemic brain injury. Exp Neurol. 2006;199(1):179-90.
19. Titomanlio L, Bouslama M, Le Verche V, Dalous J, Kaindl AM, Tsenkina Y, Lacaud A, Peineau S, El Ghouzzi V, Lelièvre V, Gressens P. Implanted neurosphere-derived precursors promote recovery after neonatal excitotoxic brain injury. Stem Cells Dev. 2011;20(5):865-79.
20. Rosenkranz K, Tenbusch M, May C, Marcus K, Meier C. Changes in Interleukin-1 alpha serum levels after transplantation of umbilical cord blood cells in a model of perinatal hypoxic-ischemic brain damage. Ann Anat. 2013;195(2):122-7.
21. Wasielewski B, Jensen A, Roth-Härer A, Dermietzel R, Meier C. Neuroglial activation and Cx43 expression are reduced upon transplantation of human umbilical cord blood cells after perinatal hypoxic-ischemic injury. Brain Res. 2012;1487:39-53
22. Meier C, Middelanis J, Wasielewski B, Neuhoff S, Roth-Haerer A, Gantert M, Dinse HR, Dermietzel R, Jensen A. Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells. Pediatr Res. 2006;59(2):244-9.
23. Geissler M, Dinse HR, Neuhoff S, Kreikemeier K, Meier C. Human umbilical cord blood cells restore brain damage induced changes in rat somatosensory cortex. PLoS One. 2011;6(6):e20194.
24. Pimentel-Coelho PM, Magalhães ES, Lopes LM, deAzevedo LC, Santiago MF, Mendez-Otero R. Human cord blood transplantation in a neonatal rat model of hypoxic-ischemic brain damage: functional outcome related to neuroprotection

in the striatum. Stem Cells Dev. 2010;19(3):351-8.

25. Tanaka N, Kamei N, Nakamae T, Yamamoto R, Ishikawa M, Fujiwara H, Miyoshi H, Asahara T, Ochi M, Kudo Y. CD133+ cells from human umbilical cord blood reduce cortical damage and promote axonal growth in neonatal rat organ co-cultures exposed to hypoxia. Int J Dev Neurosci. 2010;28(7):581-7
26. Yasuhara T, Matsukawa N, Yu G, Xu L, Mays RW, Kovach J, Deans RJ, Hess DC, Carroll JE, Borlongan CV. Behavioral and histological characterization of intrahippocampal grafts of human bone marrow-derived multipotent progenitor cells in neonatal rats with hypoxic-ischemic injury. Cell Transplant. 2006;15(3):231-8.
27. Webber DJ, van Blitterswijk M, Chandran S. Neuroprotective effect of oligodendrocyte precursor cell transplantation in a long-term model of periventricular leukomalacia. Am J Pathol. 2009;175(6):2332-42
28. Lee et al. Safety and feasibility of countering neurological impairment by intravenous administration of autologous cord blood in cerebral palsy. Journal of Translational Medicine 2012, 10:58
29. Yang et al. Effect of Umbilical Cord Mesenchymal Stem Cell Transplantation Therapy for Cerebral Palsy on Motor Function. Progress in modern Biomedicine. 2012-02
30. Fernando Ramirez, David A. Steenblock, Anthony G. Payne And Lyn Darnall. Umbilical Cord Stem Cell Therapy For Cerebral Palsy. Med Hypotheses Res 2006; 3: 679-686
31. Papadopoulos KI, Low SS, Aw TC, Chantarojanasiri T. Safety and feasibility of autologous umbilical cord blood transfusion in 2 toddlers with cerebral palsy and the role of low dose granulocyte-colony stimulating factor injections. Restor Neurol Neurosci. 2011; 29(1): 17-22.
32. Jensen A, Hamelmann E. First autologous cell therapy of cerebral palsy caused by hypoxic-ischemic brain damage in a child after cardiac arrest-individual treatment with cord blood. Case Rep Transplant. 2013;2013:951827.
33. Wang L, Ji H, Zhou J, Xie J, Zhong Z, Li M, Bai W, Li N, Zhang Z, Wang X, Zhu D, Liu Y, Wu M. Therapeutic potential of umbilical cord mesenchymal stromal cells.
34. Min K, Song J, Kang JY, Ko J, Ryu JS, Kang MS, Jang SJ, Kim SH, Oh D, Kim MK, Kim SS, Kim M. Umbilical cord blood therapy potentiated with erythropoietin for children with cerebral palsy: a double-blind, randomized, placebo-controlled trial. Stem Cells. 2013 Mar;31(3):581-91
35. Li et al. Treatment of one case of cerebral palsy combined with posterior visual pathway injury using autologous bone marrow mesenchymal stem cells Journal of Translational Medicine 2012, 10:100
36. Purandare C, Shitole DG, Belle V, Kedari A, Bora N, Joshi M. Therapeutic potential of autologous stem cell transplantation for cerebral palsy. Case Rep Transplant. 2012;2012:825289

37. Wang X, Cheng H, Hua R, Yang J, Dai G, Zhang Z, Wang R, Qin C, An Y. Effects of bone marrow mesenchymal stromal cells on gross motor function measure scores of children with cerebral palsy: a preliminary clinical study. Cytotherapy. 2013 Dec;15(12):1549-62.
38. Hassan et al. Stem Cell Transplantation in Egyptian Patients with Cerebral Palsy. Egypt J Neurol Psychiat Neurosurg. 2012; 49(2): 117-122
39. V. I. Seledtsov, M. Yu. Kafanova, S. S. Rabinovich et al. Cell Therapy of Cerebral Palsy. Cell Technologies in Biology and Medicine, Vol. 1, No. 2, April, 2005. pp. 84-88
40. Chen G, Wang Y, Xu Z, Fang F, Xu R, Wang Y, Hu X, Fan L, Liu H. Neural stem cell-like cells derived from autologous bone mesenchymal stem cells for the treatment of patients with cerebral palsy. J Transl Med. 2013;11:21.
41. Luan Z, Liu W, Qu S, Du K, He S, Wang Z, Yang Y, Wang C, Gong X. Effects of neural progenitor cell transplantation in children with severe cerebral palsy. Cell Transplant. 2012; 21 Suppl 1:S91-8.
42. http://www.clinicaltrials.gov
43. http://www.sciencedaily.com/releases/2013/12/131210172443.htm
44. Alok Sharma, Hemangi Sane, Amruta Paranjape, Nandini Gokulchandran, Pooja Kulkarni and Anjana Nagrajan, Prerna Badhe. Positron Emission Tomography - Computer Tomography scan used as a monitoring tool following cellular therapy in Cerebral Palsy and Mental Retardation - A Case Report. Case Reports in Neurological Medicine. Volume 2013, Article ID 141983, 6 pages
45. Dr. Alok Sharma, Ms. Pooja Kulkarni, Dr. Hemangi Sane, Dr. Nandini Gokulchandran, Dr. Prerna Badhe, Dr. Mamta Lohia, Dr. Priti Mishra. Positron Emission Tomography- Computed Tomography scan captures the effects of cellular therapy in a case of cerebral palsy. Journal of clinical case reports. 2012 J Clin Case Rep 2:195.

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

– Werner Erhard

10

Role of Stem Cells In Muscular Dystrophy

Muscular dystrophy (MD) is a heterogeneous group of genetic disorders primarily affecting the striated muscles of the body. It is characterized by progressive weakness and wasting of these muscles (1) In MD, there is mutation in different components of dystrophin-glycoprotein complex (DGC) which links the extracellular matrix in muscle to the intracellular cytoskeleton. (2) This results in destabilization of the muscle membrane, increased muscle fragility and degeneration, and muscle wasting. (3)

The types of MD vary according to severity, age of onset, and selective involvement of muscle groups. The most common types are Duchenne, Becker, limb girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal and Emery-Dreifuss (4) Abnormal gait (waddling gait) with frequent falls, difficulty in rising from the floor and climbing stairs, pseudohypertrophy of calves, positive Gowers' sign and scoliosis or kyphosis are a few common symptoms presented by the affected population of MD. (5)

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

Unmet Medical Needs

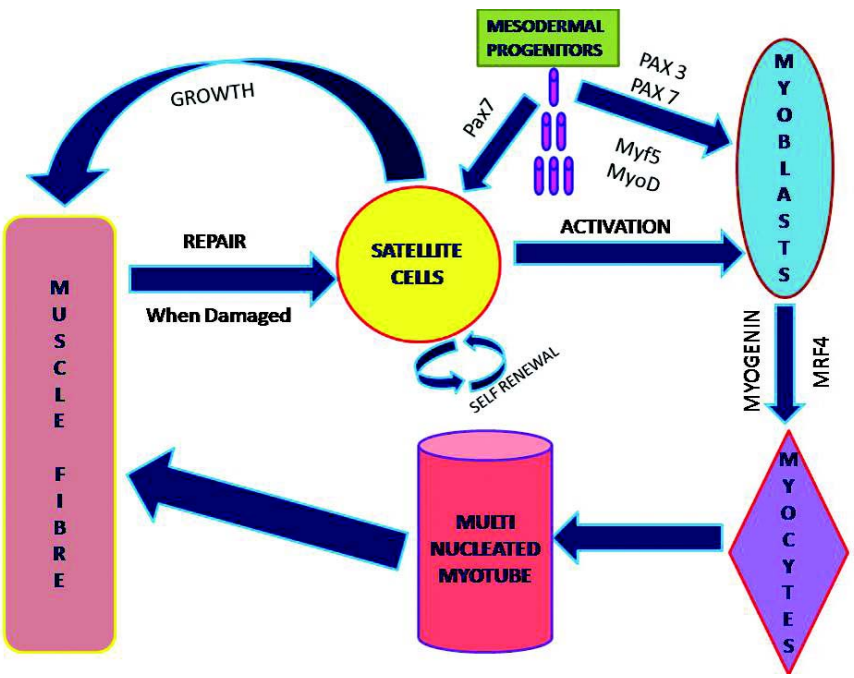
The available treatments address only the symptoms but fail to act at a cellular level. They do not regenerate the lost muscles or reverse the pathology of the disease. The muscle power progressively decreases and no treatment modality helps to improve it. Also, as MD is a genetic disorder, no treatment repairs the core mutation of the genes involved. Gene therapy is being explored but has not yet been established as a clinical

application. No standard therapeutic modality has been successful to halt the progression of the disease or increase the survival.

Role of stem cell therapy in MD

Wallace et al postulated the underlying pathogenic mechanism of muscular dystrophy to be an imbalance between muscle damage or degeneration and muscle repair through stem-cell mediated regeneration. (7) Continuous damage to the cytoskeleton of muscle fibres leads to premature exhaustion of the muscle stem cell pool that maintains muscle integrity during normal use and exercise. Stem cell therapy holds promise as a treatment for muscular dystrophy by providing cells that can both deliver functional muscle proteins and replenish the stem cell pool (8)

The mechanisms by which stem cells may function and reverse the effects of cell death include differentiation, cell fusion, and secretion of cytokines or paracrine effects. (9-11) These cells have the capacity to mobilize and exert their reparative effects at the site of injury. They are known to enhance angiogenesis and contribute to neovascularization by producing signaling molecules such as vascular endothelial growth factors (VEGF) and fibroblast growth factors (FGF2). (19) Along with increase in angiogenesis ,they also promote tissue remodeling, prevent apoptosis, decrease inflammation, release growth factors and activate the satellite cells. (16) In animal studies, these cells have shown to produce the deficient proteins and make new muscle cells which fuse with the host fibers. Satellite cells, the adult skeletal muscle progenitor cells, are commonly considered to be the main cell type involved in skeletal muscle regeneration.



Animal studies

Preclinical studies in mouse models of various muscular dystrophies have demonstrated that myoblasts on transplantation into dystrophic muscle; could repair damaged myofibres. Although, myoblast transplantation did not show effective results due to rapid death of most injected myoblasts and the failure of injected myoblasts to migrate more than ~0.5 mm away from the injection site (12) Hence other cells such as adult-derived stem cells, including bone marrow-derived stem cells, blood- and muscle-derived CD133+ cells, muscle-derived stem cells (MDSC), side population (SP) cells and mesoangioblasts have been tested in animal models (13-20)

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

A study carried out to track the fate of bone marrow derived stem cells (BMSC) in mouse models of muscular dystrophy using green fluorescent protein-positive (GFP+) demonstrated that transplanted BMSC differentiate into muscle cells via repopulation of the muscle stem cell compartment.(23) Similar test was carried out using 3H-thymidine labeled human bone marrow derived MSCs. (24) Embryonic stem cells (ESC) have also shown its potential in muscle regeneration. On injecting wild type ESCs into the mdx blastocysts, mice with improved pathology and function were produced. (25-27) However, due to ethical issues and immune rejection not many studies have been carried out on humans using ESCs. Experimental studies have also been carried out where human umbilical cord blood (HUCB) cells have shown to differentiate into muscle cells. (28,29)

Human studies

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

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

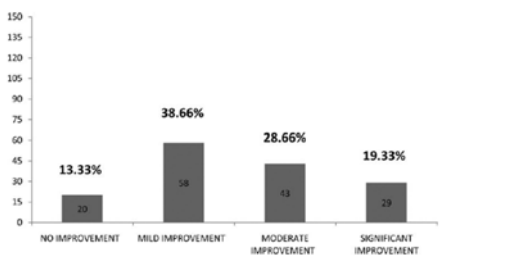
the feasibility of employing double transplantations of autologous bone marrow mesenchymal stem cells (BMSC) and umbilical cord mesenchymal stem cells (UMSC) in the treatment of progressive muscular dystrophy (PMD). Total effective rate was 82.9% concluding it as a safe and effective treatment. (42)

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

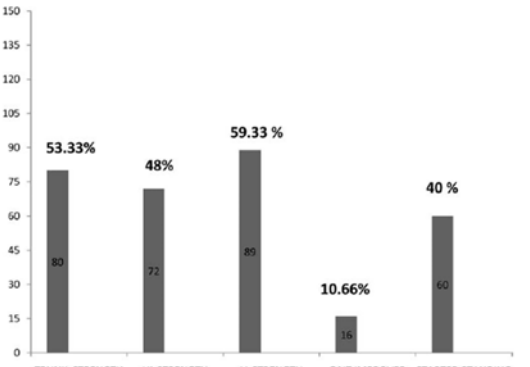
Our results

Published data

A study was carried out on 150 patients diagnosed with Muscular Dystrophy. These included Duchenne Muscular Dystrophy, Limb Girdle Muscular Dystrophy and Becker Muscular Dystrophy variants. They were administered with autologous bone marrow derived mononuclear cells intrathecally and intramuscularly at the motor points of the antigravity weak muscles followed by vigorous rehabilitation therapy. No significant adverse events were noted. Assessment after transplantation showed neurological improvements in trunk muscle strength, limb strength on Manual Muscle Testing (MMT), with Gait improvements and a shift on assessment scales such as Functional Independence Measure (FIM) ; Brooke and Vignos scale. Further, Imaging and Electrophysiological studies also showed significant changes in selective cases. On a mean follow up of 12 months ± 1 month, overall 86.67% cases showed symptomatic and functional improvements, with 6 patients showing changes with respect to muscle regeneration and decrease in fatty infiltration on musculoskeletal Magnetic Resonance Imaging (MRI) and 9 showing improved muscle electrical activity on Electromyography (EMG). 53% cases showed increase in trunk muscle strength, 48% showed increase



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



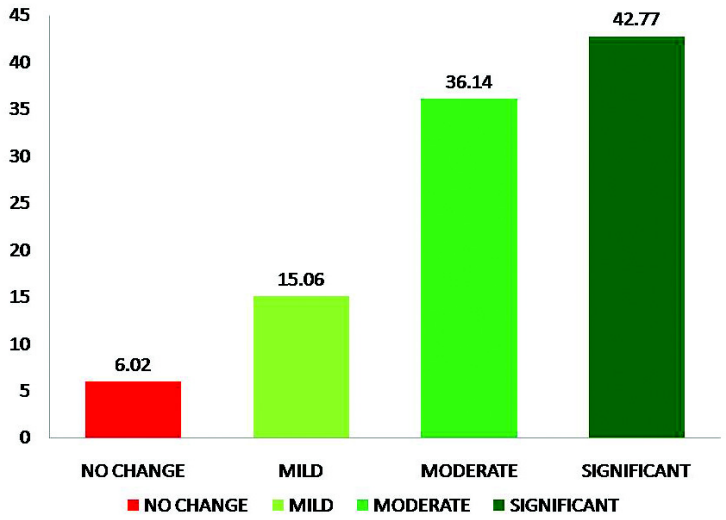
upper limb strength, 59 % in lower limb strength and about 10 % showed improved gait. This data was statistically analyzed using student's paired t-test and found to be significant. The results show that this treatment is safe, efficacious and also improves the quality of life of patients suffering from Muscular Dystrophy

Unpublished data

332 patients diagnosed with muscular dystrophy were analyzed. These patients had reached a plateau stage in the course of their disease. 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. Mild improvement was defined as improvements till 2 of the symptoms mentioned. Moderate was considered when 3 to 4 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 5 to 6 of the symptoms. In case of patients with muscular dystrophy, out of 332 patients, 6.02% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 15.06% of patients, moderate in 36.14% of patients, whereas, 42.77% of patients showed significant improvements. Further analysis revealed that patients with Limb Girdle Muscular Dystrophy contributed to the majority of moderate to significant improvements and around more than 90% of patients showed halting of progression of their disease. Patients with Duchenne Muscular Dystrophy contributed to the mild to moderate amount of improvements, with slowing down of progression of the disease in many of them.

Future Directions

In disorders involving muscular damage, the side population (SP) cells are responsible for production of fibro-adipogenic precursors (FAPs), fibroblasts and ultimately



adipocytes as a response to the injury. (50) Hence, fibrosis and fat deposition is observed in most chronic muscular dystrophies. This may hinder the repair and regenerative potential of the transplanted stem cells which may decrease the efficacy of intervention. Hence, the future research should be focused on manipulating the cells so as to bypass the fat generation and to stimulate muscle regeneration. Recently, dental pulp stem cells have also proved their capability in regenerative research. Thus, more clinical studies for MD should be designed involving them. Studies carried out so far lack the inclusion of imaging techniques. These techniques may monitor the disease activity and assess the effectiveness of therapeutic intervention. Furthermore, facilitating the translation of stem cell therapy from bench to bedside.

REFERENCES

1. Rahimov F, Kunkel LM. The cell biology of disease: cellular and molecular mechanisms underlying muscular dystrophy. J Cell Biol. 2013 May 13;201(4):499-510.
2. Rando, T.A. The dystrophin-glycoprotein complex, cellular signaling, and the regulation of cell survival in the muscular dystrophies. Muscle Nerve. 24(12):1575; 2001
3. Zejing Wang, Jeffrey S. Chamberlain, Stephen J. Tapscott, Rainer Storb,Gene Therapy in Large Animal Models of Muscular Dystrophy ILAR J. 2009 ; 50(2): 187-198
4. Emery AE. The muscular dystrophies. Lancet 2002; 359 (9307): 687-695.
5. Goyenvalle, A.; Seto, J.T.; Davies, K.E.; Chamberlain, J. Therapeutic approaches to muscular dystrophy. Hum Mol Genet. 20(R1):R69-78; 2011
6. Emery AEH. Duchenne muscular dystrophy. Oxford: Oxford university press, 1993.
7. Wallace, G.Q.; McNally, E.M. Mechanisms of muscle degeneration, regeneration, and repair in the muscular dystrophies. Annual Review of Physiology. 71:37-57; 2009
8. Meregalli, M.; Farini, A.; Colleoni, F.; Cassinelli, L.; Torrente, Y. The Role of Stem Cells in Muscular Dystrophies.Curr Gene Ther. 12(3):192-205; 2012
9. D. Orlic, J. Kajstura, S. Chimenti, D. M. Bodine, A. Leri, and P. Anversa. Bone marrow stem cells regenerate infarcted myocardium. Pediatric Transplantation 2003; 7(3): 86-88
10. E. Y. Plotnikov, T. G. Khryapenkova, and T. G. Khryapenkova. Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. Journal of Cellular and Molecular Medicine. 2008;12(5):1622-1631.
11. A. Cselenyák, E. Pankotai, E. M. Horváth, L. Kiss, and Z. Lacza. Mesenchymal stem cells rescue cardiomyoblasts from cell death in an in vitro ischemia model via direct cell-to-cell connections. BMC Cell Biology 2010;11(29).
12. Fan Y et al. Rapid death of injected myoblasts in myoblast transfer therapy. Muscle

Nerve. 1996;19(7):853-860.

13. Gussoni, E. et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 1999; 401: 390-394
14. Minasi MG, Riminucci M, De Angelis L et al. The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development. 2002;129(11):2773-83
15. Jiang, Y. et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41-49.
16. Torrente, Y. et al. Human circulate AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. J. Clin. Invest. 2004; 114: 182-195
17. Dezawa, M. et al. (2005) Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science 2005; 309:314-317
18. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell. 2005 Jul 29;122(2):289-301
19. Dellavalle, A. et al. Pericytes of human post-natal skeletal muscle are committed myogenic progenitors, distinct from satellite cells, and efficiently repair dystrophic muscle. Nat. Cell Biol. 2007; 9: 255-267.
20. Flix B, Suárez-Calvet X, Díaz-Manera J, Santos-Nogueira E, Mancuso R, Barquinero J, Navas M, Navarro X, Illa I, Gallardo E. Bone marrow transplantation in dysferlin-deficient mice results in a mild functional improvement. Stem Cells Dev. 2013 Nov 1;22(21):2885-94.)
21. Wallace GQ, Lapidos KA, Kenik JS, McNally EM. Long-term survival of transplanted stem cells in immunocompetent mice with muscular dystrophy. Am J Pathol. 2008 Sep;173(3):792-802
22. Bachrach E, Li S, Perez AL et al. Systemic delivery of human microdystrophin to regenerating mouse dystrophic muscle by muscle progenitor cells. Proc Natl Acad Sci U S A. 2004 Mar 9;101(10):3581-6
23. LeBarge MA, Blau HM. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. Cell 2002;111:589-601
24. Liu TY, Li JL, Yao XL, et al. Transplantation of 3H-thymidine-labeled human bone marrow-derived mesenchymal stem cells in mdx mice. Di Yi Jun Yi Da Xue Xue Bao. 2005 May;25(5):498-502. Chinese.
25. Darabi R, Gehlbach K, Bachoo RM,et al. Functional skeletal muscle regeneration from differentiating embryonic stem cells. Nat Med. 2008 Feb;14(2):134-43.
26. Darabi R, Baik J, Clee M, Kyba M, Tupler R, Perlingeiro RC. Engraftment of embryonic stem cell-derived myogenic progenitors in a dominant model of muscular dystrophy. Exp Neurol. 2009 Nov;220(1):212-6

27. Stillwell E, Vitale J, Zhao Q, et al. Blastocyst injection of wild type embryonic stem cells induces global corrections in mdx mice. PLoS One. 2009;4(3):e4759. Epub 2009 Mar 11

28. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol. 2000 Apr;109(1):235-42.

29. Zhang C, Chen W, Xiao LL, Tan EX, Luo SK, Zheng D, Ye X, Li Z, Lu XL, Liu Y. Allogeneic umbilical cord blood stem cell transplantation in Duchenne muscular dystrophy. Zhonghua Yi Xue Za Zhi. 2005 Mar 2;85(8):522-5.

30. Briggs D, Morgan JE. Recent progress in satellite cell/myoblast engraftment -- relevance for therapy. FEBS J. 2013 Sep;280(17):4281-93

31. Huard J, Bouchard JP, Roy R, Malouin F et al. Human myoblast transplantation: preliminary results of 4 cases. Muscle Nerve. 1992;15(5):550-60.

32. Gussoni E, Pavlath GK, Lanctot AM et al. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. Nature. 1992;356(6368):435-8.

33. Emanuela Gussoni, Helen M. Blau & Louis M. Kunkel et al. The fate of individual myoblasts after transplantation into muscles of DMD patients. Nature Medicine 1997; 3: 970 - 977 (1997)

34. Emanuela Gussoni, Richard R. Bennett, Kristina R. Muskiewicz.et al Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. J Clin Invest. 2002;110(6):807-814

35. Karpati G, Ajdukovic D, Arnold D. et al Myoblast transfer in Duchenne muscular dystrophy. Ann Neurol. 1993;34(1):8-17

36. Mendell JR, Kissel JT, Amato AA et al.Myoblast transfer in the treatment of Duchenne's muscular dystrophy. N Engl J Med. 1995;333(13):832-8.

37. Tremblay JP, Malouin F, Roy R et al. Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. Cell Transplant. 1993;2(2):99-112

38. Neumeyer AM, Cros D, McKenna-Yasek D, et al. Pilot study of myoblast transfer in the treatment of Becker muscular dystrophy. Neurology. 1998 Aug;51(2): 589-92

39. Vilquin JT, Marolleau JP, Sacconi S et al. Normal growth and regenerating ability of myoblasts from unaffected muscles of facioscapulohumeral muscular dystrophy patients. Gene Ther. 2005;12(22):1651-62.

40. J.P. Tremblay, J.P. Bouchard, F. Malouin et al. Myoblast transplantation between monozygotic twin girl carriers of Duchenne muscular dystrophy. Neuromuscular Disorders. 1993;3(5-6): 583-592

41. Daniel Skuk, Brigitte Roy, Marlyne Goulet et al. Dystrophin Expression in Myofibers of Duchenne Muscular Dystrophy Patients Following Intramuscular Injections of Normal Myogenic Cells. Molecular Therapy 2004; 9: 475-482

42. Zhang C, Chen W, Xiao LL, et al. Allogeneic umbilical cord blood stem cell transplantation in Duchenne muscular dystrophy. Zhonghua Yi Xue Za Zhi. 2005;85(8):522-5

43. Yang XF, Xu YF, Zhang YB, Functional improvement of patients with progressive muscular dystrophy by bone marrow and umbilical cord blood mesenchymal stem cell transplantations. Zhonghua Yi Xue Za Zhi. 2009;89(36):2552-6.

44. Asakura A. Skeletal Muscle-derived Hematopoietic Stem Cells: Muscular Dystrophy Therapy by Bone Marrow Transplantation. J Stem Cell Res Ther. 2012 Nov;Suppl 11.

45. Y. Torrente, M. Belicchi, C. Marchesi, et al. Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. Cell Transplantation. 2007;16(6):563-577.

46. Dr. A. Sharma, Ms. P. Kulkarni, Dr. G. Chopra, Dr. N. Gokulchandran, Dr. M. Lohia, Dr. P. Badhe. Autologous Bone Marrow Derived Mononuclear Cell Transplantation In Duchenne Muscular Dystrophy-A Case Report. Indian journal of Clinical Practice 2012; 23 (3): 169-72

47. Alok Sharma, Amruta Paranjape, Hemangi Sane, Khushboo Bhagawanani, Nandini Gokulchandran, and Prerna Badhe. Cellular Transplantation Alters the Disease Progression in Becker's Muscular Dystrophy. Case Reports in Transplantation. Volume 2013, Article ID 909328, 7 pages

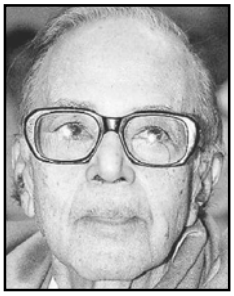
48. Sharma A., Sane, H., Paranjape, A., Badhe, P., Gokulchandran, N., & Jacob, V. (2013). Effect of Cellular Therapy seen on Musculoskeletal Magnetic Resonance Imaging in a Case of Becker's Muscular Dystrophy.Journal of Case Reports, 3(2), 440-447.

49. Alok Sharma, Hemangi Sane, Amruta Paranjape, Khushboo Bhagwanani, Nandini Gokulchandran, Prerna Badhe. Autologous bone marrow mononuclear cell transplantation in Duchenne muscular dystrophy - a case report. American journal of case reports (Ahead of Print

50. Penton CM, Thomas-Ahner JM, Johnson EK, McAllister C, Montanaro F. Muscle side population cells from dystrophic or injured muscle adopt a fibro-adipogenic fate. PLoS One. 2013;8(1):e54553.

