# Stem Cell Therapy In Neurological Disorders



Dr. Alok Sharma, M.S., M.Ch. Dr. Nandini Gokulchandran, MD

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#### ISBN 81-86876-06-5

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

Cover Page by Movewell Media Pvt. Ltd. Ahmedabad

Printed by

Surekha Press, A-20, Shalimar Industrial Estate, Matunga Labour Camp, Mumbai 400 019. Tel. : 2409 3877, 2404 3877

Price : ₹ 1,500/-

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### 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 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 Dr. Nandini Gokulchandran



The ethical basis of offering stem cell therapy as a treatment option is based on the Paragraph no. 35 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 a 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 be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available."

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## **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

Regenerative medicine is a newly evolving branch of modern medicine that deals with cell based therapies which use healthy cells cultured in the laboratory to replace damaged cells in adult organisms to treat disease. This could therefore potentially hold the key for addressing ailments which currently have no proven treatments or cures, such as, neurological disorders (spinal cord injury, cerebral palsy, brain stroke, muscular dystrophy, Alzheimer's disease, multiple sclerosis, etc.), diabetes, cardiovascular disorders, bone disorders, hematopoietic disorders, cancers, hepatic, renal and dermatological disorders.

One of the building blocks of this therapy are stem cells. Regenerative medicine aims to repair or re-grow parts or tissues which are lost as a consequence of disease or injury. Stem cells have the capability to multiply manifolds and convert or differentiate into any specialized cell types of the body. Hence, the potential of these invaluable assets could even be projected as far as, sometime in the near future, to replace organ transplantation.

Depending on the source, the potency or plasticity of stem cells varies. Stem cells procured from the 5-6 day embryo (usually from wasted or excess fertilized embryos from IVF clinic), referred to as embryonic stem cells, have theoretically the capacity to give rise to the whole embryo and cells of all the germ layers (pluripotent). However, they are surrounded by hordes of ethical issues regarding the source of these cells. Also, formation of "teratomas' is a serious possibility in the long-term with these cells.

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. A body of work has been ongoing on the use of these cells, in various specialties and disorders.

This book endeavors to the assimilate all the current information on understanding stem cells, its potential and more specifically its role in treating incurable and intractable disorders of the brain, spinal cord and the muscular system.

The nervous system is like the central processing unit of the animal body. In humans, it is more evolved and specialized. Since, disorders and injuries affecting the nervous system lead to irreparable damage and disability, this area has become a major focus point in the arena of regenerative medicine. 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.



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## 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.

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	

Stem Cell Timeline

### 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 Historical Review : Evolution of Stem Cell Therapy

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 cowinners of the 1990 Nobel Prize in Physiology of Medicine for their contribution to discoveries concerning cell and organ transplantation in the treatment of human diseases. Performing marrow transplants in humans was not attempted on a larger scale until a French medical researcher made a critical medical discovery about the human immune system. In 1958 Jean Dausset identified the first of many human histocompatibility antigens. A bone marrow transplant between identical twins guarantees complete HLA compatibility between donor and recipient. These were the first kinds of transplants in humans. It was not until the 1960's that physicians knew enough about HLA compatibility to perform transplants between siblings who were not identical twins. (13)

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

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

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

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

#### **Recent history**

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

#### **Embryonic Stem Cells:**

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

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

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

McDonald J W et al. in a seminal paper showed that transplanted neural differentiated mouse embryonic stem cells into a injured rat spinal cord after traumatic injury home onto the site and differentiate into astrocytes, oligodendrocytes and neurons, and migrated as far as 8 mm away from the lesion edge. (13) This lead to an explosion

of new thoughts and avenues for research into possible application of this newfound development, especially into treatment of spinal cord injury and other neurological disorders and papers.