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

Sustained efforts in this direction are the Immediate need of the future."



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

11

Role Of Stem Cells In Spinal Cord Injury

Spinal cord injury (SCI), a devastating disease, often results in a severe neurological deficit. It is either caused due to trauma such as road traffic accidents (RTAs), fall from height or non-traumatic events such as infection, loss of blood supply, compression by a cancer or through slow degeneration of the spinal bones (vertebrae). 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).

Currently, there is no cure for SCI. Recovery of the injured spinal cord is 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 further limiting the repair process. (3)

The conventional treatment alternatives available are surgical interventions, medicines and rehabilitation. Their main goal is to stabilize the spine and prevent any secondary complications. But, these treatments fail to repair the neurological damage completely leaving behind few deficits.

Unmet Medical Needs

Presently, all modalities aim at repairing the spine but no surgery or medication repairs the spinal cord. There is no treatment in medical field which helps in neuronal or axonal regeneration. In SCI, due to lost functions, the affected patient has a high level of dependency on the care taker. Rehabilitation and assistive devices are used to improve the ambulation and hand functions but, still the patients' dependence level does not reduce to a great extent. Also, there are no treatment modalities that help improve the sensations which are highly affected by the injury. Loss of bladder and

bowel control is one of the major complications of SCI. Medical intervention available for this fails to exert its effect in case of severe injuries. It is important to track the cellular changes occurring in the cord over the period of any intervention. However, there is no potent investigative monitoring tool available currently to record these changes.

Since, there is a global increase in the incidence of spinal cord injuries, establishing a standard treatment is the need of the hour.

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

Stem cell therapy in spinal cord injury

Animal Studies

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

These pre-clinical studies have helped translate the use of stem cells in humans initiating an array of human clinical studies.

Human Studies

One of the earliest studies used cells from the fetal nervous and haemopoietic tissues in 15 SCI patients with no side effects. (55) However, due to various ethical and medical concerns over embryonic and fetal stem cells, adult stem cells have been tried extensively. In a comparison between a) transplantation of autologous bone marrow cells directly into the SCI sites and administered granulocyte macrophage colony stimulating factor (GM-CSF) {n=2} and b) only administration of GM-CSF{n=1}, sensorymotor improvements were noticed in all three patients at varied time points (56) Safety and feasibility studies for different cells showed these cells to be safe (57-60) Comparitive studies carried out to find the optimum route of administration. Syková et al (61,62) revealed intra-arterial transplants to show more improvements as compared to those intravenous transplants. Chernykh et al, reported neurological improvements in 66.7% of chronic SCI patients who underwent autologous BMSCs transplantation

intravenously as well as at the site of injury. (63) Whereas, Saberi et al. in a similar clinical trial carried out in 4 patients, found improvement in only 1 patient. (64). Geffner et al reported administration of BMSCs via multiple routes to be safe and feasible improved the quality of life in most patients. (65) O.S Abdelaziz administered autologous adult bone marrow mesenchymal stem cell through open surgical intraparenchymal and intralesional injection into the site of cord injury followed by monthly intrathecal injection of stem cells through lumbar or cisternal punctures. Clinical improvement was observed in 30% treated patients. (66) Intramedullary direct injection of MSCs into the injured spinal cord also resulted in changes in MRI and electrophysiological tests along with other functional improvements (67) However, direct injection is an invasive procedure involving risk of secondary injury. Saito et al, Pal et al and Kumar et al reported intrathecal administration to be the optimum route of administration. (68-70) Series of studies also demonstrated the benefits of bone marrow stem cells in SCI. (71-77) In a novel method, using combination of BM mesenchymal stem cells (MSC) and patient's autoimmune T cells, Moviglia et al demonstrated the regeneration phenomenon based on the controlled inflammatory activity at the injured site. Both the patients of the study showed both motor and sensory recovery with no adverse effects. (78) Peripheral stem cells and macrophages have also been reported to show improvements of motor and sensory functions without any critical complications (79,80). Other sources such as cord blood, olfactory ensheathing cells, adipose tissue derived stem cells, etc also showed improvement in sensory-motor functional improvements (81-85) Saberi et al enrolled 33 SCI cases to study the safety of intramedullary Schwann cell transplantation. After a 2 year follow up, there were no tumor formations or other adverse events recorded. (86) Co-transplantation techniques have also been tested and found to be safe. (87) Tianshen Sun et al, in their recent study have reported The synergistic effects of the combined use of olfactory ensheathing cells and Schwann cells enhancing functional recovery in SCI. (88)

To track the behaviour and the fate of the transplanted cells, the cells are labelled with magnetic particles before administration. Callera et al administered CD34+ cells labeled with nanoparticles via lumbar puncture and 6 patients received magnetic beads without stem cells. MRI done 20 and 35 days after transplantation showed that the magnetically labeled CD34+ cells were visible at the lesion site in 5 patients out of 10. These signals were not visible in the control group (89).

Our Results

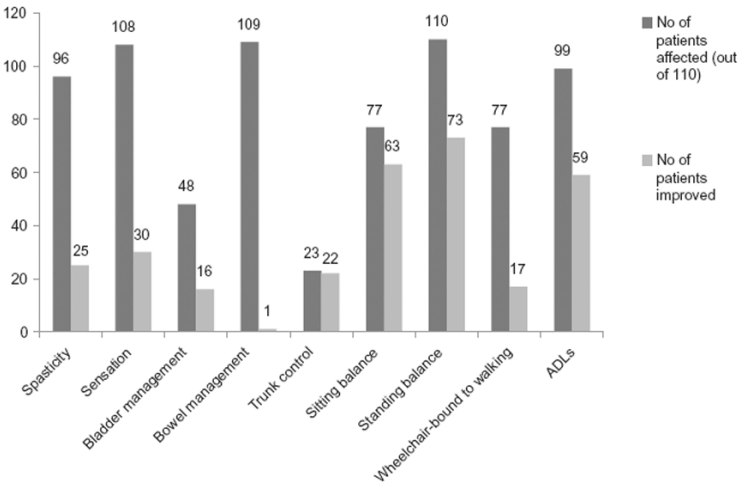
Published data:

1. A detailed analysis of chronic thoracolumbar SCI patients who underwent intrathecal administration of autologous bone marrow mononuclear cells followed by neurorehabilitation was conducted. The study sample included 110 thoracolumbar SCI patients. The outcome was recorded at a mean follow up of 2 years ±1 month. The outcome measures were Functional Independence Measure (FIM) score, American Spinal Injury Association scale (ASIA) and detailed

neurological assessment. Data was statistically analyzed using McNemar's Test to establish significance between the change in symptoms and the intervention.

100 out of 110 (91%) patients showed improvements. Improvement in trunk control was observed in 95.6% cases, bladder management in 33% with respect to shift from indwelling and condom catheter to self intermittent catheterization, partial sensory recovery in 27% and reduction of spasticity in 26%. All the patients showed improvement in postural hypotension. 38% wheelchair bound patients started walking with assistance. Functionally, 27% showed improved activities of daily living (ADLs) and 53.6% showed a positive change in FIM score. 10% cases showed a shift in ASIA scale. A statistically significant association of these symptomatic improvements with the cell therapy intervention was established using McNemar's Test. On electrophysiological studies, 2 showed improvement and 1 showed change in functional MRI. (90)

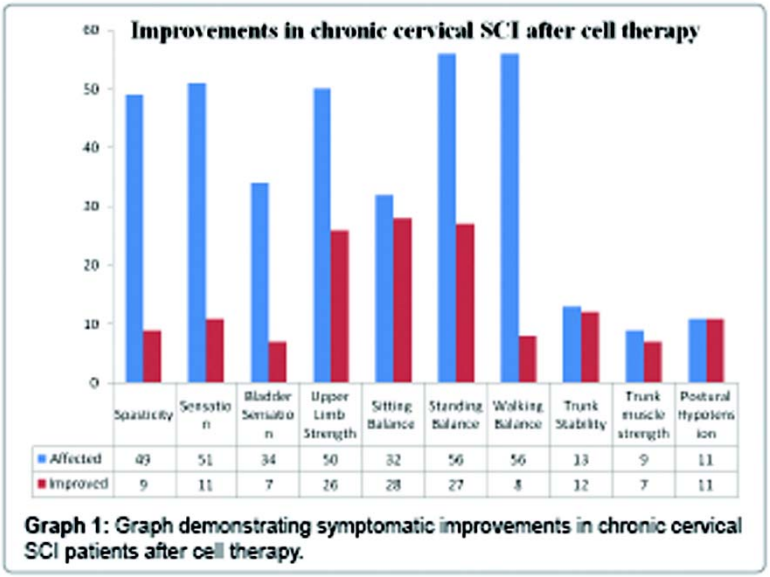
2. A detailed analysis of chronic cervical SCI patients who underwent intrathecal



administration of autologous bone marrow mononuclear cells followed by neurorehabilitation was conducted. This study includes 50 patients of chronic cervical SCI. The outcome was recorded at a mean follow up of 2 years ±1 month. The outcome measures were Functional Independence Measure (FIM) score, American Spinal Injury Association scale (ASIA) and detailed neurological assessment. Data was statistically analyzed using McNemar's Test to establish significance between the change in symptoms and the intervention.

37 out of 50 (74%) showed improvements. Sensation recovery was observed in 26% cases, improved trunk control in 22.4%, spasticity reduction in 20% and bladder sensation recovery in 14.2%. All the 50 cases had improvement in postural hypotension. 12.24% wheelchair bound patients started walking with assistance. Functionally, 20.4% patients showed improved ADLs and 48% showed a positive

change in FIM score. 6% cases showed a shift in ASIA scale. A statistical analysis using McNemar's test established a significant association of these symptoms with the intervention. (91)



Graph 1: Graph demonstrating symptomatic improvements in chronic cervical SCI patients after cell therapy.

No major side effects were noted in the duration of 2 years in both the studies. A better outcome was observed in thoracolumbar injury as compared to the cervical injury suggesting that the level of SCI greatly influences the recovery of the patient. Both studies demonstrated statistically significant clinical and functional outcome.

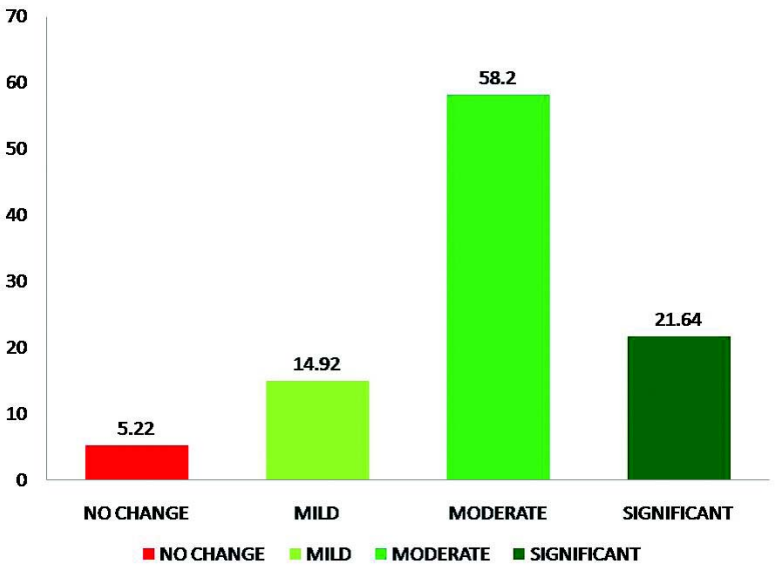
Symptoms improved	Cervical SCI	Thoracolumbar SCI
Spasticity	18.37%	26%
Sensation	21.57%	28%
Bladder Sensation	20.59%	33%
Bowel Sensation	5.66%	0.9%
Sitting Balance	87.50%	81.81%
Standing Balance	48.21%	66.36
Trunk Stability	92.31%	95.65%
Postural Hypotension	100.00%	100%

Unpublished data

Thoracic Spinal Cord Injury:

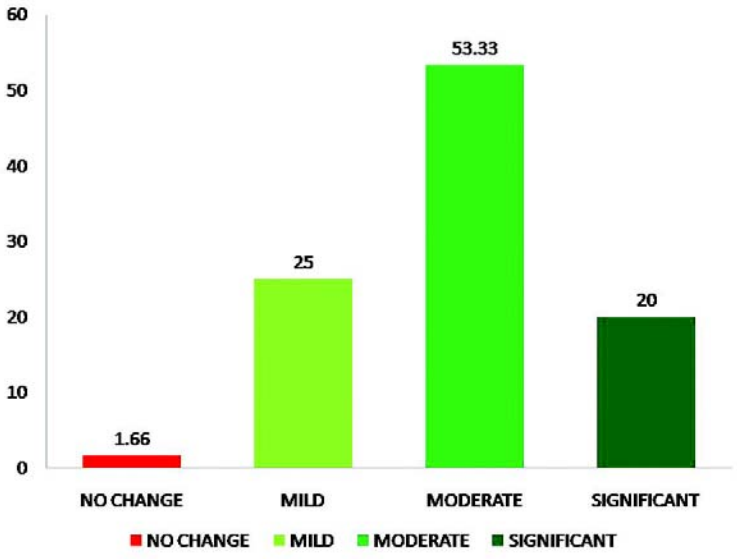
134 patients with diagnosis of thoracic spinal cord injury were included in the analysis. These patients had attained a plateau stage with respect to symptomatic and functional improvements. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant

improvements. The symptoms included were muscle tone, lower limb activity, sensory changes, bowel/bladder function, trunk activity, balance, standing, ambulation and activities of daily living. Mild improvement was defined as improvements till 3 of the symptoms mentioned. Moderate was considered when 4 to 6 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 7 to 9 of the symptoms. Analysis revealed that out of 134 patients, 5.22% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 14.92% of patients, moderate in 58.20% of patients, whereas, 21.64% of patients showed significant improvements.



Cervical Spinal Cord Injury:

60 patients with diagnosis of cervical spinal cord injury were included in the analysis. These patients had attained a plateau stage with respect to symptomatic and functional improvements. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant improvements. The symptoms included were muscle tone, upper limb activity, lower limb activity, sensory changes, bowel/bladder function, trunk activity, balance, standing, ambulation and activities of daily living. Mild improvement was defined as improvements till 3 of the symptoms mentioned. Moderate was considered when 4 to 6 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 7 to 10 of the symptoms. Analysis revealed that out of 60 patients, 1.66% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 25% of patients, moderate in 53.33% of patients, whereas, 20% of patients showed significant improvements.



Future Directions

In SCI, rapid loss of the oligodendrocytes is recorded. The quiescent endogenous ependymal cells which are activated after the injury, are unable to differentiate into the required cells of oligodendrocyte lineage failing to limit the damage. Also, the microenvironment of the injured spinal cord prevents neuronal differentiation of the transplanted cells due to the progliogenic signals. Hence, the future research should focus on manipulating the cells before transplantation or infusing growth factors manipulating the endogenous cells and modulating them towards producing more oligodendrocytes. (92) Future of regenerative medicine is the use of stem cells along with nanodrug in SCI. (93) 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. (94-96)

Many clinical trials are being conducted in the USA, China, India, Switzerland to optimize the intervention, find the appropriate time of injection, type of cells, route of administration, etc. (97)

REFERENCE

1. Kraus KH. The pathophysiology of spinal cord injury and its clinical implications. Semin Vet Med Surg (Small Anim). 1996 Nov;11(4):201-7.
2. Deumens R, Koopmans GC, Honig WM, Maquet V, Jérôme R, Steinbusch HW, Joosten EA. Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing

cell/olfactory nerve fibroblast-biomatrix bridges. J Neurosci Res. 2006 Apr;83(5):811-20.

3. Wanner IB, Deik A, Torres M, Rosendahl A, Neary JT, Lemmon VP, Bixby JL. A new in vitro model of the glial scar inhibits axon growth. Glia. 2008 Nov 15;56(15):1691-709
4. Wright KT, Masri WE, Osman A, Chowdhury J, Johnson WEB (2011) Concise Review: Bone Marrow for the Treatment of Spinal Cord Injury: Mechanisms and Clinical Applications. Stem Cells 29: 169-178.
5. Coutts M, Keirstead HS (2008) Stem cells for the treatment of spinal cord injury. Exp Neurol 209: 368-377.
6. Reier PJ (2004) Cellular transplantation strategies for spinal cord injury and translational neurobiology. NeuroRx 1: 424-451
7. John W. Mcdonald, Xiao-Zhong Liu, Yun Qu et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nature Medicine. 1999; 5(12).1410-12
8. Bottai D, Cigognini D, Madaschi L, et al. Embryonic stem cells promote motor recovery and affect inflammatory cell infiltration in spinal cord injured mice. Experimental Neurology. 2010;223(2):452-463. (PubMed)
9. Nicolas Granger, Helen Blamires, Robin J. M. Franklin, and Nick D. Jeffery Autologous olfactory mucosal cell transplants in clinical spinal cord injury: a randomized double-blinded trial in a canine translational model Brain (2012) 135 (11): 3227-3237
10. Kumagai G, Okada Y, Yamane J, et al. Roles of ES cell-derived gliogenic neural stem/progenitor cells in functional recovery after spinal cord injury. PLoS ONE. 2009;4(11)e7706 (PMC free article) (PubMed)
11. Lowry N, Goderie SK, Adamo M, et al. Multipotent embryonic spinal cord stem cells expanded by endothelial factors and Shh/RA promote functional recovery after spinal cord injury. Experimental Neurology. 2008;209(2):510-522. (PubMed)
12. Fujimoto Y, Abematsu M, Falk A, et al. Treatment of a mouse model of spinal cord injury by transplantation of human iPS cell-derived long-term self-renewing neuroepithelial-like. Stem Cells. 2012;30(6):1163-1173. (PubMed)
13. Chen J, Bernreuther C, Dihné M, Schachner M. Cell adhesion molecule L1-transfected embryonic stem cells with enhanced survival support regrowth of corticospinal tract axons in mice after spinal cord injury. Journal of Neurotrauma. 2005;22(8):896-906. (PubMed)
14. Cui YF, Xu JC, Hargus G, Jakovcevski I, Schachner M, Bernreuther C. Embryonic stem cell-derived L1 overexpressing neural aggregates enhance recovery after spinal cord injury in mice. PLoS ONE. 2011;6(3)e17126 (PMC free article) (PubMed)
15. Perrin FE, Boniface G, Serguera C, et al. Grafted human embryonic progenitors expressing neurogenin-2 stimulate axonal sprouting and improve motor recovery after severe spinal cord injury. PLoS ONE. 2010;5(12)e15914 (PMC free article)

(PubMed)

16. Hatami M, Mehrjardi NZ, Kiani S, et al. Human embryonic stem cell-derived neural precursor transplants in collagen scaffolds promote recovery in injured rat spinal cord. Cytotherapy. 2009;11(5):618-630. (PubMed)
17. Niapour A, Karamali F, Nemati S, et al. Co-transplantation of human embryonic stem cell-derived neural progenitors and Schwann cells in a rat spinal cord contusion injury model elicits a distinct neurogenesis and functional recovery. Cell Transplant. 2012;21(5):827-843. (PubMed)
18. Rossi SL, Nistor G, Wyatt T, et al. Histological and functional benefit following transplantation of motor neuron progenitors to the injured rat spinal cord. PLoS ONE. 2010;5(7)e11852 (PMC free article) (PubMed)
19. Kim DS, Jung Jung SE, Nam TS, et al. Transplantation of GABAergic neurons from ESCs attenuates tactile hypersensitivity following spinal cord injury. Stem Cells. 2010;28(11):2099-2108. (PubMed)
20. Keirstead HS, Nistor G, Bernal G, et al. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. Journal of Neuroscience. 2005;25(19):4694-4705. (PubMed)
21. Kerr CL, Letzen BS, Hill CM, et al. Efficient differentiation of human embryonic stem cells into oligodendrocyte progenitors for application in a rat contusion model of spinal cord injury. International Journal of Neuroscience. 2010;120(4):305-313. (PubMed)
22. Sharp J, Frame J, Siegenthaler M, Nistor G, Keirstead HS. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. Stem Cells. 2010;28(1):152-163. (PMC free article) (PubMed)
23. Erceg S, Ronaghi M, Oria M, et al. Transplanted oligodendrocytes and motoneuron progenitors generated from human embryonic stem cells promote locomotor recovery after spinal cord transection. Stem Cells. 2010;28(9):1541-1549. (PMC free article) (PubMed)
24. Salehi M, Pasbakhsh P, Soleimani M, et al. Repair of spinal cord injury by co-transplantation of embryonic stem cell-derived motor neuron and olfactory ensheathing cell. Iranian Biomedical Journal. 2009;13(3):125-135.
25. Nakajima H, Uchida K, Guerrero AR, et al. Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. Journal of Neurotrauma. 2012;29(8):1614-1625. (PMC free article) (PubMed)
26. Karaoz E, Kabatas S, Duruksu G, et al. Reduction of lesion in injured rat spinal cord and partial functional recovery of motility after bone marrow derived mesenchymal stem cell transplantation. Turkish Neurosurgery. 2012;22(2):207-217. (PubMed)
27. Park WB, Kim SY, Lee SH, Kim HW, Park JS, Hyun JK. The effect of mesenchymal

stem cell transplantation on the recovery of bladder and hindlimb function after spinal cord contusion in rats. BMC Neuroscience. 2010;11:p. 119. (PMC free article) (PubMed)

28. Abrams MB, Dominguez C, Pernold K, et al. Multipotent mesenchymal stromal cells attenuate chronic inflammation and injury-induced sensitivity to mechanical stimuli in experimental spinal cord injury. Restorative Neurology and Neuroscience. 2009;27(4):307-321. (PubMed)
29. Kang ES, Ha KY, Kim YH. Fate of transplanted bone marrow derived mesenchymal stem cells following spinal cord injury in rats by transplantation routes. Journal of Korean Medical Science. 2012;27(6):586-593. (PMC free article) (PubMed)
30. Osaka M, Honmou O, Murakami T, et al. Intravenous administration of mesenchymal stem cells derived from bone marrow after contusive spinal cord injury improves functional outcome. Brain Research C. 2010;1343:226-235. (PubMed)
31. Mothe AJ, Bozkurt G, Catapano J, et al. Intrathecal transplantation of stem cells by lumbar puncture for thoracic Spinal cord injury in the rat. Spinal Cord. 2011;49(9):967-973. (PubMed)
32. Boido M, Garbossa D, Fontanella M, Ducati A, Vercelli A. Mesenchymal stem cell transplantation reduces glial cyst and improves functional outcome following spinal cord compression. World Neurosurgery. World Neurosurg. 2014 Jan;81(1):183-190
33. Gu W, Zhang F, Xue Q, Ma Z, Lu P, Yu B. Transplantation of bone marrow mesenchymal stem cells reduces lesion volume and induces axonal regrowth of injured spinal cord. Neuropathology. 2010;30(3):205-217. (PubMed)
34. Alexanian AR, Fehlings MG, Zhang Z, Maiman DJ. Transplanted neurally modified bone marrow-derived mesenchymal stem cells promote tissue protection and locomotor recovery in spinal cord injured rats. Neurorehabilitation and Neural Repair. 2011;25(9):873-880. (PubMed)
35. Ban DX, Ning GZ, Feng SQ, et al. Combination of activated Schwann cells with bone mesenchymal stem cells: the best cell strategy for repair after spinal cord injury in rats. Regenerative Medicine. 2011;6(6):707-720. (PubMed)
36. Cho SR, Kim YR, Kang HS, et al. Functional recovery after the transplantation of neurally differentiated mesenchymal stem cells derived from bone barrow in a rat model of spinal cord injury. Cell Transplantation. 2009;18(12):1359-1368. (PubMed)
37. Pedram MS, Dehghan MM, Soleimani M, Sharifi D, Marjanmehr SH, Nasiri Z. Transplantation of a combination of autologous neural differentiated and undifferentiated mesenchymal stem cells into injured spinal cord of rats. Spinal Cord. 2010;48(6):457-463. (PubMed)
38. Liu WG, Wang ZY, Huang ZS. Bone marrow-derived mesenchymal stem cells expressing the bFGF transgene promote axon regeneration and functional recovery after spinal cord injury in rats. Neurological Research. 2011;33(7):686-693. (PubMed)

39. Zhang YJ, Zhang W, Lin C-G, et al. Neurotrophin-3 gene modified mesenchymal stem cells promote remyelination and functional recovery in the demyelinated spinal cord of rats. Journal of the Neurological Sciences. 2012;313(1-2):64-74. (PubMed)

40. Zeng X, Zeng YS, Ma YH, et al. Bone marrow mesenchymal stem cells in a three dimensional gelatin sponge scaffold attenuate inflammation, promote angiogenesis and reduce cavity formation in experimental spinal cord injury. Cell Transplantation. 2011;20(11-12):1881-1899. (PubMed)

41. Kang KN, Kim da Y, Yoon SM, et al. Tissue engineered regeneration of completely transected spinal cord using human mesenchymal stem cells. Biomaterials. 2012;33(19):4828-4835. (PubMed)

42. Park SS, Lee YJ, Lee SH, et al. Functional recovery after spinal cord injury in dogs treated with a combination of Matrigel and neural-induced adipose-derived mesenchymal Stem cells. Cytotherapy. 2012;14(5):584-597. (PubMed)

43. Guo YW, Ke YQ, Li M, et al. Human umbilical cord-derived schwann-like cell transplantation combined with neurotrophin-3 administration in dyskinesia of rats with spinal cord injury. Neurochemical Research. 2011;36(5):783-792. (PubMed)

44. Shang AJ, Hong SQ, Xu Q, et al. NT-3-secreting human umbilical cord mesenchymal stromal cell transplantation for the treatment of acute spinal cord injury in rats. Brain Research. 2011;1391:102-113. (PubMed)

45. Lee JH, Chung WH, Kang EH, et al. Schwann cell-like remyelination following transplantation of human umbilical cord blood (hUCB)-derived mesenchymal stem cells in dogs with acute spinal cord injury. Journal of the Neurological Sciences. 2011;300(1-2):86-96. (PubMed)

46. Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A. 1997 Apr15;94(8):4080-5.

47. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A. 1999 Sep 14;96(19):10711-6.

48. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. Science. 2000 Dec 1;290(5497):1775-9.

49. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science. 2000 Dec 1;290(5497):1779-82.

50. Priller J, Flügel A, Wehner T, Boentert M, Haas CA, Prinz M, Fernández-Klett F, Prass K, Bechmann I, de Boer BA, Frotscher M, Kreutzberg GW, Persons DA,Dirnagl U. Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. Nat Med. 2001 Dec;7(12):1356-61.

51. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic

stem cells into motor neurons. Cell. 2002;110(3):385-397.

52. Hofstetter, C. P., et al. "Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery." Proceedings of the National Academy of Sciences 99.4 (2002): 2199-2204.
53. Quertainmont R, Cantinieaux D, Botman O, Sid S, Schoenen J, et al. (2012) Mesenchymal Stem Cell Graft Improves Recovery after Spinal Cord Injury in Adult Rats through Neurotrophic and Pro-Angiogenic Actions. PLoS ONE 7(6): e39500.
54. Cho SR, Kim YR, Kang HS, Yim SH, Park CI, Min YH, Lee BH, Shin JC, Lim JB. Functional recovery after the transplantation of neurally differentiated mesenchymal stem cells derived from bone barrow in a rat model of spinal cord injury. Cell Transplant. 2009;18(12):1359-68
55. Samuil S. Rabinovich, Victor I. Seledtsov, Olga V. Poveschenko. Transplantation treatment of spinal cord injury patients. Biomedicine and Pharmacotherapy. 2003;57(9): 428-433
56. Yoon Ha, Seung Hwan Yoon, So Ra Park. Treatment of Complete Spinal Cord Injury Patients Receiving Autologous Bone Marrow Cell Transplantation and Bone Marrow Stimulation with Granulocyte Macrophage-Colony Stimulating Factor - Report of Three Cases. J Korean Neurosurg Soc 2004; 35: 459-464.
57. Feron F, Perry, C, Cochrane, J, Licina, P, Nowitzke, A, Urquhart, S, Geraghty, T, Mackay-Sim, A. Autologous olfactory ensheathing cell transplantation in human spinal cord injury. Brain 2005;128(12):2951-60.
58. Mackay-Sim, F. Féron, J. Cochrane et al. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain. 2008; 131(9): 2376- 2386.
59. G. P. V. Subbaiah, V. Adavi, L. K. Chelluri, S. Laxman, K. S. Ratnakar, P. B. N. Gopal and K. Ravindranath. Preliminary report on the safety, efficacy and functional recovery of spinal cord injury with autologous bone marrow derived mesenchymal stem cells - a clinical trial. The Internet Journal of Spine Surgery. 2009;5(1)
60. Jarocha D, Milczarek O, Kawecki Z, Wendrychowicz A, Kwiatkowski S, Majka M. Preliminary Study of Autologous Bone Marrow Nucleated Cells Transplantation in Children With Spinal Cord Injury. Stem Cells Transl Med. 2014 Feb 3. (Epub ahead of print)
61. Syková, P. Jendelová, L. Urdzíková, P. Lesný, and A. Hej?l, "Bone marrow stem cells and polymer hydrogels-two strategies for spinal cord injury repair," Cellular and Molecular Neurobiology. 2006; 26(7-8):1113-1129.
62. Eva Syková, Aleš Homola, Radim Mazanec et al. Autologous Bone Marrow Transplantation in Patients With Subacute and Chronic Spinal Cord Injury. Cell Transplantation.2006;15:1-100.
63. R. Chernykh, V. V. Stupak, G. M.Muradov et al., Application of autologous bone marrow stem cells in the therapy of spinal cord injury patients. Bulletin of

Experimental Biology and Medicine 2007;143(4):543-547.

64. H. Saberi, P. Moshayedi, H.-R. Aghayan et al. Treatment of chronic thoracic spinal cord injury patients with autologous Schwann cell transplantation: an interim report on safety considerations and possible outcomes. Neuroscience Letters, 2008;443(1): 46-50.
65. L. F. Geffner, P. Santacruz, M. Izurieta. Administration of Autologous Bone Marrow Stem Cells Into Spinal Cord Injury Patients Via Multiple Routes Is Safe and Improves Their Quality of Life: Comprehensive Case Studies. Cell Transplantation2008;17:1277-1293.
66. Abdelaziz, Osama S. MD. Feasibility, Safety, and Efficacy of Directly Transplanting Autologous Adult Bone Marrow Stem Cells in Patients With Chronic Traumatic Dorsal Cord Injury: A Pilot Clinical Study. Neurosurgery Quarterly: 2010; 20(3):216-226.
67. J. H. Park, D. Y. Kim, I. Y. Sung, et al., "Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans," Neurosurgery, vol. 70, no. 5, pp. 1238-1247, 2012.
68. Saito F, Nakatani T, Iwase M, et al. Spinal cord injury treatment with intrathecal autologous bone marrow stromal cell transplantation: the first clinical trial case report. J Trauma. 2008;64(1):53-9.
69. R. Pal, N. K. Venkataramana, A. Bansal et al. Ex vivo expanded autologous bone marrow-derived mesenchymal stromal cells in human spinal cord injury/ paraplegia: a pilot clinical study. Cytotherapy. 2009;11(7): 897-911.
70. A. Kumar, S. R. Kumar, R. Narayanan, K. Arul, and M. Baskaran. Autologous bone marrow derived mononuclear cell therapy for spinal cord injury: a phase I/ II clinical safety and primary efficacy data. Experimental and Clinical Transplantation 2009; 7(4): 241-248.
71. Zhou Q, Zhang SZ, Xu RX, Xu K. Neural stem cell transplantation and postoperative management: report of 70 cases. Di Yi Junyi Daxue Xuebao 2004;24(10):1207-9
72. Moviglia GA, Fernandez Viña R, Brizuela JA.et al. Combined protocol of cell therapy for chronic spinal cord injury. Report on the electrical and functional recovery of two patients. Cytotherapy. 2006; 8(3):202-9.
73. Hyung Chun Park, Yoo Shik Shim, Yoon Ha et al. Treatment of Complete Spinal Cord Injury Patients by Autologous Bone Marrow Cell Transplantation and Administration of Granulocyte-Macrophage Colony Stimulating Factor. Tissue Engineering. 2005, 11(5-6): 913-922.
74. Deda H, Inci MC, Kürekçi AE, Kayihan K, Ozgün E, Ustünsoy GE, Kocabay S. Treatment of chronic spinal cord injured patients with autologous bone marrow-derived hematopoietic stem cell transplantation: 1-year follow-up. Cytotherapy. 2008;10(6):565-74.
75. Frolov AA, Bryukhovetskiy AS. Effects of hematopoietic autologous stem cell

transplantation to the chronically injured human spinal cord evaluated by motor and somatosensory evoked potentials methods. Cell Transplant. 2012;21 Suppl 1:S49-55

76. Dai G, Liu X, Zhang Z, Yang Z, Dai Y, Xu R. Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury. Brain Res. 2013 Oct 2;1533:73-9.
77. Jiang PC, Xiong WP, Wang G, Ma C, Yao WQ, Kendell SF, Mehling BM, Yuan XH, Wu DC. A clinical trial report of autologous bone marrow-derived mesenchymal stem cell transplantation in patients with spinal cord injury. Exp Ther Med. 2013 Jul;6(1):140-146
78. Moviglia GA, Fernandez Viña R, Brizuela JA.et al. Combined protocol of cell therapy for chronic spinal cord injury. Report on the electrical and functional recovery of two patients. Cytotherapy. 2006; 8(3):202-9.
79. N. Knoller, G. Auerbach, V. Fulga et al., Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: phase I study results. Journal of Neurosurgery Spine. 2005; 3(3): 173-181.
80. F. Cristante, T. E. P. Barros-Filho, and T. E. P. Barros-Filho. Stem cells in the treatment of chronic spinal cord injury: evaluation of somatosensitive evoked potentials in 39 patients. Spinal Cord. 2009; 47(10):733-738.
81. Thomas E Ichim, Fabio Solano, Fabian Lara et al. Feasibility of combination allogeneic stem cell therapy for spinal cord injury: a case report. International Archives of Medicine 2010; 3:30.
82. Lima, P. Escada, J. Pratas-Vital et al. Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. Neurorehabilitation and Neural Repair 2010;24(1):10-22.
83. Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans.Stem Cells Dev. 2011 Aug;20(8):1297-308.
84. Raisman G. Repair of spinal cord injury by transplantation of olfactory ensheathing cells. C R Biol. 2007 Jun-Jul;330(6-7):557-60. Epub 2007 May 9.Review.
85. Huang H, Xi H, Chen L, Zhang F, Liu Y. Long-term outcome of olfactory ensheathing cell therapy for patients with complete chronic spinal cord injury.Cell Transplant. 2012;21 Suppl 1:S23-31.
86. Saberi H, Firouzi M, Habibi Z, Moshayedi P, Aghayan HR, Arjmand B, Hosseini K, Razavi HE, Yekaninejad MS. Safety of intramedullary Schwann cell transplantation for postrehabilitation spinal cord injuries: 2-year follow-up of 33 cases. J Neurosurg Spine. 2011 Nov;15(5):515-25.
87. Yazdani SO, Hafizi M, Zali AR, Atashi A, Ashrafi F, Seddighi AS, Soleimani M. Safety and possible outcome assessment of autologous Schwann cell and bone marrow mesenchymal stromal cell co-transplantation for treatment of patients

with chronic spinal cord injury. Cytotherapy. 2013 Jul;15(7):782-91.

88. Sun T, Ye C, Zhang Z, Wu J, Huang H. Cotransplantation of olfactory ensheathing cells and Schwann cells combined with treadmill training promotes functional recovery in rats with contused spinal cords. Cell Transplant. 2013;22 Suppl 1:S27-38
89. Callera and R. X. do Nascimento. Delivery of autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique in patients with spinal cord injury: a preliminary safety study. Experimental Hematology. 2006;34(2): 130-131.
90. Sharma A, Gokulchandran N, Sane H, Badhe P, Kulkarni P, Lohia M, Nagrajan A, Thomas N. Detailed analysis of the clinical effects of cell therapy for thoracolumbar spinal cord injury: an original study. Journal of Neurorestoratology. 2013;1:13-22
91. Sharma A, Sane H, Gokulchandran N, Kulkarni P, Thomas N, et al. (2013) Role of Autologous Bone Marrow Mononuclear Cells in Chronic Cervical Spinal Cord Injury-A Longterm Follow Up Study. J Neurol Disord 1: 138.
92. Panayiotou E, Malas S. Adult spinal cord ependymal layer: a promising pool of quiescent stem cells to treat spinal cord injury. Front Physiol. 2013 Nov 28;4:340. eCollection 2013
93. Sharma HS, Muresanu DF, Sharma A. Novel therapeutic strategies using nanodrug delivery, stem cells and combination therapy for CNS trauma and neurodegenerative disorders. Expert Rev Neurother. 2013 Oct;13(10):1085-8
94. Yamamoto A, Sakai K, Matsubara K, Kano F, Ueda M. Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury. Neurosci Res. 2014 Jan;78:16-20
95. Kokai LE, Marra K, Rubin JP. Adipose stem cells: biology and clinical applications for tissue repair and regeneration. Transl Res. 2013 Dec 4. pii: S1931-5244(13) 00426-X.
96. Fu X. The immunogenicity of cells derived from induced pluripotent stem cells. Cell Mol Immunol. 2014 Jan;11(1):14-6.
97. Clinicaltrials.gov

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

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

– Marilyn Ferguson

12

Stem Cell Transplantation In Stroke

Stroke is defined as neurological deficit caused by a sudden interruption of the blood supply to the brain leading to reduced oxygen and nutrient supply in that area. The two major types of stroke are ischemic and hemorrhagic. In ischemic stroke, decreased or absent circulating blood deprives neurons of necessary substrates. Intracerebral hemorrhage originates from deep penetrating vessels and causes injury to brain tissue by disrupting connecting pathways and causing localized pressure injury. The extent of neurological involvement may range from mild motor deficit to gross involvement of various function namely sensorimotor, perceptual, emotional, behavioral, memory intelligence, speech and language function, ultimately affecting the activities of daily living.

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

Unmet medical needs

With the current treatment approaches, be it medical, surgical or rehabilitative, the pathophysiological processes and the resultant damage occurring at the microcellular level cannot be reversed. This permanent change in the structure of CNS leads to long lasting physical impairments, seen as residual problems, which translate

gradually into activity limitation and restricts these individuals to participate in the community. There have been many advances in the medical management of acute stroke but, little has changed to address the residual deficits of chronic stroke. A treatment approach, which changes the physiology at the neuronal level, is the need of the hour. Cell therapy offers hope for stroke patients, especially for those who have missed the "window".

Role of stem cell therapy in chronic stroke:

Stem cells impersonate the natural process of recovery after stroke, which is mobilization of stem cells, originally present in the bone marrow, to the area of injury in the brain. This occurs with the release of certain factors. This mobilization of stem cells to the injured brain initiates the process of neurorestoration. These stem cells secrete various growth factors like VEGF, bFGF and BDNF which support and amplify angiogenesis, neurogenesis and synaptic plasticity at the penumbral region. Along with the above neuroreparative processes, the stem cells also decrease the glial scar formation and promote glial-axonal remodeling which is seen in chronic stroke (1-4). The number of stem cells mobilized after acute stroke starts decreasing as chronic stage approaches. Therefore as time passes by the rate of recovery also reduces in the chronic stage. This forms the rationale that if more number of stem cells are supplied to the injured area in the chronic stage, it may hasten and increase the chances of recovery.

Stem cell therapy in Stroke

Animal studies:

There are various clinical studies performed on animals, to assess the effects of stem cell therapy in improving the outcomes post stroke. The findings of these studies included increased angiogenesis at the site of the infarct, increased modulation of neurotrophic growth factors, and reduction in the infarct volumes. They exhibited improved functional performance and restore neurological deficits (5-11).

Zhao et al. in 2002 conducted a trial to assess whether transplantation of human MSCs into the brain of ischemic rats demonstrated any changes functionally. Purified human MSCs were grafted into the cortex surrounding the ischemia 1 week after cortical brain ischemia in rats. Two and 6 weeks after transplantation animals were assessed for sensorimotor function. Ischemic rats that received human MSCs exhibited significantly improved functional performance in limb placement test. The authors concluded that the observed functional improvement might have been mediated by proteins secreted by transplanted human MSCs, which could have upregulated host brain plasticity in response to experimental stroke (5).

Shyu et al. in 2006 performed a clinical trial where intracerebral transplantation of peripheral blood hematopoetic stem cells was introduced in one group of rats with chronic cerebral ischemia, and compared with vehicle-treated control rats. PBSC implantation promoted the formation of new vessels, thereby increasing the local cortical

blood flow in the ischemic hemisphere, enhancing the angiogenic architecture over the ischemic brain. quantitative reverse transcription-PCR analysis showed significantly increased modulation of neurotrophic factor expression in the ischemic hemisphere of the PBSC-transplanted rats compared with vehicle-treated control rats (6).

Human studies

There are a few clinical trials conducted of humans, to find the efficacy of cell transplantation after stroke. Clinical improvement was seen in the form of decreased spasticity and paresis, resulting in improved walking, improved functional recovery, and restoration of neurological deficits by the process of increased neural plasticity (12-18).

Prasad et al. in 2012 conducted a non-randomized clinical trial to evaluate the feasibility, safety and clinical outcome of administering bone marrow mononuclear cell (MNCs) intravenously to patients with sub acute ischemic stroke. 11 patients with ischemic stroke were included in this study. Intravenous administration of bone marrow MNCs was carried out. They were assessed on National Institute of Health Stroke Scale, Barthel Index, modified Rankin Scale, MRI, EEG and PET. Results demonstrated favorable clinical outcomes. The authors thus concluded that intravenous bone marrow mononuclear cell therapy appears feasible and safe in patients with sub acute ischemic stroke (16).

Friedrich eta l. in 2012 conducted a clinical trial where intra-arterial autologous BMMCs were infused in 20 patients with moderate to severe acute middle cerebral artery infarcts. Mononuclear cells were isolated from bone marrow aspirates and infused at the proximal middle cerebral artery of the affected hemisphere. National Institutes of Health Stroke Scale (NIHSS) scores, seizures, epileptogenic activity on electroencephalogram, and neuroimaging complications including new ischemic, hemorrhagic, or neoplastic lesions were the outcomes and tests on which all the patients were monitored. Satisfactory clinical improvement occurred in (30%) patients at 90 days. 40% showed a good clinical outcome. Infusion of intra-arterial autologous BMMCs appears to be safe in patients with moderate to severe acute middle cerebral artery strokes (17).

Lee et al. in 2010 undertook a study to evaluate the long-term safety and efficacy of intravenous MSCs transplantation in a larger population. an open-label, observer-blinded clinical trial of 85 patients with severe middle cerebral artery territory infarct was conducted. Patients were randomly allocated to one of two groups, those who received intravenous autologous ex vivo cultured MSCs (MSC group) or those who did not (control group), and followed for up to 5 years. 16 were included in the MSC group and 36 were in the control group. Clinical improvement was observed in the patients of MSC group on modified Rankin scale. The authors correlated this clinical improvement with serum levels of stromal cell-derived factor-1 and the degree of involvement of the subventricular region of the lateral ventricle (18).

Bang et al. in 2005 examined the feasibility, efficacy, and safety of cell therapy

using culture-expanded autologous MSCs in 30 patients with ischemic stroke with middle cerebral artery infarct and severe neurological deficits. These 30 patients were divided into one of two treatment groups- the MSC group (n = 5) received intravenous infusion of autologous MSCs, whereas the control group (n = 25) did not receive MSCs. Changes in neurological deficits and improvements in function were compared between the groups for 1 year after symptom onset. Patients in the MSCs treated group showed improved outcomes in the Barthel index and modified Rankin score. Thus the authors concluded that in patients with severe cerebral infarcts, the intravenous infusion of autologous MSCs appears to be a feasible and safe therapy that may improve functional recovery (19).

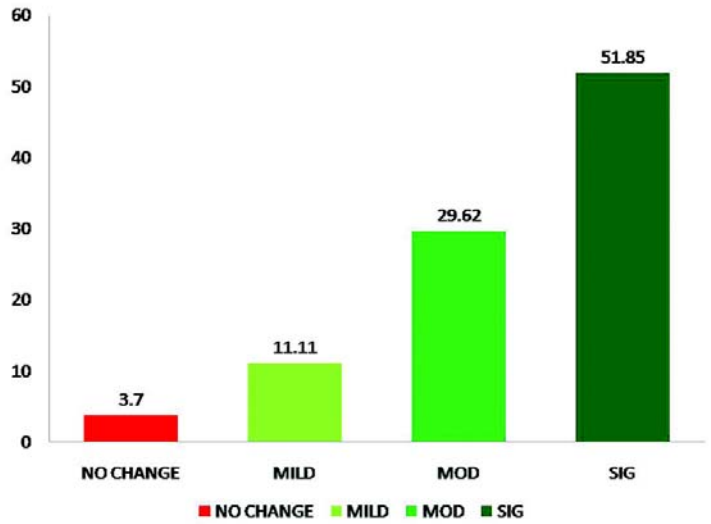
Our results

Published data

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

Unpublished data

27 patients with diagnosis of chronic stroke were included in the analysis. These patients had attained a plateau stage with respect to symptomatic and functional improvements. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant improvements. The symptoms included were upper limb activity/hand functions, lower limb activity, trunk activity, balance, higher mental functions/speech, ambulation and Activities of Daily Living. Mild improvement was defined as improvements till 2 of the symptoms mentioned. Moderate was considered when 3 to 4 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 5 to 7 of the symptoms. Analysis revealed that out of 27 patients, 3.7% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 11.11% of patients, moderate in 29.62% of patients, whereas, 51.85% of patients showed significant improvements. The majority of patients showing significant improvements can be attributed to earlier intervention from the time of stroke and other factors affecting the recovery process of the disease. The changes seen clinically were also evidently observed on PET-CT (Positron Emisssion Tomography- Computerized Tomography) scan of brain.



Future directions

There are many areas which needs to be analyzed in depth, to gain the best outcomes out of cell therapy. The question of best cell type for transplantation with stroke needs to be addressed. To optimize cell therapies in stroke, it is also necessary to elucidate the molecular mechanisms controlling the interaction of the grafted cells with the ischemic brain, as the post ischemic environment can affect the function of transplanted stem cells, which in turn can modulate the inflammatory response and the local microenvironment. Timing of transplantation in different time windows needs

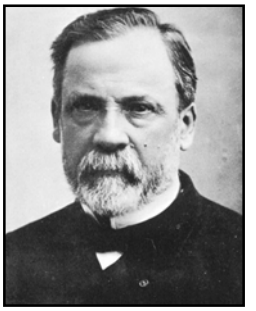
to be assessed in detail, as most of the studies takes into account acute, sub acute and chronic stroke. This is crucial to analyze the effect of cell therapy at various stages. Appropriate dosage remains unclear. A dose-response correlation is an important aspect of cell therapy. Routes of administration are an important area which decides the intensity of effect of cell therapy. Objective imaging needs to be introduced into clinical trials, to get an insight into the physiological processes occurring at the cellular level after cell therapy, to strengthen the results obtained (20).

REFERENCES:

1. Carmeliet P, Storkebaum E. Vascular and neuronal effects of VEGF in the nervous system: implications for neurological disorders. Semin Cell Dev Biol 2002;13:39 - 53.
2. Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci U S A 2002; 99:11946-11950.
3. Chen J, Zhang ZG, Li Y, et al. Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ Res 2003;92: 692-699.
4. Li Y, Chen J, Zhang CL, et al. Gliosis and brain remodeling after treatment of stroke in rats with marrow stromal cells. GLIA 2005;49:407- 417.
5. Li-Ru Zhao Wei-Ming Duan Morayma Reyes C. Dirk Keene Catherine M. Verfaillie Walter C. Low Human Bone Marrow Stem Cells Exhibit Neural Phenotypes and Ameliorate Neurological Deficits after Grafting into the Ischemic Brain of Rats Experimental Neurology 174, 11-20 (2002)
6. Woei-Cherng Shyu, Shinn-Zong Lin Ming-Fu Chiang Ching-Yuan Su Hung Li Intracerebral Peripheral Blood Stem Cell (CD34_) Implantation Induces Neuroplasticity by Enhancing _1 Implantation Induces Neuroplasticity by Enhancing _1 The Journal of Neuroscience, March 29, 2006 o 26(13):3444 -3453
7. Natalia Pavlichenko, Irina Sokolova, Svetlana Vijde, Evgenia Shvedova, Georgy Alexandrov, Peter Krouglyakov, Olga Fedotova, Elena G. Gilerovich, Elena G. Gilerovich, Vladimir A. Otellin Mesenchymal stem cells transplantation could be beneficial for treatment of experimental ischemic stroke in rats BRAIN Research 1233 (2 0 0 8) 2 0 3 - 2 1 3
8. Jieli Chen, Zheng Gang Zhang, Yi Li, Lei Wang, Yong Xian Xu, Subhash C. Gautam, Mei Lu, Zhenping Zhu and Michael Chopp Intravenous Administration of Human Bone Marrow Stromal Cells Induces Angiogenesis in the Ischemic Boundary Zone After Stroke in Rats Circ Res. 2003;92:692-699
9. Y. Li, MD; J. Chen, MD; L. Wang, MD; M. Lu, PhD; and M. Chopp, PhD Treatment of stroke in rat with intracarotid administration of marrow stromal cells NEUROLOGY 2001;56:1666-1672
10. Sang-Wuk Jeong, Kon Chu, Keun-Hwa Jung, Seung U. Kim, Manho Kim and Jae-

Kyu Roh Human Neural Stem Cell Transplantation Promotes Functional Recovery in Rats With Experimental Intracerebral Hemorrhage Stroke. 2003;34:2258-2263

11. David C. Hess, William D. Hill, Angeline Martin-Studdard, James Carroll, Joanna Brailer and Jo Carothers Bone Marrow as a Source of Endothelial Cells and NeuN-Expressing Cells After Stroke Stroke. 2002;33:1362-1368
12. Oh Young Bang MD, PhD Jin Soo Lee MD1, Phil Hyu Lee MD, PhD1, Gwang Lee PhD Autologous mesenchymal stem cell transplantation in stroke patients Annals of Neurology Volume 57, Issue 6, pages 874-882, June 2005
13. Jin Soo Lee Ji Man Hong Gyeong Joon Moon2 Phil Hyu Lee Young Hwan Ahn4, Oh Young Bang2 A Long-Term Follow-Up Study of Intravenous Autologous Mesenchymal Stem Cell Transplantation in Patients With Ischemic Stroke STEM CELLS Volume 28, Issue 6, pages 1099-1106, June 2010
14. Carlos Suárez-Monteagud1, Porfirio Hernández-Ramírez, Lázaro Álvarez-González et al. Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study Restorative Neurology and Neuroscience. 2009;27(3):151-161
15. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010;28(6):1099-106.
16. Prasad K, Mohanty S, Bhatia R, Srivastava MV, Garg A, Srivastava A, Goyal V, Tripathi M, Kumar A, Bal C, Vij A, Mishra NK. Autologous intravenous bone marrow mononuclear cell therapy for patients with subacute ischaemic stroke: a pilot study. Indian J Med Res. 2012 Aug;136(2):221-8. PubMed PMID: 22960888; PubMed Central PMCID: PMC3461733.
17. Friedrich MA, Martins MP, Araújo MD, Klamt C, Vedolin L, Garicochea B, Raupp EF, Sartori El Ammar J, Machado DC, Costa JC, Nogueira RG, Rosado-de-Castro PH, Mendez-Otero R, Freitas GR. Intra-arterial infusion of autologous bone marrow mononuclear cells in patients with moderate to severe middle cerebral artery acute ischemic stroke. Cell Transplant. 2012;21 Suppl 1:S13-21. doi: 10.3727/096368912X612512. PubMed PMID: 22507676.
18. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY; STARTING collaborators. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010 Jun;28(6):1099-106. doi: 10.1002/stem.430. PubMed PMID: 20506226.
19. Oh Young Bang MD, PhD Jin Soo Lee MD1, Phil Hyu Lee MD, PhD1, Gwang Lee PhD Autologous mesenchymal stem cell transplantation in stroke patients Annals of Neurology Volume 57, Issue 6, pages 874-882, June 2005
20. Rosado-de-Castro PH, Pimentel-Coelho PM, da Fonseca LM, de Freitas GR, Mendez-Otero R. The rise of cell therapy trials for stroke: review of published and registered studies. Stem Cells Dev. 2013 Aug 1;22(15):2095-111. doi: 10.1089/scd.2013.0089. Epub 2013 Apr 25. Review. PubMed PMID: 23509917; PubMed Central PMCID: PMC3715770.



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

–Louis Pasteur

13

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

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive degeneration of upper motor neurons in the cerebral cortex and lower motor neurons in medulla and anterior horn of spinal cord (1). It has a poor prognosis with life expectancy of 3 to 5 years since onset of the disease in western countries (2,3,4,5). The disease selectively affects motor neurons sparing the sensory system. The etiology of the disease poorly understood and several evidential theories fail to determine the exact cause. Disease presentation is a mixture of both UMN and LMN signs and symptoms (1). It manifests as progressive muscle weakness which begins in either limbs or bulbar regions and slowly spreads to other regions. The eventual fatality due to the disease is caused by weakness of the respiratory muscles and respiratory insufficiency (1). Some of the other symptoms of the disease are cramps and fasciculations present in multiple regions of the body, emotional disturbances, dysarthria, dysphagia, fatigue and spasticity. Reflexes may be exaggerated and Hoffmann's sign may also be positive. There are various presentations of the disease and the prognosis of the diseases is worse with bulbar onset, old age, presence of LMN features, low forced vital capacity (FVC) and low scores on revised ALS- functional rating scale (ALSFRSr) (7,8). ALS presents with variable clinical features which may appear similar to many other UMN and LMN diseases and hence to better categorize these patients revised El-escorial criteria are used. According to these patients are categorized into definite ALS, probable ALS and possible ALS. Diagnosis of definite ALS is made upon presence of both UMN and LMN symptoms in more than one body regions and/or elemyographic evidence of anterior horn involvement (9). ALS significantly hampers the quality of life of patients due to increased dependence for performing activities of daily living as the disease progresses.

Currently, there is no cure for ALS. A multi disciplinary management is the best approach. This includes pharmacological intervention, rehabilitation, artificial ventilator support in the later stages of the disease, Percutaneous endoscopic gastrotomy (PEG) preventing dysphagia related complications, etc.

Unmet medical needs

Because of the rapid progression and unknown etiology, ALS remains undefeated. All the conventional treatments available manage the symptoms and associated conditions, failing to address the core pathology of ALS.

Stem cell therapy for ALS

Stem cell transplantation is an attractive management strategy for ALS. Various types of cells, routes of administration and different protocols of administration are being studies widely world over. The safety of autologous stem cell transplantation has been established.

Marked progressive axonal degeneration of motor neurons in the spinal cord and motor cortex is noted in ALS (15). Multitude of mechanisms, have been thought to contribute. Non-neuronal glial cell and astrocyte involvement is also suggested in some studies (16). Up regulation of superoxide dismutase causes cascade of events and thereby oxidative stress. Whereas upregulation of glutamate cause excitotoxicity. Autoimmunity and widespread neuroinflammation are also stipulated contributors to the pathophysiology of ALS (17).