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

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

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

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



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

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

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 public and private research institution engaged in stem cell research 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

Major Stem Cell Companies in India

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

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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

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

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

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

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

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

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

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

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

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

As we restore our commitment to science, and resume funding for promising stem cell research,

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

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

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

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

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

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

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

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### Summary

The history of stem cell research had a benign, embryonic beginning in the mid 1800's with the discovery that some cells could generate other cells. Stem cells existence was discovered by the eminent German biologist Ernst Haeckel, he used the term "Stammzelle" (German for stem cell) to describe the ancestor unicellular organism from which he presumed, all multicellular organisms evolved In the early 1900's, the first real stem cells were discovered when it was found that some cells generate blood cells. The use of stem cell medicine was first reported in 1956 by Dr. E. Donnall Thomas, a bone marrow transplant specialist. Cord blood stem cells have been used in the treatment of blood cancers and/or blood diseases since 1988. 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. Stem cell research got embroiled in a controversy over the use of human embryonic stem cells for research, following ethical issues raised over its source. However a lot of work has now been done on adult stem cells for the treatment of many other conditions.

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3

Every cell in the human body can be traced back to a fertilized egg that came into existence from the union of the egg and the sperm. The body is made up of over 200 different types of cells. All of these come from a pool of stem cells in the early embryo. During early development as well as later in life, the stem cells give rise to the specialized or differentiated cells that make up our body. Over the past 2 decades scientists have been gradually deciphering 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 is the property that makes them so appealing as a method for creating 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 properties. First, it is a cell that can divide indefinitely, producing a population of identical offspring. Second, stem cells can, on cue, undergo an asymmetric division to produce two dissimilar daughter cells. One is identical to the parent and continues to contribute to the original stem cell line. The other varies in some way. This 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 or muscle cells. (1)

#### **Developmental Hierarchy In Stem Cell (SC) Compartment:**

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 extraembryonic 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 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 classified as embryonic stem cells, umbilical cord stem cells and adult stem cells on the basis of their origin.

#### **Embryonic Stem cells:**

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

Developmental studies in mouse revealed that the fertilized oocyte, the zygote, has the capacity to form the whole embryo. 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 "funny little tumors" produced benign teratomas or malignant teratocarcinomas. (5)



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



Figure 2 : Mesenchymal stem cells



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



Figure 4 : Wharton's Jelly - Mesenchymal stem cells

Clonally isolated EC cells retained the capacity for differentiation and could produce derivatives of all three primary germ layers: ectoderm, mesoderm, and endoderm. More importantly, EC cells demonstrated an ability to participate in embryonic development, when introduced into the ICM of early embryos to generate chimeric mice. EC cells, however, showed chromosomal aberrations, lost their ability to differentiate, or differentiated in vitro only under specialized conditions and with chemical inducers .The EC cells did not retain the pluripotent capacities of early embryonic cells and had undergone cellular changes during the transient tumorigenic state in vivo.(6) In order to disconnect from the tumorogenic elements of the EC lines, cell lines from mouse ICM or epiblast were derived in 1981 using a) mouse feeder and b) EC cell conditioned medium.

These cell lines, termed as 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 showed the capacity to reproduce the various somatic cell types were found to develop into cells of the germ line

Primordial germ (PG) cells, which form normally within the developing genital ridges, represent a third embryonic cell type with pluripotent capabilities. Isolation and cultivation of mouse PG cells on feeder cells led to the establishment of mouse embryonic germ (EG) cell lines .In most respects, these cells are indistinguishable from blastocyst-derived ES cells and are characterized by high proliferative and differentiation capacities in vitro, and the presence of stem cell markers typical of other embryonic stem cell lines. (7) However, have a limited proliferation capacity, and currently can only be propagated as embryoid body derivatives.

#### **Properties of ES Cell Lines:**

Mouse ES cell lines were first derived in 1980s, were grown on feeder layer of mouse embryonic fibroblasts (MEFs). Once established, murine ES cell lines displayed an almost unlimited proliferation capacity in vitro and retained the ability to contribute to all cell lineages neurons, cardiomyocytes, smooth muscle cells, hematopoietic cells, osteogenic cells, hepatocytes, insulin-producing cells, keratinocytes. In vitro, mES cells maintained a relatively normal and stable karyotype, even with continued passaging. ES cells were also characterized by a relatively short generation time of 12-15 h with a short G1 cell cycle phase. (7)

Undifferentiated mES cells express specific cell surface antigens, SSEA-1 and membrane-bound receptors, gp130 and possess enzyme activities for alkaline phosphatase (ALP) and telomerase activity.ES cells also contain the epiblast/germ cell-necessary for maintenance of pluripotency.