Replacement of degenerated motor neurons due to these causal factors is the ultimate goal of transplantation therapy but various factors influence the outcome of the transplanted cells. Survival of the cells in the host environment, their neurogenic potential, actual neurogenesis at the target site and formation of neuronal connections over long distances are some of the factors(18). As the transplantation science evolves these factors could be monitored to gain appropriate outcome but currently the aim of transplantation is to protect the existing motor neurons and attempt to bring out regeneration and repair in the damaged motor neurons. Although stem cells have neurogenic potential their fate is dependent on various factors. They have a neurotrophic influence on the nervous system and can home onto the site of injury (19). They further demonstrate immomodulatory, anti-inflammatory and cytoprotective properties (20). The factors secreted by these cells bring about neoangiogenesis (21). These paracrine effects lead to neuroprotection and subsequent alteration in the disease course and progression.

Animal Studies

The pre-clinical animal studies have shown benefits in the motor function. Human clinical trials are currently being undertaken. Safety of allogenic cells is also being studied and for the use of manipulated allogenic cells strict laboratory guidelines and clinical protocols must be followed (14)

Human studies

Frontal motor cortex transplantation suggested significant increase in survival. However the procedure of administration is extremely complex and can be performed only by a skilled surgeon(22).

Intra-spinal transplantation post laminectomy at the level of C1-C2 showed functional recovery of respiratory function, muscle strength and bulbar impairment. Another trial with spinal transplantation arrested the drop in the respiratory function and improvement on neurological scales. Intra-spinal administration also showed neurotrophic effect and preservation of existing neurons (23,24,25).

Intrathecal transplantation was found to reduce the rate of progression of the disease and drop in the ALS-FRS scores (26).

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

A phase I safety trial conducted by Mazzini et al. 2010 showed that autologous mesenchymal stem cells are safe to use for the treatment of ALS. The cells were injected intraspinally at the thoracic level and motor function improvement was observed (27).

Karussis et al. 2010 conducted a safety and efficacy trial with intrathecal and intravenous administration of autologous mesenchymal stem cells in 19 patients of ALS. These patients were followed up for 25 months. This trial reiterated the safety of autologous mesenchymal stem cells and also showed the immunomodulatory effects of MSCs in ALS (28).

A long term safety study with a follow up of over 9 years by Mazzini et al. 2012 showed that the treatment with autologous mesenchymal stem cells was safe but clinical could not be determined (29).

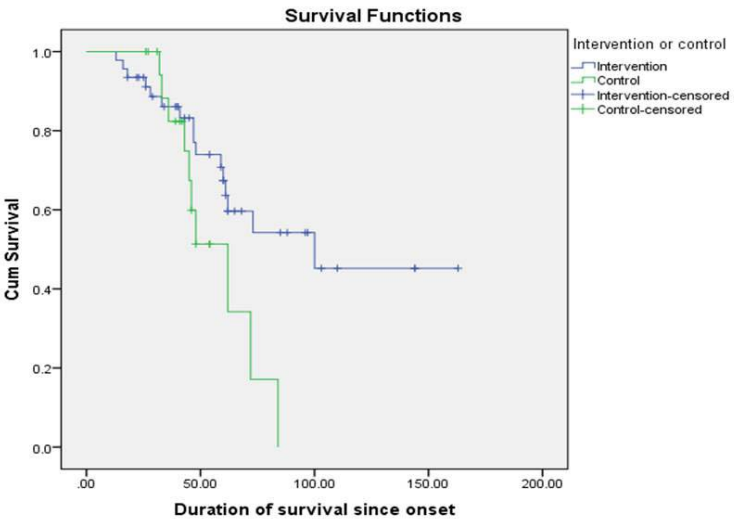
Our results

We analyzed the survival duration of the ALS patients treated with intrathecal autologous bone marrow mononuclear cells transplantation since August 2008 till February 2014. The survival duration of these patients was then compared to the survival duration of patients that did not undergo intrathecal autologous BMMNCs transplantation using Kaplan-Meier survival analysis. There were total 46 patients in the intervention group and 20 patients in the control group. Both these groups shared similar baseline demographic characteristics. Comparison of the survival duration suggested that the mean survival duration of the patients treated with intrathecal autologous BMMNCs transplantation was longer than those who were not treated (Table 1). The mean survival duration of the patients who received treatment was 104.069 (10.985) months and those who did not was 57.38(5.31). The difference between the two was statistically significant ($p=0.43$). A clinically significant difference of 47 months in the survival duration suggests the potential of intrathecal autologous BMMNCs transplantation in the treatment of ALS.

Table 1: Survival analysis

Survival analysis	Intervention group	Control group
Total mortality	35%	50.00%
Range of survival duration (months)	13 - 158	26-84
Mean survival duration (months)	104.069 (10.985)	57.38(5.31)

Figure 2: Kaplan Meier survival analysis comparison between intervention and control group



Future directions

Gene therapy: Suspected genetic causality of ALS and some evidence to support the genetic alterations in ALS has led to emergence of gene therapy as a future management strategy for ALS. A clinical trial using Antisense Oligonucleotides to reduce the toxic protein aggregates in ALS is currently being undertaken (11).

Nur-Own cells transplantation

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

Cellular therapy provides a promising future in the management of ALS. Prospective trials with rigorous methodology making use of randomization, blinding and larger sample size need to be carried out for conclusive evidence. It is of importance to compare the effects of different cell types. Combination of cellular transplantation

with various other neuroprotective regimens should also be studied to find the treatment option that gives best possible results in ALS.

REFERENCE

1. Gordon PH. Amyotrophic Lateral Sclerosis: An update for 2013 Clinical Features, Pathophysiology, Management and Therapeutic Trials. Aging Dis. 2013 Oct 1;4(5):295-310.
2. Haverkamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. Brain. 1995 Jun;118 (Pt 3):707-19.
3. Forbes RB, Colville S, Cran GW, Swingler RJ; Scottish Motor Neurone Disease Register. Unexpected decline in survival from amyotrophic lateral sclerosis/motor neurone disease. J NeurolNeurosurg Psychiatry. 2004 Dec;75(12):1753-5.
4. Millul A, Beghi E, Logroscino G, Micheli A, Vitelli E, Zardi A. Survival of patients with amyotrophic lateral sclerosis in a population-based registry. Neuroepidemiology. 2005;25(3):114-9
5. Norris F, Shepherd R, Denys E, U K, Mukai E, Elias L, Holden D, Norris H. Onset, natural history and outcome in idiopathic adult motor neuron disease. J Neurol Sci. 1993 Aug;118(1):48-55.
6. Lee CT, Chiu YW, Wang KC, Hwang CS, Lin KH, Lee IT, Tsai CP. Riluzole and prognostic factors in amyotrophic lateral sclerosis long-term and short-term survival: a population-based study of 1149 cases in Taiwan. J Epidemiol. 2013;23(1):35-40
7. Chiò A, Logroscino G, Hardiman O, Swingler R, Mitchell D, Beghi E, Traynor BG;Eurals Consortium. Prognostic factors in ALS: A critical review. Amyotroph Lateral Scler. 2009 Oct-Dec;10(5-6):310-23.
8. Kaufmann P, Levy G, Thompson JL, Delbene ML, Battista V, Gordon PH, Rowland LP, Levin B, Mitsumoto H. The ALSFRSr predicts survival time in an ALS clinic population. Neurology. 2005 Jan 11;64(1):38-43.
9. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000 Dec;1(5):293-9
10. Hardiman O. Multidisciplinary care in ALS: expanding the team. Amyotroph Lateral Scler. 2012 Feb;13(2):165.
11. Phukan J, Hardiman O. The management of amyotrophic lateral sclerosis. J Neurol. 2009 Feb;256(2):176-86.
12. Majmudar S, Wu J, Paganoni S. Rehabilitation in ALS: Why it matters. Muscle Nerve. 2014 Feb 9.
13. Mechanical ventilation for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev. 2013 Mar 28;3:CD004427.

14. Lunn JS, Sakowski SA, Federici T, Glass JD, Boulis NM, Feldman EL. Stem cell technology for the study and treatment of motor neuron diseases. Regen Med. 2011 Mar;6(2):201-13.

15. Al-Chalabi A, Jones A, Troakes C, King A, Al-Sarraj S, van den Berg LH. The genetics and neuropathology of amyotrophic lateral sclerosis. Acta Neuropathol.2012 Sep;124(3):339-52.

16. Bruijn LI, Miller TM, Cleveland DW. Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu Rev Neurosci. 2004;27:723-49

17. Walczak P, Chen N, Hudson JE, Willing AE, Garbuzova-Davis SN, Song S, Sanberg PR, Sanchez-Ramos J, Bickford PC, Zigova T. Do hematopoietic cells exposed to a neurogenic environment mimic properties of endogenous neural precursors? J Neurosci Res. 2004 Apr 15;76(2):244-54

18. Jiang C, Wang J, Yu L, Ou C, Liu X, Zhao X, Wang J. Comparison of the therapeutic effects of bone marrow mononuclear cells and microglia for permanent cerebral ischemia. Behav Brain Res. 2013 Aug 1;250:222-9.

19. Gnecchi, Massimiliano, et al. "Paracrine mechanisms in adult stem cell signaling and therapy." Circulation research 103.11 (2008): 1204-1219

20. Sharma S, Yang B, Strong R, Xi X, Brenneman M, Grotta JC, Aronowski J, Savitz SI. Bone marrow mononuclear cells protect neurons and modulate microglia in cell culture models of ischemic stroke. J Neurosci Res. 2010 Oct;88(13):2869-76.

21. Brenneman M, Sharma S, Harting M, Strong R, Cox CS Jr, Aronowski J, GrottaJC,Savitz SI. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. J Cereb Blood Flow Metab. 2010 Jan;30(1):140-9.

22. Martínez HR, Molina-Lopez JF, González-Garza MT, Moreno-Cuevas JE, Caro-Osorio E, Gil-Valadez A, Gutierrez-Jimenez E, Zazueta-Fierro OE, Meza JA, Couret-Alcaraz P, Hernandez-Torre M. Stem cell transplantation in amyotrophic lateral sclerosis patients: methodological approach, safety, and feasibility. Cell Transplant. 2012;21(9):1899-907.

23. Blanquer M, Pérez Espejo MA, Iniesta F, Gómez Espuch J, Meca J, Villaverde R, Izura V, de Mingo P, Martínez-Lage J, Martínez S, Moraleda JM. (Bone marrow stem cell transplantation in amyotrophic lateral sclerosis: technical aspects and preliminary results from a clinical trial). Methods Find Exp Clin Pharmacol. 2010 Dec;32 Suppl A:31-7.

24. Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-yearfollow-up H Deda, MC Inci , AE Ku¨rekc , A Sav , K Kay?han , E O¨ zgu¨n , GE U¨ stunsoy1 and S KocabayCytotherapy (2009) Vol. 11, No. 1, 18_25

25. Blanquer M, Moraleda JM, Iniesta F, Gómez-Espuch J, Meca-Lallana J, Villaverde R, Pérez-Espejo MÁ, Ruíz-López FJ, García Santos JM, Bleda P, Izura V, Sáez M, De Mingo P, Vivancos L, Carles R, Jiménez J, Hernández J, Guardiola J, Del Rio

ST,Antúnez C, De la Rosa P, Majado MJ, Sánchez-Salinas A, López J, Martínez-LageJF,Martínez S. Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: a pilot safety study. Stem Cells. 2012 Jun;30(6):1277-85.

26. Prabhakar S, Marwaha N, Lal V, Sharma RR, Rajan R, Khandelwal N. Autologous bone marrow-derived stem cells in amyotrophic lateral sclerosis: a pilot study. Neurol India. 2012 Sep-Oct;60(5):465-9
27. Mazzini L, Ferrero I, Luparello V, Rustichelli D, Gunetti M, Mareschi K, Testa L, Stecco A, Tarletti R, Miglioretti M, Fava E, Nasuelli N, Cisari C, Massara M, Vercelli R, Oggioni GD, Carriero A, Cantello R, Monaco F, Fagioli F. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. Exp Neurol. 2010 May;223(1):229-37.
28. Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, Bulte JW, Petrou P, Ben-Hur T, Abramsky O, Slavin S. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol. 2010 Oct;67(10):1187-94.
29. Mazzini L, Mareschi K, Ferrero I, Miglioretti M, Stecco A, Servo S, Carriero A, Monaco F, Fagioli F. Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: a long-term safety study. Cytotherapy. 2012 Jan;14(1):56-60.

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

– Mahatma Gandhi

14

Role of Stem Cells in Traumatic Brain Injury

Traumatic brain injury (TBI) is mostly caused by an external physical impact producing an altered state of consciousness resulting in impairment of physical functions or cognitive abilities (1) It is one of the leading causes of morbidity and mortality in the world. The damage to the brain could be 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) Alterations in cerebral blood flow and oxygenation, edema, excitotoxicity, cell death, disruption of the blood brain barrier, and generalized atrophy is commonly observed in TBI. (3) The damage to the brain could result in temporary or permanent behavioral and/or emotional disturbances leading to functional disability.

Very few treatment alternatives are currently available to treat TBI. Pharmacological and surgical intervention along with rehabilitation are used for the management of the symptoms.

Unmet medical needs

In chronic TBI, the life expectancy of the affected is normal, but there is high prevalence of the residual disability arising from the injury. These include hemiparesis, spasticity, cognitive, emotional and behavioral issues, etc. The available pharmacological modalities manage these disabilities, but their effect wears off gradually. The rehabilitation resources are inadequate for the increasing number of survivors of TBI. There is diffuse white matter damage which cannot be addressed by current medical treatments. Also, the gliotic areas in the brain cannot be reversed.

Stem cell therapy in TBI

Due to the brain's limited capacity to regenerate the damaged neaurons, the intervention should aim at halting the degeneration and replacing the lost and damaged neurons. (4) In past few years, cell therapy has gained attention as a prospective therapeutic options for neurological disorders. Stem cells migrate towards the damaged areas of the brain and initiate the repair process. They promote angiogenesis, axonal remodeling, neurogenesis and synaptogenesis, which may help reverse the pathology of TBI. (5) These cells differentiate into various cells including neural cells, oligodendrocytes, etc. (6) In TBI, there is loss of myelin which disrupts the signal transduction and damages the axons. The oligodendrocytes help in remyelination of the damaged axons and repair the disrupted neural connections. Bone marrow cells also produce various growth factors and neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF), which stimulate the endogenous neuroprotection and repair. (7,8)

Animal studies

Various experiments on animal models have been carried out to test the safety and feasbility of different types of cells. Reiss et al, transplanted embryonic cells in experimental rats and recorded dramatic improvements. But, over the period of observation they also recorded tumor formation in the rats, raising serious safety concerns about the use of these cells. (9) Series of experiments were conducted to study the neural stem cells in TBI which reported improved neurological functions in the injected rat models via various mechanism. (10-13) Bone marrow stem cells were also found to be efficacious. (14) In rat models, these cells modulated the inflammation-associated immune cells and cytokines in TBI-induced cerebral inflammatory responses. (15) Recently umbilical cord cells have also been tested in rats. In an experimental study, rats were injected with brain-derived neurotrophic factor (BDNF) gene-modified umbilical cord mesenchymal stem cell (UCMSC). These cells survived and migrated to the cerebral tissues. They led to dramatic improvements in behavior and other neurological functions. (16)

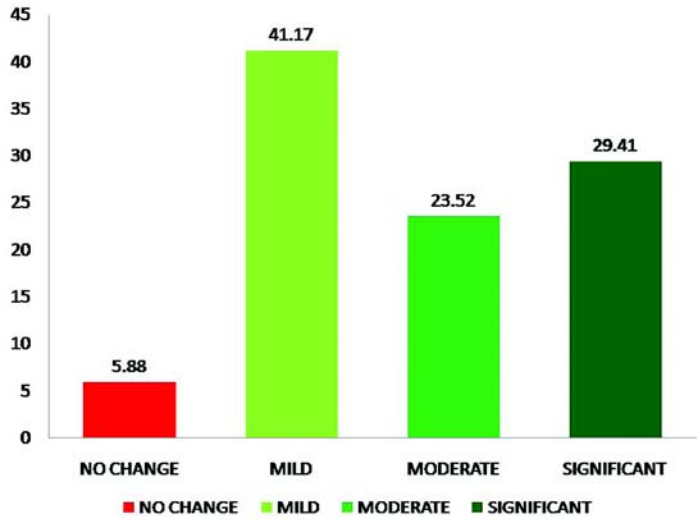
Human Studies

Stem cell therapy for TBI is still in an experimental stage. Not many clinical trials have been conducted on human subjects. Wang et al published the results of his study conducted on patients with sequelae of TBI. He administered 40 patients with umbilical cord mesenchymal stem cells. They observed improved neurological functions and self care in these patients as compared to the controls. (17)

Our Results

17 patients with confirmatory diagnosis of Traumatic brain Injury were included in the analysis. These patients had attained a plateau stage with respect to symptomatic

and functional improvements. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant improvements. The symptoms included higher mental functions, posture, trunk activity, upper limb activity, lower limb activity, coordination, oromotor, ambulation and Activities of Daily Living. Mild improvement was defined as improvements till 3 of the symptoms mentioned. Moderate was considered when 4 to 6 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 7 to 9 of the symptoms. Analysis revealed that out of 17 patients, 5.88% of patients showed no improvements in any of the symptoms. Mild improvements were observed in 41.17% of patients, moderate in 23.52% of patients, whereas, 29.41% of patients showed significant improvements. Improvement in brain metabolism was seen on PET CT scan of brain in 4 patients after the intervention. Traumatic Brain Injury generally characterized by more diffuse and global brain injury, leading to damage to multiple areas of the CNS. This can be the reason for most of the patients contributing mainly to mild improvements.



Future directions

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

References

1. Prins M, Greco T, Alexander D, Giza CC. The pathophysiology of traumatic brain injury at a glance. Dis Model Mech. 2013; Nov;6(6):1307-15
2. C. Werner and K. Engelhard. Pathophysiology of traumatic brain injury Br. J. Anaesth. (2007) 99 (1): 4-9
3. Greve MW, Zink BJ. Pathophysiology of traumatic brain injury. Mt Sinai J Med. 2009 Apr;76(2):97-104
4. Horner PJ, Gage FH. Regenerating the damaged central nervous system. Nature.2000 Oct 26;407(6807):963-70.
5. Longhi L, Zanier ER, Royo N, Stocchetti N, McIntosh TK. Stem cell transplantation as a therapeutic strategy for traumatic brain injury. Transpl Immunol. 2005 Dec;15(2):143-8
6. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol. 2000;164:247-256
7. Zhong C, Qin Z, Zhong CJ, Wang Y, Shen XY. Neuroprotective effects of bone marrow stromal cells on rat organotypic hippocampal slice culture model of cerebral ischemia. Neurosci Lett. 2003;342:93-96.
8. Hsu YC, Chen SL, Wang DY, Chiu IM. Stem cell-based therapy in neural repair.Biomed J. 2013 May-Jun;36(3):98-105
9. Riess P, Molcanyi M, Bentz K, Maegele M, Simanski C, Carlitscheck C, Schneider A, Hescheler J, Bouillon B, Schäfer U, Neugebauer E. Embryonic stem cell transplantation after experimental traumatic brain injury dramatically improves neurological outcome, but may cause tumors. J Neurotrauma. 2007 Jan;24(1):216-25
10. Zhang H, Zheng X, Yang X, Fang S, Shen G, Zhao C, Tian M. 11C-NMSP/ 18F-FDG microPET to monitor neural stem cell transplantation in a rat model of traumatic brain injury. Eur J Nucl Med Mol Imaging. 2008 Sep;35(9):1699-708.
11. Lee DH, Lee JY, Oh BM, Phi JH, Kim SK, Bang MS, Kim SU, Wang KC. Functional recovery after injury of motor cortex in rats: effects of rehabilitation and stem cell transplantation in a traumatic brain injury model of cortical resection. Childs Nerv Syst. 2013 Mar;29(3):403-11.
12. Wang JY, Liou AK, Ren ZH, Zhang L, Brown BN, Cui XT, Badylak SF, Cai YN, Guan YQ, Leak RK, Chen J, Ji X, Chen L. Neurorestorative effect of urinary bladder matrix-mediated neural stem cell transplantation following traumatic brain injury in rats. CNS Neurol Disord Drug Targets. 2013 May 1;12(3):413-25.
13. Yu B, Ma H, Kong L, Shi Y, Liu Y. Enhanced connexin 43 expression following neural stem cell transplantation in a rat model of traumatic brain injury. Arch Med Sci. 2013 Feb 21;9(1):132-8.

14. Bakhtiary M, Marzban M, Mehdizadeh M, Joghataei MT, Khoei S, Pirhajati Mahabadi V, Laribi B, Tondar M, Moshkforoush A. Comparison of transplantation of bone marrow stromal cells (BMSC) and stem cell mobilization by granulocyte colony stimulating factor after traumatic brain injury in rat. Iran Biomed J. 2010 Oct;14(4):142-9.
15. Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, Chen FF, Jiang XD. Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation. 2013 Aug 23;10(1):106
16. Yuan Y, Pan S, Sun Z, Dan Q, Liu J. Brain-derived neurotrophic factor-modified umbilical cord mesenchymal stem cell transplantation improves neurological deficits in rats with traumatic brain injury. Int J Neurosci. 2013 Dec 9. [Epub ahead of print]
17. Wang S, Cheng H, Dai G, Wang X, Hua R, Liu X, Wang P, Chen G, Yue W, An Y. Umbilical cord mesenchymal stem cell transplantation significantly improves neurological function in patients with sequelae of traumatic brain injury. Brain Res. 2013 Sep 26;1532:76-84.

SECTION C

Important Related Aspects

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

– Rudyard Kipling

15

Radiological Imaging in Stem Cell Therapy

Stem cell therapy is being widely explored as a therapeutic treatment modality. At present, there are many ongoing clinical studies to understand the encouraging outcomes of stem cell therapy. Currently, there are many ongoing clinical studies to understand the therapeutic effects of stem cell therapy. However, the safety and efficacy needs to be validated and effectively documented with the help of imaging. So, one of the most available or non-invasive means to study the effects of cell based therapy is functional imaging. There have been evidences substantiating the positive therapeutic outcomes of cell therapy.

In stem cell therapy, the major effect is exerted by the mechanisms [1] that translate into functional changes; more than structural changes. The structural changes may take a longer time to appear and appreciated objectively; as opposed to functional changes that appear earlier. Hence, these early functional changes need to be studied with the help of newer, more sophisticated imaging techniques.

Functional Neuroimaging

The basic principle of Functional neuro-imaging is that the changes in the blood flow and the energy metabolism is associated with the activity of the nervous tissue [2]. There are various modalities used in functional imaging, out of which, the ones that have been used in our studies have been:

(I) **PET CT Scan of the brain:** It is a functional imaging technique that produces a three dimensional image that reveals the physiological activity and reflects the functional processes in the body. The FDG (fluoro-deoxyglucose), which is an analogue of Glucose, is used to measure this metabolic activity of the tissue. Glucose transporter proteins transport FDG to the cells. It undergoes common metabolic changes as that of glucose molecules however once it has been converted to FDG

- 6 Phosphate it cannot be further metabolized. Because the cell membrane is impermeable to this molecule it gets trapped in the cell [3]. This trapping is directly proportional to the rate of Glycolysis in the tissue. Glycolysis is a metabolic pathway used to release energy from glucose molecule. PET measures the retention of FDG per volume of the tissue. Increased retention of FDG therefore indicates better metabolic activity of the tissue [4].

In the studies that we had performed, measurements were taken before and six months after the transplantation. PET studies were performed using the Siemens Biographmct with 64 slice high speed scanner- 3D PET True V wide detector [5] which has an intrinsic resolution of 0.6-mm full width at half maximum (FWHM) and the images of 45-50 contiguous transverse planes with a field of view of 21.6 cm axial PET FOV with True V. Standard conditions were maintained during all of the [18F1] FDG PET scans. Time duration between injection of the dye and scanning was constant at 30 minutes for all the patients and at all instances. The scan room was dimly lit and there was minimal auditory stimulation during injection and scanning period. PET scan was performed with patients lying in supine position with eyes closed to reduce any activity related confounding effect. Imaging data were processed using proprietary Scenium Software before and final image reconstruction.

We used PET - CT scan to observe the metabolic activity of the brain before and six months after cellular therapy. The scan was done in a standardized manner, maintaining similar conditions pre-scanning to ameliorate confounding factors and therefore the changes in the 18-FDG uptake may be attributed to the cell therapy based intervention. We followed robust protocol based on the European Association of Nuclear Medicine (EANM) guidelines [12] for administration of radiotracer, PET measurement, and image reconstruction to reduce the effect of various confounding factors that affect brain metabolism, image reconstruction, and SUV. This ensured the pre- and post therapy comparability of the PET-CT scans. As per the EANM guidelines the interpretation was based on the Standard uptake values (SUV), visual interpretation of the absolute and statistical reconstruction of the image, and value of standard deviations away from the mean as compared to baseline data. PET measures the retention of FDG per predetermined volume, standard uptake value (SUV). Standard uptake value is the ratio of the actual concentration of glucose in brain tissue and the hypothetical concentration of the glucose in brain tissue if it was distributed evenly in all the areas of brain. SUV is calculated for specifc region of interest (ROI) based on the image acquisition and is only the best estimate of the absolute uptake [13]. Increased SUV indicates better metabolic activity of the tissue [14]. The following were the conditions in which a functional recovery was documented in Autism, Cerebral Palsy and stroke using the PET imaging modality.

(A) PET CT scan in Cerebral Palsy:

Given below is an example of beneficial outcomes of cell therapy in a case of cerebral

palsy [5] in a 2 year old girl with spastic cerebral palsy who underwent stem cell therapy and showed that (Fig 1) the black arrow signifies reduced FDG uptake in the left mesial temporal structures as compared to the right side. The white arrow signifies reduced signifies reduced FDG uptake in the right basal ganglia seen as blue areas, as compared to the left side which are seen as the green areas. In Fig 2, the PET images show a relative hypo-metabolism of the left mesial temporal structures and right basal ganglia structures and also show that there is same metabolism throughout the rest of the areas of the brain. In Fig 3, the focused slice of mesial temporal lobes show equal FDG uptake on both sides post stem cell therapy. On comparing it with the earlier scan, it was noted that the morphological abnormality was more or less stable. The relative reduction of FDG uptake in the left medial temporal lobe and right basal ganglia had appeared to be resolved and they showed increased FDG uptake (Figure 2). Increase in FDG uptake was also recorded in the right medial temporal structures. The frontal, temporal, parietal and occipital lobes also showed increased uptake of FDG. (Figure 3)

The clinical improvements six months after mononuclear cell transplantation, she showed progressive significant improvements in the sitting balance and the spasticity of all limbs had reduced. She could balance herself while standing erect. Her head control improved and she was more co-operative than before. She was now able to speak sentences.

Fig 1 : PET images in cerebral Palsy pre cell therapy.

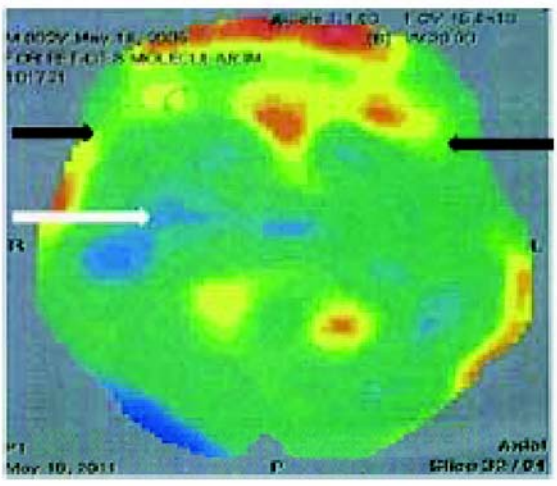


Figure 1: PET-CT scan before the stem cell therapy: Black arrow signifies reduced FDG uptake in the left mesial temporal as compared to the right side. The white arrow signifies reduced FDG uptake in the right basal ganglia seen as blue areas, as compared to the left side which are seen as green areas.

Fig 2 and Fig 3 : PET images in Cerebral Palsy post cell therapy

Fig 2 and Fig 3 : PET images in Cerebral Palsy post cell therapy

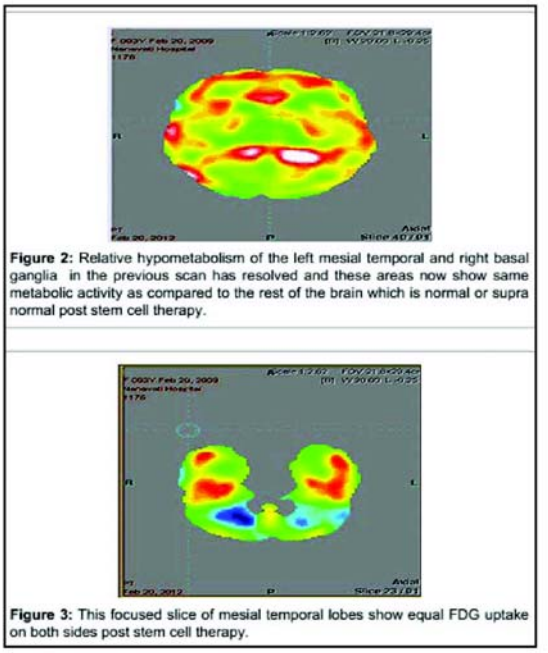


Figure 2: Relative hypometabolism of the left mesial temporal and right basal ganglia in the previous scan has resolved and these areas now show same metabolic activity as compared to the rest of the brain which is normal or supra normal post stem cell therapy.

Figure 3: This focused slice of mesial temporal lobes show equal FDG uptake on both sides post stem cell therapy.

(B) PET CT scan in Stroke:

As analysed in the clinical study on stroke patients, functional improvements were seen that were objectively assessed on PET images as given below. In the below images, the pre stem cell therapy images (Fig 1) and the six months post stem cell therapy images(Fig 2), there was an increased FDG uptake seen in the right cerebral hemisphere that shows a reduction in the gliotic areas, which co-related with the clinical improvements.

Fig 1: Pre cell therapy in stroke

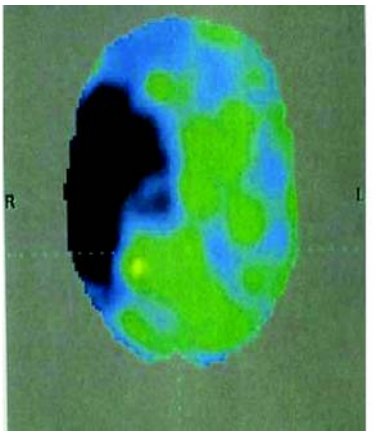
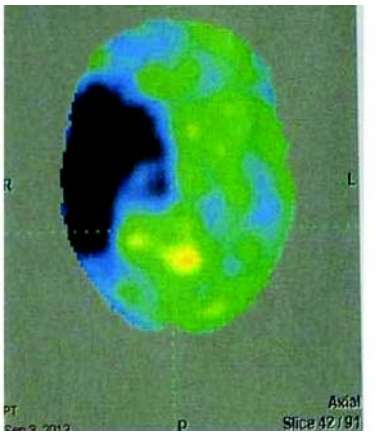


Fig 2: Post Cell Therapy in stroke



As seen in another case of stroke and put forth in the images given below, the pre cell therapy (Fig 3) and the post cell therapy images (Fig 4) show a reduction in the gliotic areas in the left cerebral hemispheres and in the right cerebral hemispheres. In the pre cell therapy images, there is a reduced FDG uptake in the Left Temporo-parietal regions. In the post cell therapy images, there is an increased FDG uptake in the left temporo parietal images.

Fig 3: Pre cell therapy in stroke

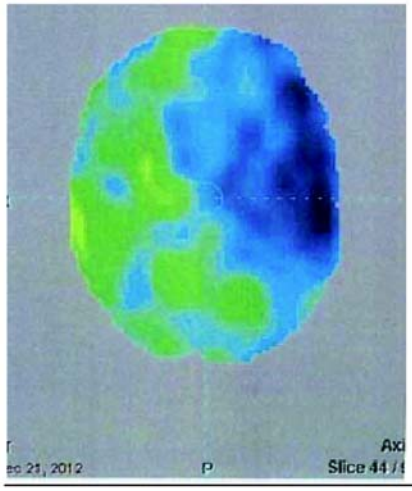
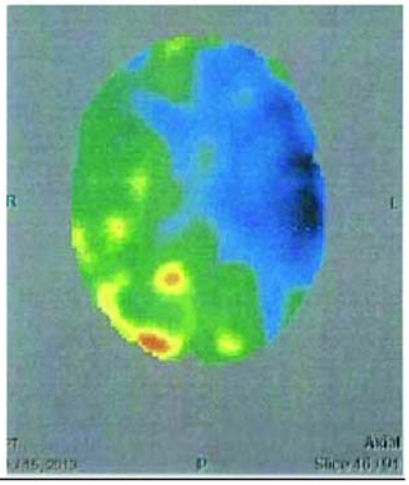


Fig 4: Post Cell Therapy in stroke



As put forth in another case of stroke and treated with cell therapy, the below given images portray changes in the PET before cell therapy (Fig 5) and six months post cell therapy (Fig 6). These were found to co-relate with clinical improvements. As put forth in the below images, in the pre cell therapy images, there is reduced FDG uptake in the left temporo-parietal areas, while in the post cell therapy scans, there is an improved FDG uptake in the same areas which shows reduced amount of gliosis.

Fig 5: Pre cell therapy in stroke

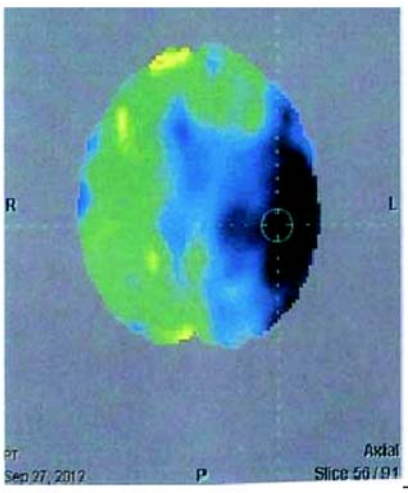
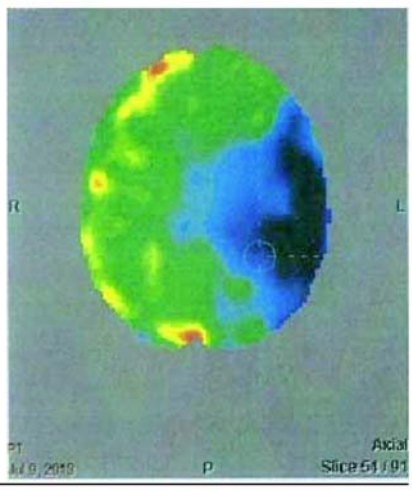


Fig 6 : Post Cell Therapy in stroke



(C) PET in Autism :

In our study we analysed a case of a 14 year old boy with autism who was found to have severe autism [7]. Given below are the PET CT Scan images pre stem cell therapy and post six months of stem cell therapy after which he was found to have mild autism. Comparative study of previous and post stem cell therapy PET CT scan showed markedly increased uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex. All the views; saggital, transverse and coronal views showed the improvements that co-related with the clinical improvements. Within a week, there was improvement in his eye contact and attention. His hand-writing and fine motor activities like buttoning had improved significantly. On follow up after six months, further improvements were observed in his behavior with respect to social interaction and emotions. Aggression in activities and hyperactivity had reduced by 45 to 50%. Improvements in impulse control, reading skills, tracing, recognition of all shapes and following commands were noted. His score on CARS reduced from 42.5 (Severely autistic) to 23.5 (Non-Autistic) but the general impression on clinical assessment showed mild autism. At one year follow up, he was found to interact more with his peers. Peer activity had increased significantly. New task learning abilities had improved which was noticed due to increased participation in household work. Comprehension and ability to follow commands had improved significantly. He had developed self insight and appropriate emotional response.

(II) **Single-photon emission computed tomography (SPECT):** is imaging technique using gamma rays. It is very similar to conventional nuclear medicine planar imaging using a gamma camera. However, it is able to provide true 3D information and this information is typically presented as cross-sectional slices through the patient, but can be freely reformatted or manipulated as required. The basic difference between PET and SPECT is that the PET measures metabolism and SPECT measures blood flow. The basic technique requires delivery of a gamma-emitting radio-isotope into the patient, normally through injection into the bloodstream. In SPECT, a marker radioisotope, which is of interest only for its radioactive properties, has been attached to a specific ligand which is of interest for its chemical binding properties to certain types of tissues. This combination of ligand and radioisotope to be carried and bound to a place of interest in the body, which then (due to the gamma-emission of the isotope) allows the ligand concentration to be seen by a gamma-camera.

(III) **Functional MRI (fMRI) :** It is a functional neuro-imaging procedure that uses magnetic reasonance technology that measures brain activity by detecting associated changes in blood flow [10]. This technique is based on the premise that cerebral blood flow and neuronal activation are coupled and a change in either would reflect a change in other. When an area of the brain is in use, blood flow to that region also increases. The primary form of fMRI uses the Blood-oxygen-level dependent (BOLD) contrast which is a type of specialized brain and body scan used to map neural activity in the brain or spinal cord of humans or other animals

FIG A

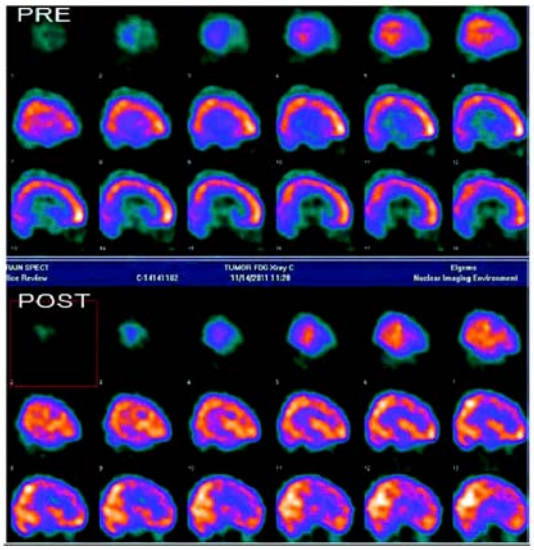


FIG B

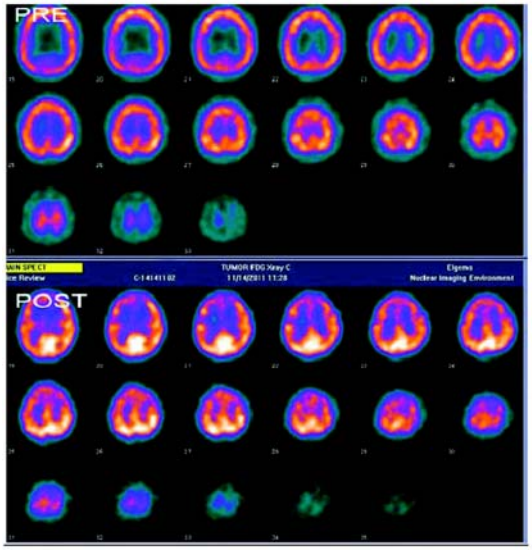


FIG C

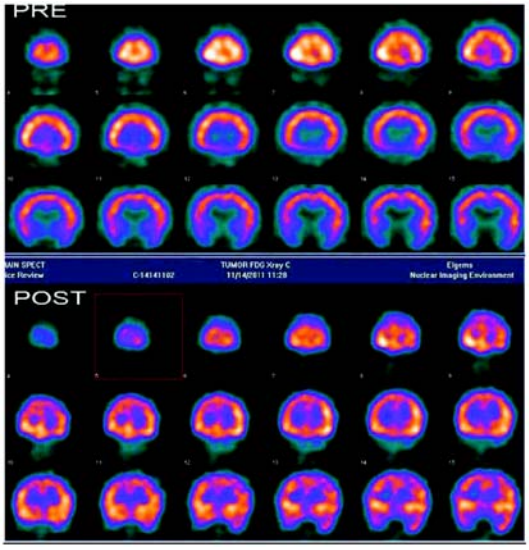


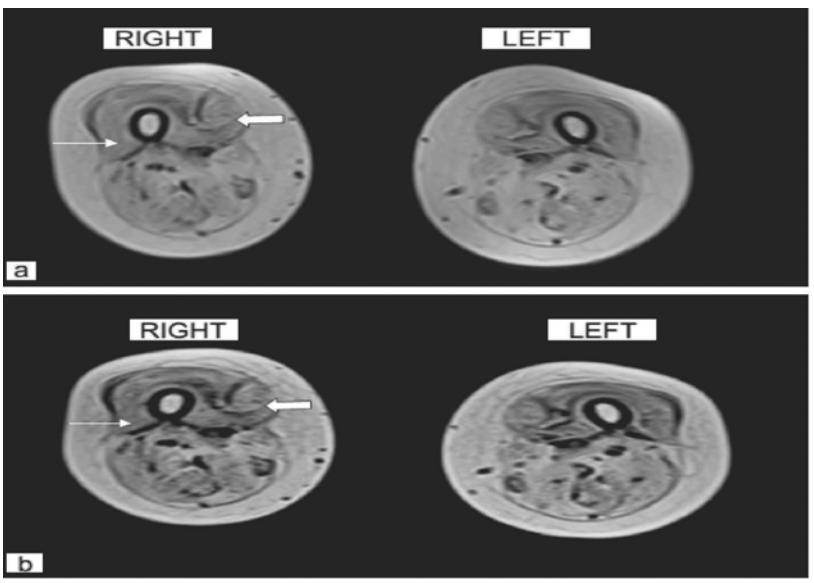
Figure 1: A, B, C: The Pre and the Post cell therapy PET CT scans. Comparative study of pre and post cell therapy PET CT scan shows increased FDG uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex as visualized below in the post scans.

by imaging the change in blood flow (hemodynamic response) related to energy use by brain cells.

The biggest advantage of fMRI is that it doesn't use radiation like X-rays, computed tomography and Positron emitted tomography. If fMRI is done correctly, it has virtually no risks. It can evaluate brain function safely, noninvasively and effectively. fMRI is easy to use, and the images it produces are very high resolution (as detailed as 1 millimeter).

Yet fMRI also has its disadvantages. First, it's expensive. Second, it can only capture a clear image if the person being scanned stays completely still. Also, one of the major drawbacks is that, it can only look at the blood flow in the brain and spinal cord. It can't home in on the activities of individual neurons, which are critical to understanding function. Each area of the tissue in brain and spinal cord, studied in fMRI is made up of thousands of individual neurons and, because certain areas of the brain that "light up" on fMRI may represent a number of different functions, it's hard to tell exactly what kind of brain activity is being represented on the scan. At present, though an effective imaging modality, but more standardized protocols are required for the same. Especially in children with cognitive deficits, it is difficult to use fMRI to assess the effects of cell therapy. Further studies are required before it can be established as a monitoring tool for effects of cell therapy.

(IV) **MRI-MSK:** Musculo skeletal MRI is an important diagnostic tool for researchers involved in for the spectrum of healthcare providers who treat musculoskeletal conditions. Magnetic resonance imaging (MRI) in particular holds great potential for clinical and research purposes due to the ability to display high definition



In Fig A and in Fig B, the images of post cell therapy are suggestive of lesser fatty infiltration and regeneration of muscle fibers six months post stem cell therapy.

images of the musculoskeletal system. It can also be used to understand the course of disease progression in muscular conditions such as Muscular Dystrophy [11] and to effectively study the process of regeneration post cellular therapy intervention [8]. In our studies, MRI MSK was used to objectively assess the level of fatty infiltration and instances of muscle regeneration pre and post cell therapy. After cell therapy, it has been found that the disease process has remained stable that has been objectively assessed on musculo-skeletal MRI. In these patients, MRI-MSK showed no increase in fatty infiltration of the muscles in the post cellular scans. Given below is the MRI-MSK images of an 18 year old male diagnosed with DMD [9] and the images of his quadriceps muscle on both sides pre and post six months after cell therapy. These images portray improvements that reflect clinical improvements.

CONCLUSION:

Cell therapy has shown effective functional outcomes [14], [15]. The encouraging outcomes of cellular therapy can be more effectively studied by means of functional imaging; as opposed to structural imaging. Functional imaging has been found to be more sensitive in picking up functional changes as seen in cellular therapy. Hence, the role of functional imaging would hold substantial advocacy in understanding and paving way for future premises of cellular therapy.

REFERENCES:

1. Anthony DF, Shiels PG (2013), Exploiting paracrine mechanisms of tissue regeneration to repair damaged organs : 2013 Jun 20;2(1):10. doi: 10.1186/2047-1440-2-10.
2. Raichle ME, Feiz JA, Videen TO, MacLeod AK, Pardo JV, Fox PT, Petersen SE (1994): Practice-related changes in human brain functional anatomy during nonmotor learning. Cereb Cortex 4:8-26.
3. E. K. J. Pauwels (1998) FDG Accumulation and Tumor Biology Nuclear Medicine and Biology Volume 25, Issue 4, May 1998, Pages 317-322.
4. S Ahmad Sarji (2006) Physiological uptake in FDG PET simulating disease. Biomed Imaging Interv J 2006; 2(4):e59
5. Siemens-CTI, Knoxville, Tenn., Imaging Life: The Magazine for Molecular Imaging Innovation, Issue Number 02/June 2011 SNM-Edition | June 4-8, 2011 USA
6. Sharma A, Kulkarni P, Sane H, Gokulchandran N, Badhe P, et al (2012) Positron Emission Computed Tomography Scan Captures the Effects of Cellular Therapy in a case of Cerebral Palsy. J Clin Case Rep 2:195. Doi: 10.4172/2165-7290.1000195.
7. Sharma A, Gokulchandran N, Badhe P, Kulkarni P, Mishra P, et al. (2013), An Improved Case of Autism as Revealed by PET CT Scan in Patient Transplanted with Autologous Bone Marrow Derived Mononuclear Cells. J Stem Cell Res Ther 3:139.

8. Liu GC, Jong YJ, Chiang CH, Jaw TS (1993) Duchenne muscular dystrophy: MR grading system with functional correlation, Radiology. 1993 Feb;186(2):475-80.
9. A Sharma, Pooja Kulkarni, G Chopra, N Gokulchandran, M Lohia, P Badhe: Autologous Bone Marrow-derived Mononuclear Cell Transplantation in Duchenne Muscular Dystrophy, Indian Journal of Clinical Practice, Vol. 23, No. 3, August 2012.
10. Huettel, S. A.; Song, A. W.; McCarthy, G. (2009), Functional Magnetic Resonance Imaging (2 ed.), Massachusetts: Sinauer, ISBN 978-0-87893-286-3
11. Arne Fischmann, Patricia Hafner , Monika Gloor, Maurice Schmid ,Andrea Klein, Urs Pohlman, et al (2012), Dirk Fischer Quantitative MRI and loss of free ambulation in Duchenne muscular dystrophy: J Neurol DOI 10.1007/s00415-012-6733-x
12. A. Varrone, S. Asenbaum, T. Vander Borght et al., "EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2," European Journal of Nuclear Medicine and Molecular Imaging, vol. 36, no. 12, pp. 2103-2110, 2009.
13. J. A. Thie, "Understanding the standardized uptake value, its methods, and implications for usage," Journal of Nuclear Medicine, vol. 45, no. 9, pp. 1431-1434, 2004.
14. Alok Sharma, Nandini Gokulchandran, Hemangi Sane, Anjana Nagrajan, Amruta Paranjape, Pooja Kulkarni, Akshata Shetty, PritiMishra, Mrudula Kali, Hema Biju, nd Prerna Badhe: (2013) Autologous Bone Marrow Mononuclear Cell Therapy for Autism: An Open Label Proof of Concept Study, Stem Cells International, Volume 2013, Article ID 623875, 13 pages
15. Alok Sharma, Nandini Gokulchandran, Guneet Chopra, Pooja Kulkarni, Mamta Lohia, Prerna Badhe, and V. C. Jacob (2012), Administration of Autologous Bone Marrow-Derived Mononuclear Cells in Children With Incurable Neurological Disorders and Injury Is Safe and Improves Their Quality of Life Cell Transplantation, Vol. 21, Supplement 1, pp. S79-S90, 2012

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

– Henry Ford

14

Importance of Neurorehabilitation – Concept of NRRT

Neurorehabilitation is the clinical subspecialty that is devoted to the restoration and maximization of functions that have been lost due to impairments caused by injury or disease of nervous system. The goals of neurorehabilitation is to help patients with impairments and disabilities and to make them functionally independent, which requires team of rehabilitation specialists, such as nurses, physical therapists, occupational therapists, speech therapist, psychologist and others. (1)

Importance of Rehabilitation:

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

Neurorehabilitation team has an understanding of neural regulation of movement patterns. As framework for typical motor behaviour is necessary to understand how motor behaviour is altered in persons with neurological dysfunction and how plastic properties of nervous system interact to produce change.

Motor control is the study of how an individual controls movements already acquired. Neuroplasticity is defined as brain's ability to adapt or use cellular adaptations to learn or relearn functions which are previously lost as result of cellular death by trauma or disease at any age. Neuronal sprouting is thought to be primary mechanism, allowing injured neurons, to reconnect in new ways and allowing intact undamaged neurons to form new connection and to enhance function. Motor learning will continue throughout life as long as environment asks for change and CNS has pliability and desire to learn. The rehabilitation team promotes this learning and facilitates neural plasticity (2)

The philosophic foundation of rehabilitation team is to promote purposeful activity

thereby preventing dysfunction and eliciting maximum adaptation. These goal-oriented activities are meant to be culturally meaningful and important to the needs of patient and their families. Activities include daily life and work skills, exercise, recreation and crafts.

Exercise tasks in animal models, have shown that specifically skilled type of exercises lead to increased angiogenesis in damaged cortical areas whereas unskilled activities did not show this positive change. It is believed that in humans too rehabilitation techniques would enhance neuroplastic changes.

Concept of Neuro Regenerative Rehabilitation Therapy (NRRT) :

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

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

Physical therapy

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

Practices in Physical Therapy includes:

1. Therapeutic exercise and reeducation.
2. Neurofacilitation techniques.
    i) Proprioceptive neuromuscular facilitation
    ii) Bobath
    iii) Brunnstrom
    iv) Rood
3. Motor skills learning.
4. Task-oriented practice.
5. Forced use.
6. Massed Practice.
7. Biofeedback.

8. Virtual environment training.
9. Musculoskeletal techniques.
10. Electromyogram-triggered neuromuscular stimulation.
11. Orthosis and assistive devices.

Occupational Therapy

Occupational Therapists bring expertise to the rehabilitation team in enhancing the independence and personal satisfaction of patients in their activities of daily living(ADL), community and leisure activities, social integration, and work performance.

They play integral part in evaluating the need for a range of assistive devices and training patients to make them independent in eating, dressing, bathing combing and other ADL.

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

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

Psychology:

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

Neuropsychological testing and evaluation is to identify the pattern of cognitive, behavioural, and emotional strengths and weaknesses and to provide specific treatment recommendations or clarify diagnostic questions. The domains and tests specified Psychological Counseling:

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

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

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

symptoms and character problems.

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

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

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

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

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

vii) Brief Therapy: It emphasizes (4) a focus on a specific problem and (5) direct intervention. It is solution-based rather than problem-oriented..

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

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

Play Therapy, Gestalt Therapy, Rational Emotive Behaviour Therapy, Solution based therapies and Reality Therapy some other forms of psychotherapy. (6)

Speech therapy:

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

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

i) Aphasia:

Aphasia is defined as loss of reception or expression of language as a result of brain stroke. It can be classified as Broca's aphasia (patient presents with intact comprehension with affected expression), Wernicke's (patient presents with affected comprehension with jargon speech), Anomia or nominal aphasia (patient presents with naming difficulties).(4)

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

a) Improving auditory comprehension using pointing tasks "point to the spoon".

b) Encouraging verbal utterances voluntarily.

c) Improving sentence formation.

d) Improving naming

A study done on aphasics concluded that combination of two inout channels - auditory plus visual, auditory plus gestural may facilitate better comprehension and performance by the patient (Darley, 82)

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

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

ii) Dysarthria:

The literal definition of dysarthria is disordered utterance (dys means disordered or abnormal; arthria means to utter distinctly). A more comprehensive definition is that dysarthria is the impaired production of speech because of disturbances in the muscular control of the speech mechanism (as cited in Freed, 2000).

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

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

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

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

a) PNF (proprioceptive and neuromuscular facilitation).

b) Improving loudness levels.

c) Improving articulatory precision by using exaggerated consonants.

iii) Apraxia:

According to Darley (1969), apraxia is an articulatory disorder resulting from impairment, as a result of brain damage of the capacity to program the positioning of speech musculature and the sequencing of muscle movement for the volitional production of phonemes. No significant weakness, slowness, or incoordination in reflex and automatic acts is seen (as cited in Freed, 2000). (7)

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

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

iv) Dysphagia:

Dysphagia means disordered swallowing. Swallowing disorders occur in all age groups from newborns to the elderly, and can occur as a result of CVA, presence of tumors and/ or progressive neurologic conditions. (9)

Swallowing consists of 4 stages namely oral preparatory, oral, pharyngeal and esophageal stage. Depending upon the stage affected, a swallowing therapist needs to make a judgement on the treatment modality.

A swallowing therapist aims to work on:

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

During swallowing therapy, the therapist should ensure airway safety and rule out any silent aspiration.

Children with autism, cerebral palsy, hearing impairment or mental retardation present with either absence of speech or deficient speech and language skills as compared to their age. The main aim of the speech therapist is to bridge the gap between the chronological age and the language age of the child. The speech and language pathologist tries to explore the areas which the child would respond in and facilitate communication within child's impairment.

Most widely used techniques for language learning are repetitions, modeling utterances, expanding a topic and role play. However, children with higher grade of severity may have to rely on alternative and augmented communication (AAC) in order to reduce the communicative burden on the caregivers.

Various Neurological Conditions (Assessment and rehabilitation protocol)

1. Spinal cord Injury

Examination and Evaluation: emphasizing on following points

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

Neurological Examination:

1. American Spinal Cord Injury Association Examination: is used for specific

neurological examination after spinal cord injury.

2. Assessment of muscle performance allows for specific diagnosis of level and completeness of injury. Examination includes each specific muscle and identifies subsititutions from other muscles.
3. Presence, absence and location of muscle tone should be assessed as a common tool to describe tone, using Modified Ashworth Scale.
4. Sensation is described by dermatome. The recommended tests include:
   i) Sharp-dull discrimination or temperature sensitivity to test the lateral spinothalamic tract.
   ii) Light touch to test the anterior spinothalamic tract and
   iii) Proprioception or Vibration to test posterior columns of spinal cord.

Sensation is indicated as intact,impaired or absent per dermatome. A dermatomal map is helpful and recommended for ease of documentation.(10)

Functional Examination:

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

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

Prevention of all secondary complications as a result of being bed ridden.
Restoration of functional independency to the maximum possible limit.
Psychological councelling
Social and Vocational Rehabilitation
Family Education and Home adaptation

1. Education:
   - Patient and caregiver education plays an integral part of rehabilitation.
   - Formal education includes group and individual instruction and family/ caregiver training.
   - Preventive skin care,bowel and bladder programs, safe ways to perform all ADLS tasks, nutritional guidelines, thermoregulation precautions, pulmonary management, cardiopulmonary resuscitation, management of autonomic dysreflexia, equipment management and maintenance, transfer techniques, wheelchair chair mobility, ambulation, proper body positioning,ROM exercises,ADL basics and leisure skills should be taught.
   - Home programs to increase strength,endurance,ROM and function are taught.
   - Energy conservation techniquesand proper body mehanics should be incorporated.
2. Health Promotion and Wellness :
   - Exercise program for persons with SCI must take into consideration the musculoskeletal, respiratory, cardiovascular and autonomic nervous system

changes that occur after SCI.