The ES cell property of self-renewal depends on a stoichiometric balance among various signaling molecules, such as, LIF, Nanog, Wnt signaling pathway, BMP expression. An imbalance in any one can cause ES cell identity to be lost. (8-9)

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. (10,11) 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. (12) 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. As of December 2004, only 22 of the cell lines listed in the NIH register have been successfully propagated in vitro.

Recently, hES cell lines have now been cultivated both on human feeder cells to avoid xenogenic (10) and in the absence of feeder cells under serum-free conditions (13) 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.

DNA can be introduced into ES cells by conventional infection, transfection, or electroporation protocols. (14) Random insertion events have been employed to over express, mutate, and tag genes in phenotype-driven screens, and the discovery that DNA (cloned or genomic) introduced into ES cell lines can undergo homologous recombination at specific chromosomal loci has revolutionized our ability to study gene function. The ability to introduce virtually any mutation into the genome following gene targeting in mouse ES cells provides a powerful approach for elucidating gene function both in vitro and in the whole animal. ES cell progeny can therefore be biased into a desired cell lineage by exposure to appropriate differentiation factors and by

genetic manipulations of key developmental genes. 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. (15)

#### Invitro Differentiation Potential of Embryonic Stem Cells:

During mouse embryogenesis, the primitive ectoderm of the epiblast forms three primary germ layers: the ectoderm, the mesoderm, and the definitive endoderm. These germ layers interact to form all tissues and organs of the developing embryo. The complex interactions that control the transition of ectoderm to visceral and parietal endoderm in the postimplantation embryo, followed by the formation of mesoderm at the gastrulation stage (days 3 to 7 post coitum), are only beginning to be defined. The in vitro differentiation potential of mES cells has facilitated the examination of these processes.

Differentiation is induced by culturing ES cells as aggregates (EBs) in the absence of the self-renewal signals provided by feeder layers or LIF, either in hanging drops, in liquid "mass culture", or in methylcellulose. (16-17) Moreover, co culture with stromal cell line activity (i.e., of PA6 cells), and recently, even adherent monolayer cultures in the absence of LIF have been used to differentiate mES cells in vitro. Scaleable production of ES-derived cells can furthermore be achieved through the use of stirred suspension bioreactors with encapsulation techniques

Human hES cells differentiate when removed mechanically ("cut and paste") or by enzymatic dissociation from feeder layers and cultured as aggregates in suspension. Cystic EBs formed under these conditions are heterogeneous and express markers of various cell types, including those of neuronal, cardiac, and pancreatic lineages. However, none of the factors known to influence mES cell differentiation directs hES cells exclusively into a single cell type. (18)

#### Uses of Embryonic Stem Cells:

#### 1. Embryonic stem cells as cellular models

Experiments designed to understand gene function in the context of an organism require genetic strategies. Enhancer and promoter traps, gene traps, random activation of gene expression (RAGE) and genome-wide cell-based knockout (GECKO) represent genome-wide strategies to identify, isolate, or determine gene function. Because of gene-targeting techniques, transgenic mice have also proven critical to the creation and evaluation of some models of human disease. Embryonic stem cell lines have proven to be useful mediums for genetic manipulation, for understanding developmental processes and correction of genetic defects. (19)

#### 2. Embryonic stem cells in pharmacology and embryotoxicology

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. (20)

Human ES cell lines may, therefore, prove clinically relevant to the development of safer and more effective drugs for human diseases. Three aspects are relevant to this

issue. 1) At present, insufficient methods exist in some areas of in vitro toxicology to predict target organ toxicity. 2) In embryotoxicology, interspecies variation complicates data analysis, and human cell systems may enhance the identification of hazardous chemicals. 3) Human ES-derived cells cultured in vitro may reduce the need for animal testing in pharmacotoxicology.

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 therapeutically applicable, any ES-based treatment must, however, show limited potentials for toxicity, immunological rejection, or tumor formation, and at present, human ES cell research has not reached this threshold.