- Components of an exercise program include flexibility,muscular strength and cardiovascular endurance.
- Frequency ranges from 2-5 times per week with atleast 1 day of rest between strengthening sessions.
- Duration of an exercise program as little as 20 minutes or as much as 90- 120 minutes.
- Intensity ranges between 40% and 85% of maximal heart rate or within 13 -15 on Borg Rate of Perceived Exertion Scale.
- Injuries can be prevented or slowed if clients perform a proper warm up with stretching/flexibility exercises, wear protective equipment (i.e helmet and padded gloves),alternate modes of exercises and get proper rest between exercises sessions.
- Equipments like weighted cuffs, elastic bands and tubing and hand cycles can be used for home exercises program.
- Health and Wellness program has potential to increase QOL, improve ADLS, decrease secondary complications, decrease depression and decrease no. of hospitalizations. (12)

3. Preventing and Managing Pressure Ulcers and Skin compromise:
   - Turning the positions at regular intervals, every 2-3 hrs.
   - Pillows and rectangular foam pads to cover bony prominence should be used.
   - Treatment like hydrotherapy, speciality wound dressings, electromodalities and thermomodalities to increase circulation can be given.
   - Surgical intervention with skin flaps or muscle flaps can be used to close the wound if not healed.
   - Patient should be educated to maintain skin integrity.
4. Prevention and Management of Joint Contracture:
   - Contracture may result in postural malalignment or impede potential function.
   - Daily ROM exercises and proper positioning will prevent contractures.
   - Use of splints for proper joint alignment techniques like wt bearing, ADLS and functional exercises prevents contracture.
   - Splinting to prevent Joint Deformity :
   - Deformity prevention is first goal for spinting. For e.g Patients with C8 andT1 injuries or incomplete injuries may have clawing or hyperextension of metacarpophalagneal joints which is due to stronger pull of finger extensors over finger flexors. Thus splints to block metacarpophalagneal joints and promote weak instrinsic muscle function.
   - Cost, time and material should be considered when deciding between custom

made and prefabricated.

- Education regarding splint wearing schedule, skin checks and splint care should be emphasized. (13)

5. Bed mobility:
   - Rolling side to side and supine to prone, coming to sit, and scooting in all the directions while either long or short sitting.
   - Compensatory strategies and assistive devices, such as bed loops, can be used to accommodate for upper limb dysfunction.
6. Pressure Relief in the Upright Position:
   - Appropriate time to maintain change in position is usually 60 seconds at intervals of 30 to 60 minutes.
   - With higher tetraplegia,speciality controls like pneumatic control switch, manual recliner or tilt wheelchair are present for pressure relief.
   - Mild to low tetraplegic, side or forward lean technique can be used.
   - For paraplegic, push ups is performed for pressure relief.
7. Wheelchairs Transfers:
   - Type of transfer depends upon the level of injury, assistance needed, patient preference and safety transfer.
   - Appropriate body mechanisms is needed for performing transfers.
   - Dependent transfers can done by power lift, hyradulic lift, manual pivot, transfer board or manual lift.
   - Transfers are performed on different surfaces starting with easiest transfer progressing to more difficult transfer with level surfaces to uneven surfaces.
   - Transfer training should proceed with mat -bed- toilet -bath-car- floor-other surfaces (sofa,theater seat,pool). (11)
8. Wheelchair Mobility Skills :
   - Safe and appropriate use of wheelchair before getting out of bed should be taught.
   - Training such as mobility on level surfaces in open areas, setup for transfers,mobility in tight spaces,mobility in crowded places,on andoff elevators, up/down ramps, in/out doors, wheelies, negotiation of rough terrain and up/down curbs and steps.
9. Ambulation :
   - Hope is important to maintain positive survival skills in SCI rehabilitation. Patients who are not candidates for ambulation should receive an explanation of why these goals are not feasible.
   - When ambulation is appropriate goal,treatment like therapeutic exercises,biofeedback, neuromuscular stimulation,balance training,standing, pregait and gait activites should be included.(14)
10. Sexual Issues:

- Altered sexual function result in impairment of erection,ejaculation, orgasm, male fertility and vaginal lubrication.
- Formal sexual counseling and education programs like group sessions to addresses general issues and individual sexual function evaluations should be addressed in areas of sexual dysfunction,alternative behaviours, precautions and other related areas.
- Coordinated effort between client, significant other, psychologist and urologist can help with treatment of sexual dysfunction.
- Options like surgical implantation of a penile prosthesis,vacuum erection devices, intracorporeal injection therapy and use of lubricants can be used to treat sexual dysfunction.(15)

Psychological Aspect in Spinal Cord Injury

Spinal Cord Injury (SCI) leaves a major impression on the person's body and mind. A new SCI patient usually has many queries regarding his future and at the same time has a sense that things are not going to be the same. A person who had been leading an independent satisfying life becomes immobilized, bowel and bladder incontinence, loss of sexual functioning and becomes dependent on others for every small necessity. The patient not only faces loss of body control but also experience changes in self worth, sense of independence, confidence, attractiveness, sexuality, and relationship with family and friends.

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

Psychological treatment of SCI often includes group psychotherapy, which is an excellent method to both maximize patient learning and efficiently use therapist time. Patient groups can provide emotional support, peer role models; teach new coping skills, and decrease social discomfort. Likewise, multiple-family group psychotherapy is a powerful and effective tool for facilitating family adjustment to SCI. Family members experience similar emotional responses to the patient and similarly benefit from psychological intervention. If not included in the team effort, a well-meaning family member could inadvertently sabotage the independence-oriented rehabilitation approach, or be too psychologically distressed to provide the emotional or physical care the patient needs.

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

2. Multiple Sclerosis:

Framework For Rehabilitation In MS:

According to the National MS Society's Medical Advisory Board, rehabilitation referral should be initiated whenever there is an abrupt or gradual worsening of the function or an increase in impairment that has significant impact on the individual's mobility, safety independence and /or quality of life.

A coordinated interdisciplinary team is necessary to oversee the comprehensive examination and management needed to address the patients complex and multifaceted problems. The team typically includes the physician, nurse, physical therapist, occupational therapist, speech -language pathologist, nutrionist, psychologist and social worker. The rehabilitation team considers the patients disease history, course and symptoms including impairments, Functional limitations and Disability.

Examination :

1. Detailed history Including current chief complaints and functional status, family history, medical and surgical history.
2. Motor Performance:
   a) Muscle Performance:

      Functional strength using Manual Muscle testing (MMT) and Dynamometers (Isokinetic, grasp and pinch dynamometers) should be examines. Spasticity is contraindication to MMT positions.

   b) Spasticity is examined using Modified Ashworth Scale.
   c) Gait Analysis and Posture.
   d) Cerebellar Signs Using coordination tests for Upper and Lower limbs.
   e) Range of Motion of all joints (active and passive)
3. Sensory system : Superficial and Deep Sensation.
4. Aerobic Capacity and Endurance
5. Visual Acuity: Acuity, tracking and Accomodation is examined, the presence of visual defects (blurred vision, field defects (scotoma), diplopia) is documented by ophthalmologist
6. Cranial Nerve Integrity: Motor and Cranial Nerve Function mainly presence of deficits like (optic pain (optic neuritis), occulomotor dyscontrol, dysphagia, impaired gag reflex, trigeminal neuralgia need to be documented.

Specific Measures for MS

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

Expanded Disability Status Scale (EDSS) and Functional Independence Measures(FIM).

Goals of rehabilitation in MS :

1. To Improve muscle performance in terms of strength, power and endurance:

Prescription is based on four interrelated elements (the FITT Equation)

a) Frequency of exercise: Daily exercises sessions should be scheduled, preferably in the morning, when body core temperature tends to be lowest and before fatigue sets in.

b) Intensity of exercise :Submaximal Exercise intensities (50 to 70 % of MVC-Maximal Contraction)

c) Time or Duration of exercise :

Exercising to the point of fatigue is contraindicated so frequent rest intervals are advised as time to fatigue varies greatly among individuals with MS.So respect patients desire to rest and allow him to rejuvenate himself between sessions.

d) Type of Exercise: Circuit training in which improved work capacity is developed through the use of various different stations that alternate work between upper and lower extremities, distributes the load among muscles and may prove best for reducing the likelihood of fatigue.

Symptomwise Management :

1. Spasticity :

   a) Topical cold or hydrotherapy can temporary reduce spasticity by decreasing tendon reflex excitability and clonus and by slowing conduction of impulses in nerves and muscles.

   b) Intermittent static stretching held for minimum of 30 to 60 seconds be applied ideally for 5 to 10 repetitions.

2. Coordination and Balance training : Frenkel's Exercises including upper and lower extremity coordination exercises.

3. Tightness:Flexibility exercises and ROM exercises to ensure adequate joint ROM.Mainly stretching advised for hip flexors, adductors,hamstrings and heel cords in lower limbs.

   In upper limbs pectoralis major / minor and lattismus dorsi as these are likely to develop shortness due to slumped posture.

4. Pain:Use Of TENS (Transcutaneious Electrical Stimulation), multidisciplinary stress pain clinic, stress management. (16)

   Psychological Treatment:

   Treatment:

   Treatment plan would be directed to:

   - Managing their mood better
   - Coping better
   - Improved levels of daily and cognitive activities
   - Better understanding of their difficulties
   - Improved relationships

- Less prone to feelings of suicide
- More confident about managing their future with MS.

Cognitive Rehabilitation Therapy: The purpose of the cognitive rehabilitation therapy is to help an individual acquire the highest level of cognitive functioning and functional independence possible for that individual. This is accomplished through treatment programs utilizing retraining strategies, teaching the use of compensatory skills for areas not amenable to retraining, counseling, environmental restructuring, utilizing the services of educational and vocational training facilities and following our patients as they go into their next placement, be it work, school or just better living at home. The main goal is that these changes result in significant improvement in functioning and meaningful participation in daily life events

3. Cerebral palsy :

Examination:

1) Medical history:
   a) Prenatal history:
      - Any prenatal exposure to illicit drugs, toxins, or infections/ maternal diabetes/ acute maternal illness/ trauma/ radiation exposure/ prenatal care/ and fetal movements.
      - A history of early frequent spontaneous abortions/ parental consanguinity/ a family history of neurological disease (eg, hereditary neurodegenerative disease) also is important.
   b) Perinatal history:
      - gestational age (ie, degree of prematurity),
      - presentation of the child and delivery type,
      - birth weight, Apgar score, and complications in the neonatal period (eg, intubation time, presence of intracranial hemorrhage on neonatal ultrasound, feeding difficulties, apnea, bradycardia, infection, and hyperbilirubinemia).
   c) Developmental history
      - Gross motor/ fine motor/ language/ and social milestones from birth until the time of evaluation.
      - delayed gross motor milestones /or show an early hand preference when younger than 1.5 years, suggesting relative weakness of one side (eg, reaching unilaterally).
      - Presence of a hereditary neurodegenerative disease than CP.
      - Current social skills, academic performance, and participation in an early intervention program (if <3 y) or school support (if >3 y) should be reviewed, including resource room assistance, physical, occupational, and speech and language therapy and adaptive physical education.
      - Standardized cognitive and educational testing and a current

individualized education plan can be used to determine whether speech therapy, occupational therapy, and physical therapy are in place or whether referrals for these are needed.

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

3) Specific Measures for CP

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

i) The Gross Motor Function Classification System (GMFCS):

The Gross Motor Function Classification System (GMFCS) for Cerebral Palsy is based on self-initiated movement, with emphasis on sitting, transfers, and mobility. The expanded GMFCS includes an age band for youth 12 to 18 years of age and emphasis the concepts inherent in the World health Organization's International Classification of Functioning, Disability, and Health (ICF).The focus of the GMFCS is on determining which level best represents the child's or youth's present abilities and limitations in gross motor functions. Emphasis is on usual performance in home, school, and community settings (i.e., what they do), rather than what they are known to be able to do at their best (capability).

ii) Functional Independence Measure (FIM):

Measure of BADL disability that includes 18 items scored on a seven-point scale; includes sub-scores for motor and cognitive function; performance areas include self-care, sphincter control, mobility, locomotion, cognition, and

socialization.

Aims of Rehabilitation:

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

Principles of Treatment:

1. Repetition and reinforcement are essential for learning and establishing of modified motor pattern.
2. Maximize sensory motor experiences.
3. Adequate consideration for developmental training and facilitation of purposeful activities: Therapist incorporates the principles of the neuro-developmental concept (Performance areas, gross and fine motor skills, quality of movement), conductive education, and sensory integration.

Integrated approach for CP:

1. Developing rapport with parents and patients:
2. Normalising tone of muscles:slow passive movement, sustained stretch, cryotherapy over muscle for 15 -20 minutes, stimulation of antagonist movement and vibration are used.

   In cases of hypotonicity:weight bearing, joint compression, rhythmic stabilization, vibration, cryothearpyin brisk manner and tapping can be used.
3. Stretching and Mobilty
4. Developing Postural Reaction:Postural reactions consists of righting reactions, protective extension and equilibrium reactions.These reactions are best developed by various exercises on vestibular ball and tilt board.
5. Sensory integration Therapy: This therapy helps to overcome problems experienced by many young children in absorbing and processing sensory information. Encouraging these abilities ultimately improves balance and steady movement. Therapies include stimulating touch sensations and pressures on different parts of the body. With the use of certain items with different textures, such as Styrofoam chips, water, or textured toys, this therapy can also motivate children to learn sequences of movements.

6. Oromotor control training (depends on good head control): Common oromotor problems are drooling, problems in sucking, swallowing,inadequate tongue movements and speech. Hence, therapy consists of good neck control, use of brush to decrease drooling and speech therapy.

Psychotherapy

Mental Retardation: It has been estimated that around 65 percent of the individuals living with cerebral palsy also have some form of mental retardation. About 50% are full mentally retarded i.e. an IQ below 70. Because cerebral palsy and mental retardation can exist at the same time in an individual, they can contribute to emotional stresses as well. Learning disabilities may be present, depending on the area of the brain that was damaged. About a third of individuals with cerebral palsy have mild intellectual impairments, a third have moderate-to-severe intellectual impairments, and another third have normal intellectual functioning. (17)

Behavioral Problems seen in Cerebral Palsy: Behavioral problems and cerebral palsy usually correlate, depending on the degree of mental retardation. The child may have behavioral problems or emotional issues that in turn, may affect psychological development and their ability to have social interaction.(18)

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

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

Neuro-cognitive therapy: A new approach to treating cerebral palsy from Snowdrop. It is based upon two proven principles. (1) Neural Plasticity. (2) Learning can lead to development.

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

4. Muscular Dystrophy :

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

Eg: lot of running, walking on stairs etc.

Conversely, concentric activities where muscle fibre shorten when they fire, stress on muscles is reduced significantly and are thus advised.
Eg: water exercises, where gravity is eliminated. (19)

1. Assessment tools :

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

Aims of Physical Rehabilitation

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

Aims of Functional Rehabilitation

1. Self-Care activities such as
   i) Eating
   ii) Grooming
   iii) Bathing
   iv) Dressing which are part of normal daily routine.
2. Mobility training:
   Transfers in and out of bed/ chairs/ Car transfers etc.

During therapy sessions patient is made to :

1. Perform weight bearing exercises that strengthen and tone the muscles. Stronger muscles can help to delay the impending weakness associated with muscular dystrophy.
2. Weight Bearing Activities to strengthen the trunk and in standing emphasizing on upper extremity strengthening activities.
3. Stretching Exercises to maintain flexibility, emphasing on intensity, as it has to be submaximal to avoid muscle fibre damage.
4. Engage in range of motion exercises and stretching mainly for tendo achlles, hamstrings and Iliotibila band. Flexibility can help ease the severity of joint contractures, a stiffening of the muscles around a joint.

   Splinting mainly advised during the night and is advisable for foot and knees to prevent contractures.

5. Emphasis is placed on mobility. The goal of rehabilitation team is to provide the patient with independence for as long as possible by focusing on movement. Developing large muscle groups to make the body stronger and give it more endurance (with assistance of KAFO /long leg brace).
6. Respiratory Muscle Strengthening for which following exercises are given:
   a) Spirometer exercises
   b) Blowing Whistle
   c) Blowing bubbles with straw in a bottle filled with water approximately 1-2 litres
   d) Sucking through straw etc.
7. Use of aquatic therapy is also advised as Many experts agree that water exercises and swimming help to tone and strengthen muscles and joints without putting stress on those parts of the body that are already weakened or weakening. Hot baths during hydrotherapy sessions also help to keep tendon and joints loose and flexible, thereby avoiding contractures.

5. Stroke:

Examination:

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

Specific Measures for Stroke :

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

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

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

Commonly observed deficit:

1. Loss of trunk and postural control.
2. Poor sitting balance.
3. Poor standing balance.
4. Cognitive- perceptual impairment.
5. Impaired hand functions.
6. Speech.
7. Activities of Daily Living.

1. Strategies to improve Sensory Function :

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

2. Strategies to improve Motor Function:

   i) Strategies to improve Flexibility and Joint Integrity:

      Soft tissue/joint mobilization and ROM exercises are initiated early to maintain joint integrity and mobility and prevent contractures. Effective positioning of hemiparetic extremities encourages proper joint alignment while positioning limbs out of abnormal postures.

   ii) Strategies to improve Strength :

      Specificity of training in strength should cover up the lack of significant transfer to functional tasks.

   iii) Strategies to manage Spasticity :

      Early mobilization combined with elongation of spastic muscles and sustained stretch through positioning, PNF techniques, activation of antagonist muscles using slow and controlled movements; active splinting, soothing verbal commandsand cognitive relaxation techniques provide an overall calming effect and relaxes the tone. (1)

   iv) Strategies to improve Initial Movement Control:
      Activities like Functional tasks, proprioceptive loading promote normal

postural alignment, and control and functional use of extremities thus focus on initiation of movement.

v) Strategies to improve Motor Learning :

Motor skill learning is based on brain's capacity for recovery through mechanisms of reorganization and adaption. Optimal motor learning can be promoted through attention to number of factors like Strategy development, feedback and practice explained by Carr and Shepherd.

vi) Strategies to improve Postural Control and Functional Mobility :
Initial treatment strategies should focus on trunk symmetry and use of both sides of the body with gentle movements to active movements till independent control comes. Functional training like rolling, supine to sit, sit to supine, sitting, Bridging, sit to stand and sit -down transfer, standing modified planti grade, standing, transfers can be administrated to foster postural control and functional mobility.

vii) Strategies to improve Upper Extremity Function :

Activities like UE postural support, reaching and manipulation, enhanced training activities like constrained induced movement therapy (CIMT), Bilateral arm training with rhythmic auditory cueing (BATRAC), Electromyographic feedback, Neuromuscular electrical stimulation along with behavioral training methods have demonstrated gains in recovery of function.

viii) Strategies to improve Lower Extremity Function :
LE training activities for appropriate gait requires breaking up obligatory synergy patterns.

ix) Strategies to improve Balance :

Stroke results in changes in balance with delayed, varied or absent responses with impairments in latency, amplitude and timing of muscle activity. Thus consistency, range and speed of self-initiated movements with symmetry and maximum use of more affected side has improved balance. Postural strategy development and enhanced training activities has been used to improve balance.(19)

x) Strategies to improve Locomotion :

Locomotor training using Body Weight Support from an overhead harness and motorized treadmill stimulates automatic walking using intense task-oriented training has improved locomotion.

Gait training with enhanced training activities, Orthotics, wheelchairs has improved in mechanics and quality of life.

3. Strategies to improve Aerobic Function :

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

4. Strategies to Improve Feeding and Swallowing :

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

Psychological Rehabilitation:

The psychological reaction to having a stroke can cause feelings of frustration, anxiety, apathy, anger or depression. Depression can seriously hinder an individual's willingness and ability to participate in rehabilitation. Alterations in identity and personality may also result from the interaction of fluctuating emotional, cognitive, and physical abilities as well as from changes in social context and family dynamics. Social isolation, or lack of access to social contact or resources, can be a consequence of difficulties in cognitive and emotional functions that influence interpersonal relationships, changes in social roles, communication difficulties, and challenges in transportation and employment. Social stigma and marginalization also contribute to isolation.

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

6. Motor neuron disease:

Examination :

1. Cognition: Impairments such as executive functioning, language comprehension, memory and abstract reasoning should be examined.Mini - Mental State examination can be used.
2. Pyschosocial Function: Can be assed by Beck's Depression Inventory, Hospital Anxiety and Depression Scale(HADS)
3. Pain : seen in ALS and can be assessed by Visual Analog Scale.
4. Joint Integrity, Range of Motion and Muscle length: should be examined using standard methods.
5. Muscle Peformance: can be measured by Manual Muscle Testing (MMT), isokinetic muscle strength testing or hand -held dynamometer. Muscle strength also can be assessed by Maximum Voluntary Isometric Contraction (MVIC).
6. Motor Function: Due to Spasticity, and weakness of muscles there could be many manifestations like Impairments in dexterity, incoordination of both gross and fine movements as well as loss of motor control. Therefore Functional assessment of both Upper and lower extremities should be done. Functional ability of hands should be done in detail.
7. Tone and Reflexes: Tone can be assessed by Modified Ashworth Scale and reflexes by deep tendon reflexes.

8. Cranial Nerve involvement should be assessed. Pseudo Bulbar and Progressive Bulbar varieties of MND only show involvement of cranial nerves.
9. Postural mal alignment and imbalance are seen which can be assessed by Tests like Tinetti Performance Oriented Mobility Assessment(POMA), Berg Balance Scale, Timed Up andGo Test and Functional Reach Test
10. Gait: Deviations due to muscle imbalance should be assessed, so also endurance.
11. Respiratory Function: There could be involvement of respiratory muscles resulting into breathlessness, Low vital capacity and lack of cough effectiveness. Therefore Respiratory Function evaluation should be done in detail by using a hand-held spirometer. Aerobic capacity and cardiovascular pulmonary endurance should also be tested to evaluate aerobic conditioning.
12. Because of being in bed for long time without mobility there are chances of getting trophic ulcers: periodic skin inspection should be done.
13. Functional Status: Functional Independence Measure (FIM) can be used to document functional status.
14. Environment Barriers: should be considered for easy accessibility and safety.
15. Fatigue: Fatigue Severity Scale to be used.

Specific Measures for MND :

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

The efficacy of therapeutic interventions is related to:

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

Rehabilitation intervention plan depends on the following: (20)

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

Phase I (Independent)

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

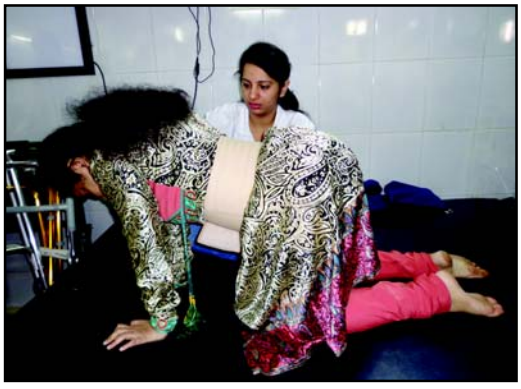
Stage 2: In case of moderate selective weakness, stretching exercises to avoid contractures.

Exercises to improve grip strength

Gait Training

Over head activity while standing with walker.

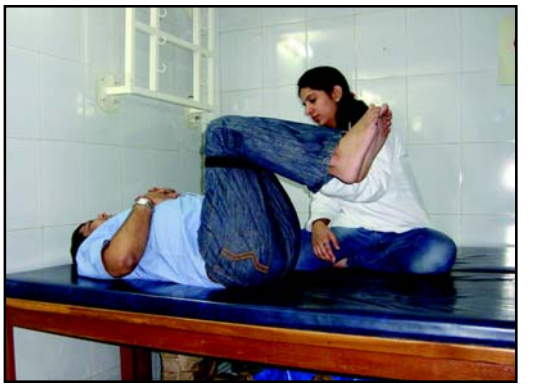
Standing on standing board with bilateral push knee splints and high boots



Quadriped for trunk balance

Strengthening of scapular muscles

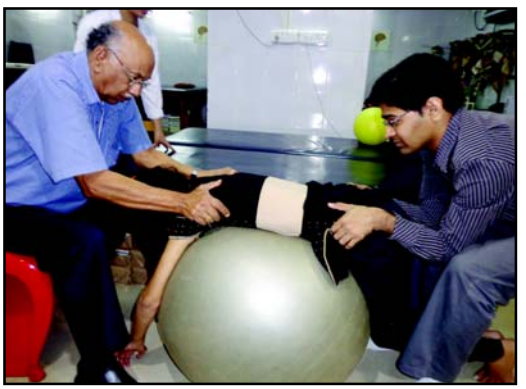
Strenthening of back extensors



Strenthening of lower abdominals

Strenthening of neck musles

Strenthening of upper abdominals



Stretching of dorso lumbar fascia

trunk strenthning act.

In case of decreased independence in ADLs like climbing, overhead activities and difficulty in buttoning etc, strengthening exercises to be prescribed avoiding fatigue.

In case of difficulty in Ambulation, Orthotic devices like AFO, hand splints to be considered.

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

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

Phase 2 - (Partially Independent)

Stage 4: In case of pain and edema in hand andfeet, consider modalities like massage, elevation and active exercises.

In case of severe weakness in extremities, caution is to be taken to support the joints while doing rotations.

In case of Fatigability in ADLS, encourage isometric upto level of tolerance and to consider slings or arm support, motorized chairs etc.,

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

In case of severe upper extremity weakness, consider modifications at home.

Phase 3 (Dependent)

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

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

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

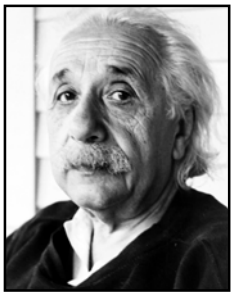
The research evidence suggests:

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

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

REFERENCES

1. The organization of neurorehabilitation services: the rehabilitation team and the economics of neurorehabilitation, Richard D. Zorowitz.
2. Neural repair and rehabilitation: an introduction, Michael E. Selzer, Stephanie Clarke, Leonardo G. Cohen, Pamela W. Duncan and Fred H. Gage.
3. Psychological Testing, An Introduction, Second Edition, - George Domino. Marla L. Domino. Pages 238 - 240.
4. Darley, F.(1982). Maximizing Input and Output. Aphasia. London: W.B Saunders Company, 186 - 231.
5. Davis, G.A (2000). Functional Therapeutics and Outcomes. Aphasiology: Disorders and Clinical Practice. Boston: Ally and Bacon, 246 - 263.
6. Application of Personality Theories and Counseling Strategies to Clients with Physical Disabilities, Hanoch Livneh and Ardis Sherwood, Journal of Counseling and Development. July/August 1991. Vol 69, Pgs 525 - 537.
7. Freed, D (2000). A Brief Historical Review of Motor Speech Disorders. Motor Speech Disorders: Diagnosis and Treatment. Canada. Singular publishing group, 1 - 12.
8. Freed, D (2000). Apraxia of Speech. Motor Speech Disorders: Diagnosis and Treatment. Canada. Singular publishing group, 277 - 306.
9. Logemann, J (1983). Evaluation and Treatment of Swallowing Disorders.
10. Occupational Therapy and Physical Dysfunction- Annie Turner, Marg Foster and Sybil E Johnson.
11. Bray, G.P (1978). Rehabilitation of Spinal Cord injured: A family approach. Journal of Applied Rehabilitation Counseling, 9, 70-78.
12. Trombly CA. Occupational Therapy for Physical Dysfunction, 4th ed. Baltimore: Williams and Wilkins, 1995
13. Darcy Umphered, Neurological Rehabiliation, 5th edition.
14. Willard and Spackman,s Occupational Therapy,10th edition.
15. Pedretti LW, ed. Occupational Therapy; Practice Skills for Physical Dysfunction, 6th ed.
16. Susan B O'Sullivan and Thomas J Schmitz. An overview of Occupational Therapy intervention for adults with Spinal Cord Injury.
17. http://www.essortment.com/articles/emotional-effects-of-cerebral-palsy_103108.html
18. http://www.treatmentofcerebralpalsy.com/01-behavioraltherapy.html
19. Understanding the concept of rehabilitation: definition, Aims and Interventions.
20. Clinical science of neurologic rehabilitation, 2nd edition Bruce H. Dobkin, M.D.



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

15

Complications of Stem Cell Therapy

Cell replacement therapy is an exciting research area and it offers potential treatment for several developmental, traumatic and degenerative neurological diseases for which there is currently no cure. The field was first brought alive by blooming of the differentiation potential of the embryonic stem cells (McDonald et al). A lot was expected from this research and very intensive work has gone behind elucidating the pathways of neuronal development and differentiation.

But, like any therapeutic modality, cellular therapy is also associated with some minor and major complications. The occurrence of these complications depends upon the type of cells used and the route of administration. Therefore, we describe the complications as cell related adverse events and procedure related adverse events.

Cell related adverse events:

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

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

Below are the major cell related adverse events reported with different cell types. It is important to note that not all the complications are associated with all cell types. There are some adverse events like teratomas which have been reported only with the use of embryonic stem cells.

(1) Tumorogenecity/ Teratomas

Embryonic Stem Cells

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

To achieve human embryonic stem (ES) cell-based transplantation therapies, allogeneic transplantation models of nonhuman primates have been useful. A model based on cynomolgus ES cells genetically marked with the green fluorescent protein has been described by researchers from Jichi Medical Centre, Japan. Primates provide a close mammalian representation to the humans. The cells were transplanted into the allogeneic fetus because the fetus is supposed to be immunologically premature and does not induce immune responses to transplanted cells. In addition, fetal tissue compartments are rapidly expanding, presumably providing space for engraftment. However, the researchers found that 3 months after transplantation, a fluorescent teratoma, which was obviously derived from transplanted ES cells, was found in the fetus. Hence, it was understood that, though the transplanted cynomolgus ES cells can engraft in allogenic fetuses, the cells may, however, form a tumor if they "leak" into an improper space, such as the thoracic cavity.(1)

Another mammalian model, a rhodopsin-knockout mice, was used to determine whether transplantation of embryonic stem (ES) cells into its subretinal space had a tumorigenic effect.

Mouse ES-cell-derived neural precursor cells carrying the sequence for the green fluorescent protein (GFP) gene were grafted subretinally into the eyes of rhodopsin-/- mice, whereas control animals underwent sham surgery. Eyes were retrieved after 2, 4, and 8 weeks after cell injection or sham surgery for histologic analysis. Gross morphologic, histologic, and immunohistochemical analysis of eyes at 2 and 4 weeks after engraftment exhibited no morphologic alterations, whereas neoplasia formation was detected in 50% of the eyes evaluated at 8 weeks after engraftment. Since, the neoplasias expressed differentiation characteristics of the different germ layers, they were considered to be teratomas. The resultant tumor formation affected almost all layers of the eye, including the retina, the vitreous and the choroid. (2)

Hence, it has been established in many mammalian models that although ES cells may provide treatment for degenerative disease in the future, their unlimited self-renewal and high differentiation potential poses the risk of tumor induction after engraftment.

Though clinical studies on use of ES cells in humans are not available, however, cell lines studied shows that human ES lines with submicroscopic genetic abnormalities can display altered growth and differentiation properties suggestive of premalignant change. In other words, a normal karyotype is not necessarily a guarantee of a normal genetic makeup within a cell line. One of the "major challenges to the field" is developing techniques that can detect rare, abnormal cells, particularly if the transformations are not due to changes in gene sequence.(3)

Thus, a lot of caution and diligent research will be required before using various human ES cell lines for cell transplantation as a therapeutic option for patients with degenerative disease.

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

(2) Seizures

Seizure is one of the possible adverse events of autologous BMMNCs intrathecal transplantation. Earlier bone marrow transplantation in children with leukemia has exhibited epilepsy as an adverse event post transplantation [1]. A case series of autologous BMMNCs transplantation in stroke also reported one patient who developed seizures post transplantation [2]. 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 [3]. 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 [4,5,6]. Also these disorders present with seizures as a co-morbidity [7,8]. 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 anti-epileptic prophylactic regimen

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

(3) Immunogenicity:

a) Autologous:

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

b) Allogenic:

These may be associated with immunological reactions.

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

Procedure related adverse events:

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

(1) Local Infection either at the bone marrow aspiration site or the CSF injection site or a more severe meningitis is always a possibility after stem cell implantation.

However, at the NeuroGen Brain and Spine Institute where over 400 stem cell implants have been done there has not been any case of local or meningeal infection. None of the other papers reviewed have reported any very serious infection leading to any morbidly or mortality.

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

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

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

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

REFERENCES:

1. Antonini G, Ceschin V, Morino S, Fiorelli M, Gragnani F, Mengarelli A, Iori AP, Arcese W. Early neurologic complications following allogeneic bone marrow transplant for leukemia: a prospective study. Neurology 1998, 50(Suppl 5):1441-1445.

2. Moniche F, Gonzalez A, Gonzalez-Marcos JR, Carmona M, Piñero P, EspigadoI,Garcia-Solis D, Cayuela A, Montaner J, Boada C, Rosell A, Jimenez MD, MayolA,Gil-Peralta A. Intra-arterial bone mzarrow mononuclear cells in ischemic stroke: a pilot clinical trial. Stroke 2012, 43(Suppl 8):2242-2244.
3. Sharma A, Gokulchandran N, Sane H, Nagrajan A, Paranjape A, Kulkarni P, Shetty A, Mishra P, Kali M, Biju H, Badhe P. Autologous bone marrow mononuclear cell therapy for autism: an open label proof of concept study. Stem Cells Int 2013, 2013:623875.
4. Simonato M, Tongiorgi E, Kokaia M. Angels and demons: neurotrophic factors and epilepsy. Trends Pharmacol Sci 2006, 27(Suppl 12):631- 8.
5. Bregola G, Frigati L, Zucchini S, Simonato M. Different patterns of induction of fibroblast growth factor-2 and brain-derived neurotrophic factor messenger RNAs during kindling epileptogenesis, and development of a herpes simplex vector for fibroblast growth factor-2 gene transfer in vivo. Epilepsia 2000, 41(Suppl 6):S122-S126.
6. Viscid EW, Triche EW, Pescosolido MF, McLean RL, Joseph RM, Spence SJ, Morrow EM. Clinical characteristics of children with autism spectrum disorder and co-occurring epilepsy. PLoS On. 2013, 8(Suppl 7):e67797.
7. Knezevi?-Pogancev M. [Cerebral palsy and epilepsy]. Med Pregl. 2010 Jul-Aug;63(7-8):527-30. Review.
8. Viscidi EW, Triche EW, Pescosolido MF, McLean RL, Joseph RM, Spence SJ, Morrow EM. Clinical characteristics of children with autism spectrum disorder and co-occurring epilepsy. PLoS One. 2013 Jul 4;8(7):e67797

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

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

16

Regulation of Stem Cell Therapy

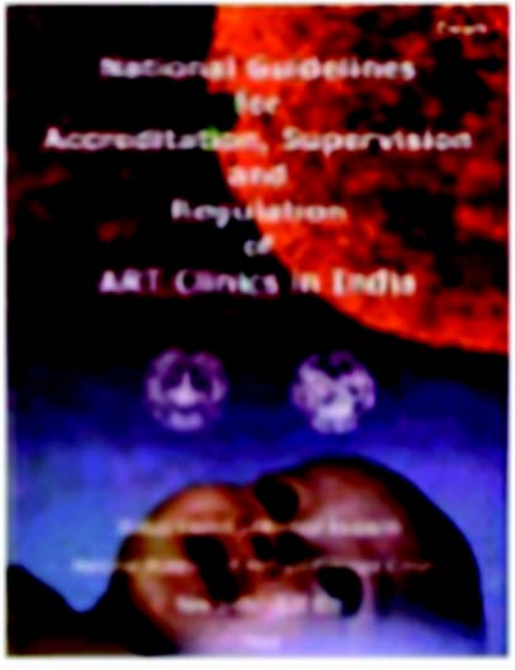
Evolution of stem cell therapy has brought forth mindboggling possibilities of finding treatment for a variety of degenerative conditions. However, it has also raised with it a host of ethical and moral implications, which has led governments to attempt regulation of both the science and funding of stem cells research. Due to a diversity of opinions and cultural viewpoints, no single policy or set of rules exist to govern stem cell research. Instead, each country has developed its own policy.

Rapid economic growth in many developing nations, especially, in Asia has also experienced a a proportionate surge in IT sectors as well as biomedical research. Moreover, with restrictions on stem cell research imposed in the US, a shift of activity in this field has been seen in, with the opportunity being explored to its earnest in countries, such as China, India, Korea, etc. However, a overview of the regulatory procedures in the global players shows that these vary from nonexistent to extremely stifling. Both ends of the spectrum are not conducive for the healthy progress of this highly promising area and we feel there needs to be a discussion so that a middle ground can be reached.

India

Government of India has drawn up a plan to effectively review and monitor the way stem cell research is being conducted in the country. Currently, there are no regulations governing stem cell research and therapy. The health ministry has approved and notified a committee to look at therapies related to stem cells and genes. The 11-member committee will be headed by V.M. Katoch, secretary, department of health research, and director-general of the Indian Council of Medical Research (ICMR). The drug controller general of India is also one of the members.

An effective surveillance on the highly complex stem cell research is yet to be in practice in India even though the country has already worked out the fundamental


National Guidelines
for
Accreditation, Supervision
and
Regulation
of
ART Clinics in India


National Guidelines
For
Stem Cell Research And
Therapy
(ICMR – DBT)
2006


ETHICAL GUIDELINES
for
BIOMEDICAL RESEARCH
on
HUMAN SUBJECTS

guidelines for stem cell research more than two years ago.

Indian Council for Medical Research (ICMR) - the apex body regulating medical research in India and the Department of Biotechnology (DBT) under the ministry of science and technology, government of India announced the guidelines for stem cell research and therapy way back in 2007.National guidelines for conducting stem cell research in India have been formulated by the Indian Council of Medical Research. These guidelines provide a mechanism to ensure that stem cell research is conducted in a responsible and ethically controlled environment. A copy of the guidelines is available on the website (http://icmr.nic.in).

Some of the salient features of the guidelines include the identification of the three sources of stem cells and categorization of stem cell studies into three groups: Permissive, Restrictive and Prohibitive research.(1)

"The prohibitive research" includes any research related to germ line genetic engineering or reproductive cloning of any in vitro culture of the intact human embryo, regardless of the method of its derivation, beyond fourteen (14) days or the formation of the primitive streak, whichever is earlier; transfer of human blastocysts generated by SCNT; or the breeding of parthenogenetic animals,in which human stem cells have been introduced at any stage of development.(1)

Human embryonic stem cell derivation and differentiation falls in "restrictive" category, whereby, these cells can only be used for research purposes.

Adult and umbilical cord blood cells are clubbed under the "permissive" group and both research and therapy using these is allowed.

As per National guidelines, every organization (academic or otherwise) interested in working on stem cells, must formulate an Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). Members of the Committee must include people with appropriate expertise (representatives of the public and persons with expertise in clinical medicine, developmental biology, stem cell research, molecular biology, assisted reproduction technology, and ethical and legal issues in stem cell research) and this Committee must function at the institutional level. Projects will be approved on the basis of scientific evaluation and ethical conduct. The IC-SCRT must also be registered with an NAC-SCRT. The NAC-SCRT is constituted by the Government of India. NAC would be comprised of experts from various fields, who would be responsible for examining the scientific, technical, ethical, legal and social issues in the area of stem cell based research and therapy. It will have around 10 members. A chairman, a deputy chairman, member secretary and nominees from DBT, DST, CSIR, ICMR, DCGI, DAE, and biomedical experts from pharmacology, immunology, cell biology, hematology, genetics, developmental biology, clinical medicine and nursing. Legal expert, social scientist, and a women's representative will also be part of NAC.NAC could also consult outside experts on a case to case basis.(1,2)

Institutions involved in stem cell research and therapy will have to be registered with the NAC through Institutional Committee for Stem Cell Research and Therapy (IC-SCRT).

NAC will set standards for procedures for collection, processing, differentiation,

preservation and storage of human tissues to their assure quality and sterility.

The function of the NAC-SCRT would be to approve, monitor and oversee research falling under the restricted category. Hence, all institutions, hospitals and private companies involved in stem cell research and therapy must be registered.

This guideline also includes specific reference for the establishment of cord blood banks and the clinical use of umbilical cord blood stem cells. There is also a specific reference to cord blood banking and clinical use of cord blood in the Ethical Guidelines for Biomedical Research in Human Participants, released by the ICMR in 2000 and updated in 2006. Establishment of an umbilical cord stem cell bank with prior approval of the IC-SCRT and Institutional Ethical Committee falls under permissible research and therapy.(1,2)

Detailed guidelines are given in this document for the collection, processing, and storage, etc., of umbilical cord blood. Appropriate standard operating procedures (SOPs) need to be prepared for the cord blood banks, which need to be registered with the Drug Controller General of India (DCGI) as per the guidelines for blood banks.(1,2)

Although the regulatory guidelines are in place, a vacuum still persists. One of the major lacunae is that there should be more clarity on how clinical research and product development should be carried out., since, though guidelines have been formulated, these are still not practically implementable. Also,the guidelines laid down in 2007 need to be revised as well as updated to suit current needs, since a lot has happened in the field of stem cells and regenerative medicine since then.The NAC has not yet been physically formed and only exists on paper.Hence, registration of the IC-SCRT under it is still not possible. Also, unless the guidelines are legalized, regulation of research will not be feasible.(1,2)

Some of the requests to conduct clinical trials using stem cells are being sent to The Drug Controller of India (DCGI) for approval. There is currently no clear-cut process flow in place and hence, systematic reviewing & monitoring procedures for clinical research are in a nascent stage or non existent.

So in practical terms what do the Indian regulations mean to someone who intends to work with stem cells.

(1) As on December 2010, there are no legal restrictions that stops any physician to either do clinical trials or to offer stem cell therapy as a treatment either all by itself or combined with other treatment modalities.

(2) The ICMR has formulated certain draft guidelines. Following these guidelines would be desirable presently for any physician starting stem cell work. These guidelines are presently not legally binding. However, they have been submitted to Parliament. Once Parliament approves it, these guidelines will be legally binding and following them will then become mandatory.

(3) As per the guidelines, if one is working adult stem cells (which includes autologous bone marrow derived stem cells) then the only permission that is required is from the local Institutional Committee for Stem Cell Research and therapy (ICSCRT). This ICSCRT has to be formed according to the guidelines and has to be registered with the National Apex Committee (NAC) of the ICMR. Unfortunately, as of

December 2010, this committee had still not started functioning. What this means is that even if one wants to follow the guidelines, at present it is not possible to do so, since registration with the NAC is not possible.

(4) If one is working with umbilical cord derived stem cells, then also approval of the ICSCRT is required. However, in addition DCGI's (Drug Controller General of India) approval is needed ,if one is dealing with a marketable product.

(5) According to the guidelines, working with embryonic stem cells is in the restrictive category and this cannot be done without the ICMR's prior approval. Cloning is prohibited altogether.

China

China has one of the most unrestrictive stem cell policies. The Chinese government allows research on human embryos and cloning to continue for therapeutic purposes. However as per the "Ethical Guidelines for Research on Human Embryonic Stem Cells" which were laid down by the Ministry of Science and Technology and the Ministry of Health of China, any research aiming at human reproductive cloning and hybridizing human germ cells with germ cells of any other species is prohibited.

Also, embryos used for stem cell research should be left over from in vitro fertilization (IVF); fetal cells from abortions; blastocytes from Somatic Cell Nuclear Transfer (SCNT); or germ line cells voluntarily donated. Interestingly, according to Chinese cultural attitudes, a person's life begins with birth. (3)

Korea

However, the Korean setup is much more permissive for stem cell research. The government allows and funds work on human embryonic stem cells. The Bioethics and Safety act lays down the legal boundaries for permissible area for stem cell research. The early guidelines made by the Ethics Committee of the Stem Cell Research Center in 2003 permitted the use of only spare embryos for hES cell line derivation. They prohibited cloning, inter-species transplantation of reproductive cells that might lead to chimeras, production of embryos for research purposes, and somatic cell nuclear transfer to prevent attempts to engage in reproductive cloning.

A further advanced version of the Bioethics and Safety Act enacted in January 2004,and enforced since 2005 as a penal law identifies criminal offenses pertaining to stem cell research. It prohibits human reproductive cloning. the transfer of embryos between two different species, embryo production other than for the purpose of pregnancy and also disallows research on spare embryos that have the embryological primitive streaks appearing in their developmental process.It only allows research on spare embryos for research aimed at curing rare or incurable diseases.

The though on surface it appears prohibitive, but in practicality provides a legal platform to allow legitimate researchers to conduct research on human embryonic stem cells, including somatic cell nuclear transfer for the purpose of conducting research aimed at curing currently incurable diseases., if they adhere to the procedures laid down by the act.

In 2006, Dr. Hwang Woo-suk scandal, raised not only ethical issues regarding procurement of the eggs, but also questions regarding scientific ethics & falsifying results brought disrepute to the stem cell " hub" which was to be lead by him..This, also, lead to enactments of stricter rules regarding embryo donor for research,which came in the form of Bioethics and safety act 2008. Nevertheless, South Korea continues to pursue research for the purposes of therapeutic cloning, with complete financial and legal backing from the government.(4)

Japan

The publication of the human iPS cell paper by Japanese researchers has renewed the vigour with regards to stem cell research in Japan.The governmental committee revised the guideline for human ES cell research in August 2009. The original guideline was split into two separate ones: one about derivation of human ES cells and the other about use of human ES cells.The renewed two-level review was abolished and now a protocol only needs an approval of the institutional ethics review committee.

The another change in policies in Japan, recently, is pertaining to research that aims to produce germ lineage, which was prohibited till this year.

In May 2010, a new guideline came into effect for germ cell research using human iPS cells and the two existing guidelines for human ES cell research were revised to allow germ cell research using human ES cells.

Further guidelines for use of induced pluripotent stem cells and human embryonic stem cells have been drafted by the Ministry of Education, Culture, Sports, Science and Technology (MEXT),allows researchers to use human iPS cells and ES cells under the strict review system included in the original guideline,although the use of human ES cells is not possible until the derivation guideline (which is under control of the MEXT) is amended to enable researchers to establish clinical grade human ES cells.(5)

Singapore

Singapore is widely considered "Asia's stem cell center". It has more than 40 stem cell research groups in the country and authorizes the use, for therapeutic purposes, of embryos that are no more than two weeks old.

Singapore does not have too many political or legislative restrictions on hESC research and has not enacted any specific legislation on the generation and use of hESCs. Instead, researchers in Singapore adhere strictly to guidelines drafted in 2002 by the Bioethics Advisory Committee (BAC:http://www.bioethics-singapore.org/) and subsequently endorsed by the Government, which were modeled on existing UK legislation.(6)

Thailand

In Thailand, similar to other countries, stem cells appear to be a hot topic for patients, scientists, researchers, physicians. Work on stem cells in general started in 2001 with the import of human ES cells, which gave way to the use of adult progenitor cells to treat ischemic heart diseases(as a pilot study), on popular public request. This therapy was then opened up and offered as a treatment for patients who had exhausted

all conventional treatment options. The treatment required the cells to be sent to an Israeli company for manipulation, where they were cultured and sent back to Thailand for later infusion into patients. There were many concerns about the ethical issues of providing stem cell therapy to the patients in private hospitals in Bangkok. Hence,in 2007, a round table discussion was convened by the Forum of Ethical Review Committee in Thailand (FERCIT), which emphasized on an urgent need to have appropriate guidelines and regulations for stem cell therapy. The Thai Medical Council took the onus of establishing a committee to draft regulation on ethical conduct of stem cell therapy, which was announced in2009. This regulation is based on the fact that hematopoietic stem cell transplantation is, at present, the only standard treatment. Stem cell therapy other than hematopoietic stem cell transplantation is currently still considered to be experimental. (7)

USA

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

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). Individual states offer private funding but have the challenge of setting up guidelines to govern stem cell research. The guidelines developed by the US National Academy of Sciences (NAS) provide a good framework but individual states have their own approaches to specific legal and ethical concerns. This has resulted in a wide variation of laws: some states have no specific regulations while others have varying degrees of restriction. For example, South Dakota has a ban on all hESC research,whereas California is working towards providing long-term state funding for such research. With each state having differing viewpoints, and therefore differing laws, a coalition of states has been established - the Interstate Alliance for Stem Cell Research. And in fact this alliance isn't restricted to the US. To promote crossborder knowledge-sharing, four meetings a year are held between state representatives and representatives from the UK and Canada. These meetings provide an open forum where common issues can be discussed (eg, tracking systems for egg donation) and where collaboration is actively encouraged.(8)

Under the auspices of the Obama administration, the National Institutes of Health plans to expand federal funding for stem cell lines that meet certain ethical requirements: the embryo was discarded after IVF; informed consent was obtained from the donors; the couple does not receive compensation (neither financial nor medical benefits) or are 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. (9)

Canada

In Canada, the Tri Council Policy Statement 18 (TCPS) is the main national reference for all publicly funded bodies undertaking research involving humans. The guidelines set ethical norms to delimit the duties and rights of all those implicated in research involving humans. Under the TCPS guidelines, the only embryos that may be used for stem cell research are those w been created as part of medically assisted reproduction, but are no longer needed for that purpose. Another government agency with competence to regulate and license stem cell research is the Canadian Institutes for Health Research (CIHR). Its Guidelines for Human Pluripotent Stem Cell Research were first drafted in 2002 and last updated in June 2010. This set of Guidelines was put into place to further interpret and make explicit the ethical standards and principles found in the TCPS guidelines. The CIHR Guidelines has created a special ethics review board - the Stem Cell Oversight Committee (SCOC) to monitor and approve all research proposals dealing with human pluripotent stem cell research. However, in some cases, in addition to SCOC, review and approval must be obtained by the local ethics review board (REB) and Animal Care Committee (ACC).(10)

European Union

The European union provides a general framework for the member states for carrying out human stem cell research.Howeever, it is not a legislation and hence, within the European countries there is discordancy in implementing those guidelines.(11)

Currently, twenty-five (25) of the European Union countries have adopted legislation that explicitly prohibits human reproductive cloning (excluding Poland, Lithuania and Ireland, as well as Croatia and Luxembourg).

However, hESC research and the derivation of new hESC lines from supernumerary IVF embryos by law is allowed in seven (7) countries (Belgium, Sweden, UK, Spain, Finland, the Czech Republic and Portugal). The same countries allow SCNT by law, except Finland and the Czech Republic, who neither prohibit nor allow it.

Another three (3) countries have adopted legislation to allow the creation of embryos for research purposes under strict conditions(Belgium, Sweden, UK). Currently, seventeen (17) countries allow the procurement of SCs from supernumerary embryos, and six (6) countries have not adopted legislation regarding hESC research(Bulgaria, Croatia, Cyprus, Luxembourg, Romania and Turkey).

Stem cell-based therapies are principally permissible all over the European Union. Like every other therapy, stem cell therapies have to be safe and reliable and must have a positive risk-benefit-balance. The legal and scientific requirements for stem cell based therapies are laid down in several European provisions, e. g. in the so-called Tissue Directive 11 or in the Regulation on Advanced Therapy Medicinal Products (ATMP).The ATMP Regulation states that principally all stem cell based therapies do

need market approval by the European Medicine Agency (EMA).There are a few exceptions for certain autologous therapies if they were performed in a hospital on a non-routine basis in accordance with specific quality standards in order to comply with an individual medical prescription for a custom-made product for an individual patient. The Tissue Directive sets the standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. However, the provisions of the Tissue Directive, as well as of the ATMP-Regulation do not apply to the (basic) research situation. These provisions have only to be considered for therapeutic usage.

Currently, the EMA is also reviewing already existing guidelines and setting up new guidelines with further requirements for the mandatory market approval of (stem) cell based therapies.(13)

United Kingdom

The UK has a strict, but permissive, regulatory framework in place, covering all forms of stem cell research and its translation into marketable therapeutic products. There are five key regulatory bodies in the UK responsible for ethical and regulatory oversight in the stem cell field, and these are outlined briefly below. In 2009, the UK Government's Department of Health and Medical Research Council published an on-line UK Stem Cells Toolkit designed to guide human stem cell researchers and translators through the UK's regulatory framework and enable them to develop their own regulatory roadmap specific to their research and/or product needs.

These regulatory authorities, some independent while some associated with the department of health are:

1. Human Fertilisation and Embryology Authority (HFEA is responsible for overseeing the use of gametes and embryos in fertility treatment and research.
2. Human Tissue Authority (HTA) is the regulatory authority responsible for licensing organisations that store and use human tissue for purposes such as research, patient treatment, post-mortem examination, teaching, and public exhibitions; it also gives approval for organ and bone marrow donations from living people.
3. Medicines and Healthcare Products Regulatory Agency (MHRA) is an executive agency of the Department of Health that is responsible for the regulation of medicines and medical devices, and equipment used in healthcare and the investigation of harmful incidents. It also looks after blood and blood products, working with UK blood services,healthcare providers, and other relevant organisations to improve blood quality and safety.
4. Gene Therapy Advisory Committee (GTAC) is a committee of the Department of Health that has UK-wide responsibility for the ethical oversight of proposals to conduct clinical trials involving gene or stem cell therapies. The Committee also advises Ministers on the development and use of gene and stem cell therapies and works with the other regulatory agencies/authorities listed above.
5. UK Stem Cell Bank Steering Committee is a high-level committee of the Medical

Research Council which oversees the operation of the UK Stem Cell Bank and reviews all applications to deposit or access the Bank's stem cell lines.

The regulatory framework in the UK has not yet been fully tested from research to the market approval of a product, though its earlier stages have recently been pioneered by the company ReNeuron Group plc, which was the first to receive regulatory approval from GTAC and the MHRA to proceed with a Phase I clinical trial of its foetal (adult) stem cell therapy for stroke damage.(12)

Germany

Research with human embryonic stem cells is permissible and possible in Germany, but only with stem cells imported from abroad to Germany and procured as the requirements laid down in the Stem Cell Act are met.

The basic requirements of the Stem Cell Act are the requirement for administrative permission(to be granted by the Robert-Koch-Institute in Berlin) for the import of and work with the imported cells and the fact that only certain stem cells can be imported. Only those ESCs which have been derived abroad under the specific conditions stated in the Act may be imported : First, those cells must have been derived before May 1, 2007,secondly those cells must be derived in accordance with the provisions in the country in which they were derived. Additionally, the stem cells must have been derived only from embryos and /or oocytes that were given to research facilities free of charge from surplus embryos created for infertility treatment and having tested negative for PGD.

For research with adult stem cells, including stem cells from the umbilical cord and fetal stem cells from medically or spontaneously aborted foetuses, there are no restrictions. All these stem cells can be used for any kind of research in Germany. The only point to consider is the requirement of the informed consent of the donor.

Research with the recently developed induced pluripotent stem cells (iPSC) is legally treated like research with adult stem cells in Germany. Therefore, there are no restrictions on iPSC research in Germany.