The availability of human ES cells, however, represents an extraordinary opportunity for cell transplantation that may be applicable to a wide range of human ailments. Three properties make ES cells relative to adult stem cells very attractive for replacement therapies.1) Human ES cells can be grown indefinitely in culture.2) ES cells can be genetically manipulated, and loss of function genes (e.g., CTFR) can theoretically be repaired by the introduction of transgenes into ES cells either by random transgenesis or through gene targeting. 3) Numerous differentiation protocols have already been established that permit the generation of almost any cell type, either through the use of established culture conditions or when coupled with genetic manipulations. In theory, hES cells could be applied to a wide range of human ailments, but the proof of principle has largely come from the use of mouse ES cells.(21,22)

#### Adult Stem Cells

Adult stem cells are pluripotent, clonogenic, self renewing, having 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. liver, brain, bone marrow, adipose tissue, heart, etc. The primary role of these adult stem cells is initiation of repair process in the organ following an injury. These cells have been, in practicality, difficult to obtain due to the following reasons:

- 1) Inaccessibility and small numbers (e.g. neural stem cells)
- Lack to markers for characterization and isolation of the "stem cell population" from various organs.(23)

The field of Regenerative medicine, which got 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) 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. Indeed, marrow-derived cells seem to retain a remarkable plasticity, since they have much wider differentiation potential than previously thought. 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. (24)

#### 2) 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. (25) The contribution of MAPC to blastocyst development, however, requires confirmation by other, independent laboratories

#### 3) 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

#### 4) 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.

#### 5) 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 (26) Direct electron microscopical analysis revealed that VSEL (2-4 ?m 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) 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.(27)

#### 2) 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. (28,29)

#### 3) 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. (30)

#### 4) 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. (31)

#### 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) 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.(32) 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. (33)

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.

The dogma regarding neuronal plasticity or rather the non existence of neuronal plasticity is being refuted. There is varied and conflicting opinion regarding whether these NSCs are neuronal progenitors or astrocytes. (34)

#### 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. (35,36)
- (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. (37)
- (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.(38,39)

(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. (40,41)

#### 5) Pancreatic stem cells

There has been controversy as to whether the pancreas contains true stem cells. It was reported that the endocrine cells of the rat pancreatic islets of Langerhans, including insulin-producing beta-cells, turn over every 40-50 days by processes of apoptosis and the proliferation and differentiation of new islet cells (neogensis) from progenitor epithelial cells located in the pancreatic ducts. The administration to rats of glucose or glucagonlike peptides resulted in the doubling of the islet cell mass, suggesting that islet progenitor cells may reside within the islet themselves. The same authors showed that rat and human pancreatic islets contained an unrecognized population of cells that expressed the neural stem cell-specific marker nestin. These nestin-positive cells were distinct from ductal epithelium. These nestin positive cells, after isolation, had an unusually extended proliferative capacity in vitro, could be cloned repeatedly and appeared to be multipotential. They were able to differentiate in vitro into cells that expressed liver and exocrine pancreas markers. The authors proposed that these nestin-positive islet derived progenitor cells were a distinct population of cells that resided within the pancreatic islets and participated in neogenesis of islet endocrine cells.(42)

#### 6) 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. (43)

#### 7) Liver stem cells

Mammals are said to survive surgical removal of at least 75% of the liver by regeneration. The original tissue can be restored in 2-3 weeks. This is in contrast to most other organs such as the kidney or pancreas. Recent evidence strongly suggests that different cell types and mechanisms are responsible for organ reconstitution, depending on the type of liver injury. (44)

#### **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 characteristics of hematopoietic stem cells in umbilical cord blood have recently been clarified. 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. This matrix termed 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 esenchymal progenitor cells that can be harvested after birth, cryogenically stored, thawed, and expanded for therapeutic uses. (45) 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-versushost 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.

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.

## **SUMMARY:**

Stem cells have been of interest and studied by biologists for decades. By definition stem cells are unspecialized cells which are capable of renewal and self division and give rise to specialized cells. Under certain physiologic and experimental conditions, they can be induced to become cells with special functions such as beating cells of the heart, muscles, nerves and insulin producing cells of the pancreas. Stem cells are classified as embryonic stem cells, umbilical cord stem cells and adult stem cells on the basis of their origin. Stem cells are increasingly seen as potential therapies for organ and tissue failure.

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## 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

#### Mechanism Of Action

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 II, 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.

### **SUMMARY**

The definitive mechanisms for protection via stem cells remains unclear. Stem cells mediate their effects via 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. They may also act via effecting remylination. Improved understanding of these paracrine and other mechanisms may allow earlier and more effective clinical therapies.