Other Countries

Australia bans all human cloning for reproduction or research. (14)

It does allow for the use of embryos remaining after assisted reproduction from before April 5, 2002.Initially, South Africa enacted legislation that banned reproductive cloning but authorized therapeutic cloning. In 2004, this country became the first African nation to create a stem cell bank(9)

The Swiss parliament is considering allowing research on stem cells derived from stored embryos remaining at the end of assisted reproduction for therapeutic purposes only. In 2004, a national referendum was put forth in which two-thirds of voters agreed to allow embryonic stem cell research. (9)

The Brazilian government passed legislation in March 2005 that allows the use of excess IVF embryos that have been frozen for more than three years. The Brazilian Catholic Church challenged the law, arguing that embryonic stem cell research violates

the right to life, but Brazil's Supreme Court rejected the petition, thus permitting embryonic stem cell research. (15). While Mexico has a flourishing stem cell industry, it does not have formal regulations (9)

Considering the varied guidelines and regulations in different countries and also the variation within a country, there is no harmony in the consensus regarding the direction that stem cell research and therapy should take. The uncertain progress in this field, hence, can be attributed partly to the moral and ethical prejudices of various groups and partly to the strict regulations or maybe lack of them.

REFERENCES

1. Guidelines for stem cell research and therapy,ICMR-DBT,2007.
2. Satish Totey and Aparna Khanna. Stem Cell Research In India -An Update, World Stem Cell Report, 2010
3. Lianming Liao, Lingsong Li and Robert Chunhua Zhao. Stem cell research in China. Phil. Trans. R. Soc. B (2007) 362, 1107-1112
4. Ock-Joo Kim Stem Cell Research in Korea: Ethical and Legal Perspectives, World Stem Cell Report,2010
5. Kazuto Kato and Masahiro Kawakami. Stem Cell Research in Japan: Policy Changes in "The Era of iPS Cells",, World Stem Cell Report,2010
6. Alan Colman. Dispatches from Singapore: Stem Cells, Regenerative Medicine and Wealth and Health Creation, World Stem Cell Report,2010
7. Surapol Issaragrisil, Pakpoom Kheolamai. The Road to Regulation of Stem Cell Therapy: Thailand as a Model, Stem Cell World Summit,2010
8. 'Bernard Siegel - The US stem cell dilemma', Pharmaceutical Technology Europe, 27 March 2009
9. Deepali Dhar* and John Hsi-en Ho. Stem Cell Research Policies around the World, Yale J Biol Med. 2009 September; 82(3): 113-115
10. http://www.stemcellnetwork.ca/index.php?page=canada-s-regulatory-oversight-of-stem-cell-research
11. EMEA/14327/2009
12. J Ben Sykes. Stem Cell Research, Funding and Regulation in the United Kingdom-A Brief History,, World Stem Cell Report,2010]
13. Timo Faltus. The German Stem Cell Law - A Balancing Act Between the Protection of Human Embryos and the Freedom of Research, Dipl.-Biol., Dipl.-Jur, World Stem Cell Report,2010
14. Rebecca Skinner and Megan Munsie. Small Continent, Big Ideas: Stem Cell Science in Australia,, World Stem Cell Summit,2010
15. Mari Cleide Sogayar. and Antonio Carlos Campos. Stem Cell Research in Brazil: Incentives, Barriers and Perspectives, World Stem Cell Summit, 2010

Difficulty of Being Good:

"In order to preserve dharma in this imperfect world of Kali Yuga, he had to commit 'smaller wrongs' for the sake of a 'bigger right'."

From the book "The Difficulty of being Good. On the subtle art of Dharma" in the chapter "Krishna's Guile" by Gurcharan Das (Penguin Allen Lane)

17

Ethics

Consensus on the potential of stem cell therapy to address various incurable, debilitating disorders is unanimous. Stem Cell research and therapy is the frontline of the biomedical field. However, no other area of biomedical research has faced the quantum of ethical, moral and political controversies that surrounds stem cell research.

Adult stem cells as an alternative source, other than embryos, have been spared of controversies and have been generally welcomed and encouraged for research and therapy.

Embryonic stem cell, on the other hand, has been hounded by objections and restrictions due to the source of its procurement, by various religious bodies of the world.

Ethical Issues Associated With Embryonic Stem Cell Research

Religion and embryonic stem cell:

The major dictum common to all religions is : 1) Human life is sacred and has to be guarded 2) Alleviation of human suffering should be strived for.

Though, there is consensus among all religions regarding the potential of stem cell research being a means towards addressing the second dictum, the opinion on what construes a human life differs vastly.

Should the 5-7 day embryo be given the status of a person and hence have the right to life or is this stage too early to confer this right?

For some religions, ensoulment of the embryo would make it a person. But, then when does ensoulment take place?

These are just a few issues surrounding the embryonic stem cell field. Contrasting opinions among various religions and even within the religion exist.

The following is a sample of this diversity among different faiths.

Greek Orthodox and Roman Catholic Churches

The official position of these churches is that a human person begins at conception and the human embryo has the same moral status as human persons. Consequently, research on human embryos, including hES derivation and subsequent use is unethical, and if it involves the willful destruction of embryos, it is homicide.

The argument that the cell lines are derived from excess embryos, after the fertility needs are dealt with, is of no consequence, since the production of excess embryos itself is unacceptable to the church. The fact that these embryos and their products would be used for the alleviation of human suffering, does not justify the destruction of the embryos.

Since the underlying belief is in the embryo's right to life, any use of the embryo that is not for its own good is immoral and therefore, impermissible. There is no consequentialist or utilitarian approach that would make this act acceptable.

The belief in the personhood of human embryos also means that it is not possible to use hES lines previously derived from human embryos or to use therapies derived from hES research. The idea is that these cell lines and therapies are tainted by the immoral act of killing the embryo. To use them would be to become complicit in the immoral act.

However, this rigid stance, especially of the Roman Church, is somewhat diluted by certain other catholic groups, who do not believe that the embryo is a human person, but believe that its ensoulment is the morally relevant time with regard to personhood.

Protestant Churches

Most protestant churches do not believe that embryos have personhood and are open to embryo research but consider that the goals of the research are of paramount importance. In addition, considerable emphasis is placed on the need for both public discussion and for oversight of the research rather than leaving it as an unregulated private enterprise. They believe that the benefits from this and other medical research be distributed evenly and justly to all those in need, regardless of resources or geography.

Official positions vary from country to country on the moral status of the embryo and therefore, on the morality of embryo research in general. These divisions show just how personal an issue stem cell research can be. For these churches like for the lay public, weighing the moral status of the embryo and the need to help ailing and suffering people is not a simple arithmetic. (1)

Judaism

Orthodox Jews believe that embryos do not have the same moral status as human persons. In fact, gametes and embryos outside a human body do not have any legal status under Jewish law. The result therefore, is that embryos created by IVF have no special moral or legal status. Under Jewish law (Halcha) the fetus does not become a person (nefesh) until the head emerges from the womb.

They believe that when the embryo is implanted it is "as water" up to the fortieth day. After that time and before the fetus emerges from the woman's body it is a potential

life and has great value. Ensoulment is generally thought to occur sometime after the fortieth day. It gains full human status, however, only once it emerges from the woman's body. Since embryos used in hES research are outside the body, according to the Jewish faith it is possible to use excess IVF embryos in research.

In addition to the Jewish views on the moral status of the human embryo, this religion places emphasis on preventing and alleviating suffering. This leads to a deep belief in the morality of and value in pursing medical research. The commitment to preserving one's body and health is joined by a commitment to helping others and alleviating suffering. So there is a moral imperative to help those who are suffering from diseases and to explore the potential of all types of stem cell research. This belief leads Jews to have a generally favorable view of stem cell research including hES research. (2)

Islam

In Iran, Turkey, Singapore (with a majority of Muslims) and other Islamic countries, embryo research policies are influenced by the religious belief that full human life with its attendant rights begins only after the ensoulment of the fetus. This is generally believed by Muslim scholars to take place at 120 days after conception (although a minority belief indicates ensoulment takes place 40 days after conception). This fact, in conjunction with the importance articulated in the Qur'an of preventing human suffering and illness, means that the use of surplus IVF embryos for stem cell research is relatively uncontroversial. What remains controversial in the Muslim world is creating embryos for the purpose of research.

As with other religions, Islam and its followers have differing point of views on these issues. For example, in Egypt, a conservative religious country, the Muslim head of the Egyptian Medical Syndicate stated that embryos are early human life and should never be used in research.

Hinduism and Buddhism

In traditional Hindu belief, conception is the beginning of a soul's rebirth from a previous life. Some Hindu traditions place the beginning of personhood between three and five months of gestation, while few believe that the soul's rebirth can occur as late as the seventh month.

Most Buddhists have adopted the classical Hindu teaching that personhood begins at conception. Though Buddhist teachings do not directly address the issue, like Hinduism there are two main tenets - the prohibition against harming or destroying others (ahimsa), and the pursuit of knowledge (prajña) and compassion (karua) - that divide Buddhists. Some Buddhists argue that embryonic stem cell research is in accordance with the Buddhist tenet of seeking knowledge and ending human suffering, while others argue that it is a violation of the notion of not harming others.

A central belief of Hinduism and Buddhism is that an individual's soul or self is eternal. In Hinduism the soul is believed to be passed from one living being to another in a process called reincarnation. In Buddhism reincarnation is described differently as the rebirth of the self. These beliefs, that the soul or the self are reborn lead to a greater

acceptance of cloning technology. Although the use of embryos in stem cell research remains a divisive issue in these religions, the use of cloning technology in stem cell research is less controversial.(3-5)

Medical And Other Ethical Issues And ES Cell Research:

Proponents of embryonic stem cell research advocate that obtaining human ES-cells from the embryos left over after successful pregnancy in the course of IVF treatment for the goal of treating diseases and saving lives justifies the symbolic loss that arises from destroying embryos in the process. They emphasize on the significance of saving life of many patients who need cell replacement therapy, as an essential reason for permission of research on embryos and obtaining ES-cells from them.

A different set of ethical issues arises once researchers have learnt safe and effective ways to direct human ES-cell to differentiate into specified cell or tissue types, and to transplant them for therapeutic effects in patients.

An important clinical issue at this point will be whether ES-cell not derived from the patient, will be rejected by the patient's immune system. The strategy for dealing with this problem, would then be to use a patient's nuclear DNA to create an embryo from which ES-cells compatible with that patient could then be derived. This process, known as somatic cell nuclear transfer could prove to be a safe and effective use of ES-cell derived replacement therapies.

However, this would raise more ethical issues beyond the destruction of left-over embryos to obtain human ES-cells. One issue would be ethical concerns about creating human embryos for the sole purpose of destroying them to obtain replacement cells for the patient who provided the nuclear DNA. Ethical debates about creating human embryos solely for research have existed since the inception of debates over embryo research. One can question; however, whether those concerns are even relevant to generating human ES-cells by somatic cell nuclear transfer, for the haplogenomes of gametes are not combined through sexual fertilization to form the blastocyst that provides the ES-cells. In addition, there is no intention of culturing the embryo beyond the blastocysts stage, nor of implanting that blastocyst in a uterus for reproduction. Given the asexual means of creating the embryo and the lack of intent of implanting it in the uterus, the embryonic entity produced in these circumstances lacks the reproductive significance that some have argued is the moral basis for valuing early embryos.

The other issue is of egg donation for therapeutic cloning and effective cell-replacement therapy. The ability to meet the therapeutic demand for oocytes would present an important problem. The ability of live, unrelated donors to meet such a demand is highly unlikely for several reasons: the hormone treatments that stimulate the production of many oocytes impose a considerable burden on women; surgery is required to retrieve the oocytes; and ethical problems now surround such donations.

Fetal Stem Cells And Ethics:

Pluripotent stem cells can be derived from fetal tissue after abortion. However, use of fetal tissue is ethically controversial because it is associated with abortion, which

many people object to. Under American federal regulations, research with fetal tissue is permitted provided that the donation of tissue for research is considered only after the decision to terminate pregnancy has been made. This requirement minimizes the possibility that a woman's decision to terminate pregnancy might be influenced by the prospect of contributing tissue to research. Currently there is a phase 1 clinical trial in Batten's disease, a lethal degenerative disease affecting children, using neural stem cells derived from fetal tissue . (6,7)

Induced Pluripotent Stem Cells (iPS Cells) - a safe and ethical alternative?

Somatic cells can be reprogrammed to form pluripotent stem cells, called induced pluripotential stem cells (iPS cells).These would match the donor cells. This was initially tried using viral vectors, followed by plasmids. Currently, the aim is to be able to induce pluripotency without genetic manipulation. Because of unresolved problems with iPS cells, which currently preclude their use for cell-based therapies, most scientists urge continued research with hESC.(8)

iPS cells avoid the heated debates over the ethics of embryonic stem cell research because embryos or oocytes are not used. Furthermore, because a skin biopsy to obtain somatic cells is relatively noninvasive, there are fewer concerns about risks to donors compared with oocyte donation. The President's Council (USA) on Bioethics called iPS cells "ethically unproblematic and acceptable for use in humans" Neither the donation of materials to derive iPS cells nor their derivation raises special ethical issues.

Evolution Of Policies On The hES Cell Research In The US:

The most keenly followed and studied policy change regarding the human ES cell research has been that of the United States. This has been mainly attributed to be influenced by the ethical, moral & religious stand of the catholic church.

In 1973 a moratorium was placed on government funding for human embryo research. In 1988 a NIH panel voted 19 to 2 in favor of government funding. In 1990, Congress voted to override the moratorium on government funding of embryonic stem cell research, which was vetoed by President George Bush. President Clinton lifted the ban, but changed his mind the following year after public outcry. Congress banned federal funding in 1995. In 1998 DHHS Secretary Sullivan extended the moratorium. In 2000, President Bill Clinton allowed funding of research on cells derived from aborted human fetuses, but not from embryonic cells. On August 9, 2001, President George W. Bush announced his decision to allow Federal funding of research only on existing human embryonic stem cell lines created prior to his announcement. His concern was to not foster the continued destruction of living human embryos. In 2004, both houses of Congress asked President George W. Bush to review his policy on embryonic stem cell research. President George W. Bush released a statement reiterating his moral qualms about creating human embryos to destroy them, and refused to reverse the federal policy banning government funding of ESC research (other than for ESC lines established before the funding ban).

In the November 2004 election, California had a Stem Cell Research Funding

authorization initiative on the ballot that won by a 60% to 40% margin. It established the "California Institute for Regenerative Medicine" to regulate stem cell research and research facilities. It authorizes issuance of general obligation bonds to finance institute activities up to $3 billion dollars subject to an annual limit of $350 million.

Under President Obama, it is expected that federal funding will be made available to carry out research with hESC lines not on the NIH list and to derive new hESC lines from frozen embryos donated for research after a woman or couple using in vitro fertilization (IVF) has determined they are no longer needed for reproductive purposes. However, federal funding may not be permitted for creation of embryos expressly for research or for derivation of stem cell lines using somatic cell nuclear transfer (SCNT)

The Korean Stem Cell Controversy

The meteoric rise and equally sudden fall of Korean scientist Woo-Suk Hwang depicts all that can possibly go awry, ethically and scientifically, in the world of stem cell research.

What would have been regarded as a seminal paper in SCNT technology and human ES therapeutics turned out to complete fraud and hogwash. Not only were the results fabricated, but also, unethical practices were employed to procure oocytes for the research.

At the end of 2005, the scientific community was shocked by one of the greatest cases of misconduct in the history of science. Two breakthrough articles about stem cell technology from a Korean laboratory headed by Woo-Suk Hwang, published in Science, appeared to be almost completely fabricated and were therefore retracted. The two fraudulent papers concentrated on the concept of therapeutic cloning in humans. In this somatic cell nuclear transfer (SCNT) technology, a nucleus from a patient's somatic cell is transplanted into an enucleated donor oocyte. The resulting blastocyst embryo is used for the isolation of embryonic stem cell (ESC) lines that possess virtually all the patient's characteristics and thus will minimize immune rejection upon transplantation. Until the publication of the fraudulent papers, therapeutic cloning was a cumbersome and inefficient technique and successful therapeutic cloning in humans had not been reported before. In their 2004 paper, Hwang and his associates claimed to have isolated the first human ESC line derived from SCNT and in their second paper they reported to have improved the efficiency to such an extent that clinical application became within reach. Two months following the first paper, criticism arose on the ethics of obtaining the human oocytes used in the study. After initial denial it became clear that egg donors had been paid and two lab members had provided oocytes. This forced Hwang to admit these unethical practices. Subsequently, the scientific content itself raised questions. Duplications of four microscopic photographs in different panels, and designated as different ESC lines, in the publication of 2005 were uncovered, but these were parried as an accidental mistake by Hwang and the Science editorial board. Furthermore, DNA fingerprint comparison of presumed donor and derived ESC lines showed no inter-experimental variety and were in fact performed on the same fingerprint profile. Hwang agreed to an independent investigation by Seoul National University. His three most important recent works were investigated:

the retracted 2004 and 2005 Science papers and a publication in Nature about a cloned dog. The conclusions were clear. The claim of being the first laboratory to create a pluripotent human ESC line through SCNT was reported to be false. Verification of the DNA fingerprints of cell lines, teratomas and donors showed that the NT-1 cell line was not derived from the designated donor. Second, no evidence was found to verify the conclusions of the report of the 11 ESC lines in the paper of 2005. The claims were based on material obtained from two ESC cell lines derived by IVF rather than SCNT. Displayed results of DNA fingerprinting, karyotyping, data of MHC-HLA isotyping and photographs of teratoma and embryoid bodies were all fabricated. (9)

Ethical Issues For Cord Blood Banking

The ethical implications of cord blood banking in the case of donated samples for the purposes of allogeneic transplantation or research are the same as for any tissue bank. This issue has been addressed in the European group on Ethics in Science and New technologies (EGE) Opinion no. 11 on the ethical aspects of tissue banking (21 July 2001). The ethical values underlined in this opinion are the following: body integrity, respect of privacy and confidentiality of data, promotion of solidarity, fairness of access to healthcare and information and consent of the donors. (10)

Umbilical cord blood banking process should comprise of a detailed consent explained clearly to the woman or to the couple of the prospective new treatments, but stress that they are still very much at the experimental stage. Principally, tissue bank activities should be reserved to public health institutions or non-profit making organizations. All public and private banks tissue banks should be monitored for quality measures and standards.

These guidelines are based on the principle of respect for human dignity and integrity which asserts the principle of non commercialization of the human body; principle of autonomy or the right to self-determination on the basis of full and correct information; principles of justice and solidarity, as regards to fair access to healthcare services; principle of beneficence, or the obligation to do good, especially in the area of health care; principle of non-maleficence, or the obligation not to harm, including the obligation to protect vulnerable groups and individuals, to respect privacy and confidentiality; and principle of proportionality which implies a balance between means and objectives. (11)

There are also some value conflicts regarding the Umbilical cord blood banking. The values of freedom and free enterprise can conflict with the principles of solidarity and justice, according to which access to healthcare should be on an equitable basis and based on realistic needs, as well as with the principle of protection of vulnerable groups.

Informed Consent:

Informed consent is a vital step to any research project. It is the process in which a patient/participant consents to participate in a research project after being informed of its procedures, risks, and benefits (12) After fully comprehending the information about the project, the patient/participant gives full and conscious consent for the

physician/scientist to continue with the procedure. The consent is obtained after giving all the information to the patient in comprehensible non-medical terms, preferably in the local language about the diagnosis; nature of treatment; risks involved, prospectus of success, prognosis if the procedure is not performed and alternative treatment. The three main aspects of the informed consent are information, voluntariness and capacity. In keeping the observations of the Supreme Court, the National Commission of India stated that all information would imply adequate information to enable the patient to make a balanced judgement to whether or not to be a part of the trial or treatment.

Current Ethical Basis Of Stem Cell Therapy:

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

In accordance to the International policies as stated in the Helsinki Declaration, our centre NeuroGen Brain & Spine Institute follows the guidelines.

There are in addition some other aspects of the Stem cell therapy debate that need further discussion. These are:-

(1) That there is a need to make a clear cut distinction between embryonic stem cells and adult stem cells whilst strict regulations for embryonic stem cell work are completely justified the same are not needed for adult stem cell work.

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

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

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

To elaborate on the above points :

(1) That there is a need to make a clear cut distinction between embryonic stem cells and adult stem cells whilst strict regulations for embryonic stem cell work are completely justified the same are not need for adult stem cell work:-

It is clear from all the above that the entire ethical debate regarding stem cell therapy revolves around the use if embryonic stem cell and cloning. There are no ethical issues with the use of autologous stem cells derived from bone marrow, Yet there are various restrictions in place for the use of any types of stem cells in different countries. Until everyone concerned starts looking at stem cells of non

embryonic origin differently from embryonic stem cells we will continue to involved in debating the issue and the price for these delays are paid for by the patients for no fault of theirs. Herein lies the tragedy. There is available a form of cellular replacement therapy that can give relief to millions of patients, for which there is enough published clinical evidence of safety and a satisfactory published evidence of efficacy yet this treatment cannot be freely used by one and all. It is our belief that by letting patient suffer and at time side when there are treatment option with stem cells that could possibly benefit them is unethical.

(2) *That there is a need to look at the whole issue from the patients point of view respecting the fact that even small functional improvements can mean a lot to a particular patient:*

We tend to judge improvements from normal peoples point of view. We don't realize that even small improvements, seemingly unimportant to us, can make a quantum difference in the lives of patients paralyzed with neurological problems. The Beijing Declaration of the International Association of Neurorestoratology (IANR) says it "recognizes the importance of small functional gains that have significant effects on quality of life". We need to stop being arm chair professors and talking only about evidence based medicine. We have to look at this from the point of view of the patients. To highlight this we highlight a case which show us how improvements that may mean nothing to us can mean the world to suffering patients. This was one of the first cases of multiple sclerosis treated with stem cells. Patient had a lot of improvements including significant improvements in her speech, ability to use her hand to hold a cup and her mobile, ability to sit without support, ability to stand with support. All of these were not possible before the stem cell therapy treatment. Yet the improvement that mattered to her more than all of these was something very small. Earlier when lying in the prone position she could not turn in bed by herself. After the stem cell therapy she could do so. Prior to the treatment every night she would have to wake up her grandmother 3-4 times a night to help her turn her position in bed. This used to upset the patient since it used to emotionally hurt and pain her that she had to wake up her grandmother multiple times in the night just to turn her. And she needed to turn since sleeping in one position would make her very uncomfortable. So despite all her other improvements with her speech and hands what made her most happy and the improvements that mattered to her the most was after the treatment she could turn in bed by herself and did not have to wake up her grandmother every night. This has been highlighted just to make one very simple point. That we must look at this entire issue from the patients point of view. We musts recognize that small improvements that do not mean anything to us can mean a lot to a patient with severe physical limitations. That at the end of the day all ethics, moral, values principles, laws and regulations have just one purpose. The well being of the common man.

What has unfortunately happened in the field of stem cell therapy is that the regulations we have made to protect ourselves are now limiting us and tying us up. These regulatory chains need to be unshackled. Physicians need to be free to

use whatever modality of treatment they believe is in the patients best interests. However the other side of the argument is that these are helpless patients and they are likely to be exploited by physicians offering stem cell therapy. We must however note that there are black sheep in every profession. That those who don't have values and principles are doing all manner of unprincipled and unethical practices with conventional treatments also. On the other had there are researchers who have been working in this field for many years both in the laboratory as well as clinically. They should be permitted to offer treatments they believe are safe and will benefit patients. Unless more physicians offer these treatments there will always be a supply demand gap with the result that fly by night operators will enter the field to make money. Therefore freeing up the field will bring more transparency and accountability to this aspect of medical treatment.

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

The Helsinki Declaration that has been discussed earlier in this chapter makes one thing very clear that for diseases for which there are no cures or the cures have been ineffective the physician is justified in using an unproven treatment if the physician believes that it will benefit the patient. This is the ethical bedrock on which we offer stem cell therapy as a form of treatment for neurological disorders for which there are no other treatments.

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

In the section on clinical aspects we have mentioned in this book numerous studies that have clearly shown the safety and efficacy of adult stem cells in various neurological disorders. A question that remains unanswered is when does a treatment that is "unproven or experimental" become a treatment that is "proven or established". How many publications documenting safety and efficacy will it take to make that shift ? Is a single publication enough, or are 10, 50 or 100 ok, or are multicentric international trials the only basis to make any treatment option an excepted form of treatment. Is it necessary to go on reinventing the wheel just to satisfy our intellectual considerations whilst millions of patients continue to suffer?

So to go back to what we have mentioned in the preface that there are two sides to the ethical debate on basing our treatment options on evidence based medicine. (1) One side of the debate is " Is it ethical for doctors to offer to patients treatment options that have not become a standard of care as yet?." (2) The other side of the debate is "Is it ethical to deny patients suffering from disabling diseases, treatments options that are safe and available, whilst we wait many years for the results of multicentric international trial to prove that these treatments work ?" Both these questions are answered differently by different people depending on what is at stake for them.

REFERENCES

1. Testimony of Ronald Cole-Turner, Ethical Issues in Human Stem Cell Research, Commissioned Papers, Volume III Religious Perspectives,2000: A1.http://www.bioethics.gov/reports/past_commissions/ nbac_stemcell3.pdf
2. Testimony of Rabbi Elliot N. Dorff, Ethical Issues in Human Stem Cell Research, Commissioned Papers, Volume III Religious Perspectives, 2000: A-1. http://www.bioethics.gov/reports/past_commissions/ nbac_stemcell3.pdf.
3. Ethical Issues in Human Stem Cell Research, Commissioned Papers, Volume III Religious Perspectives, 2000; http://www.bioethics.gov/reports/past_commissions/nbac_stemcell3.pdf
4. Holland, S., Lebacqz, Karen., and Zoloth, L., eds. The Human Embryonic Stem Cell Debate: Science, Ethics, and Public Policy. (Cambridge: MIT Press, 2001).
5. The Pew Forum on Religion and Public Life, Religious Groups' Official Positions on Stem Cell Research, July 17, 2008, http://pewforum.org/docs/?DocID=319
6. Clinical trial overview: neuronal ceroid lipofuscinosis (NCL, often called Batten disease).Available at: http://www.stemcellsinc.com/clinicaltrials/clinicaltrials.html. Accessed March 4,2009.
7. 18 December 2008 StemCells, Inc. receives FDA approval to initiate clinical trial of HuCNS-SC cells in a myelin disease. Available at: http://www.stemcellsinc.com/news/081218.html. Accessed March 3, 2009.
8. International Society for Stem Cell Research 2008 Endorse the open letter. Support all forms of stem cell research. Available at: http://www.isscr.org/ScienceStatementEndorsers.cfm. Accessed January 7, 2009.
9. Fraud and misconduct in science: the stem cell seduction. Implications for the peer-review process. M.A.G. van der Heyden, T. van de Ven and T. Opthof. Neth Heart J. 2009 January; 17(1): 25-29.
10. Ethical aspects of umbilical cord blood banking. Opinion Of The European Group On Ethics In Science And New Technologies To The European Commission. No.19
11. Ethical aspects of umbilical cord blood banking. Official Journal L 281, 1995; 0031 - 0050.
12. Bulger, R.E. (2002). Research with Human Beings. In Bulger, R.E., Heitman, I., & Reiser, J. (Ed.), The Ethical Dimensions of the Biological and Health Sciences (pp 117-125). New York: Cambridge University Press.

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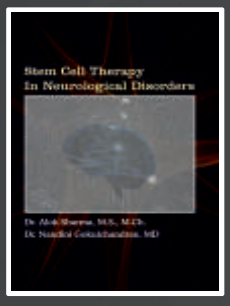

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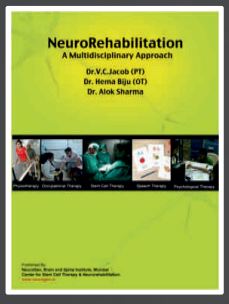

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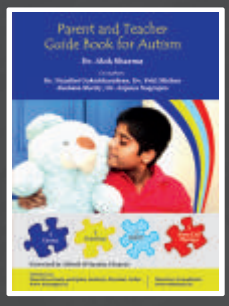

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