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

## 5

## Laboratory Aspects Of Stem Cell Therapy

Stem cell harvesting is preliminary and important part of the whole process of stem cell therapy. Various sources of stem cells have already been discussed in the previous chapters. Stem cells have been sourced for treatment primarily from hematopoietic 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.

Bone-marrow transplantation is the standard treatment for several haematological malignant disorders and is being assessed for the treatment of severe forms of several autoimmune disorders such as Multiple Sclerosis. Hematopoietic progenitor cells might re-establish the defective immune system in patients with autoimmune disorders. The cells can be obtained from a sibling or an unrelated donor who is closely matched on HLA (allogeneic transplantation), an identical twin (syngeneic transplantation), or the patient before chemotherapy (autologous transplantation). The haematopoietic progenitor cells can be directly harvested from the bone marrow or collected from peripheral blood; the term haematopoietic-stem-cell transplantation (HSCT) includes both sources. (1)

#### Source of haematopoietic stem cells

Haematopoietic stem cells are mainly found in the bone marrow but they can be mobilised to the peripheral blood, in large numbers, by the administration of recombinant granulocyte colony-stimulating factor. Hematological (neutrophil, red cell, and platelet) and immune recovery is faster with peripheral blood cells than with bone marrow cells, owing to more rapid engraftment.

The protocols for cell transplants are varied. Various researchers have tried different conditioning regimens (myeloablative versus non-myeloablative), use of stimulating factors (G-CSF and GM-CSF) for mobilization of stem cells, purifying/enriching methods.



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)

#### 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 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. (2)

#### Bone marrow harvesting

#### **Open** Method

Bone marrow blood (100-150 mL) aspirated from the iliac bone(generally either anterior or posterior superior iliac spine) and is diluted in Hanks' balanced salt solution (HBSS) at a ratio of 1:1. After centrifugation of samples at 1000 x g for 30 min through a density gradient (Ficoll-Paque Plus, 1.077 g/L; Amersham Biosciences, Piscataway, NJ), the mononuclear cell layer is recovered from the gradient interface and washed with HBSS. The cells are centrifuged at 900 xg for 15 min and resuspended in 1.8 mL of phosphate buffered saline (PBS) at a density of  $1.1 \times 106$  cells/L. (3)

#### **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<sup>TM</sup> (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.

In Harvest's European studies, injected autologous adult stem cell concentrates from bone marrow have shown promise in achieving tissue regeneration in vascular, orthopedic and cardiovascular disease. In the U.S., Harvest Technologies is sponsoring a multi-centered randomized controlled double-blind study evaluating the therapeutic effect of BMAC for treating patients with non-reconstructable CLI. (4)

#### 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 has avoided these potential problems by working in a completely closed system eliminating excess air contamination and, most importantly, allowing for up to 99% recovery of necessary cells for transplantation.

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. (5)

#### 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 our published procedure [1]. 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).(6)

#### 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. (6)

## Summary

Currently, stem cells used in human studies are either adult stem cells or umbilical cord stem cells. Adult Haematopoietic stem cells(bone marrow derived or peripheral blood stem cells) have to be obtained by various methods.Bone marrow derived stem cells can be obtained via a closed or an open method.Peripheral blood stem cells are generally obtained through apheresis or leukopheresis.Processing of cord blood stem cells are harvested by either manual or automated methods.

Methods of harvesting , expansion and differentiation for clinical purposes is still undergoing further refinement.

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# 6

## Surgical aspects of stem cells therapy

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 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.



Figure 1: Bone marrow J needle



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



Figure 2: Bone marrow aspiration



Figure 4: Intrathecal Injection step 1



Figure 5: Intrathecal Injection step 2



Figure 7: Intrathecal Injection step 4



*Figure 6 : Intrathecal Injection step 3* 



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.* 

Surgical aspects of stem cells therapy

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,  $2 \times 10^8$  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  $\mu$ L of stem cells. In his planned trials, Wise Young is intending to inject an escalating dose of 4  $\mu$ L, 8  $\mu$ L and 16  $\mu$ L.
- 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.

#### 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

#### Surgical aspects of stem cells therapy

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 perioperative 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.

## Summary

Along with varied sources of stem cells, routes of delivery are critical aspects of stem cell therapy. Apart from systemic delivery ,other routes are more local ones, such as, intrathecal, intraspinous, intraarterial (into blood vessels), intracerebral and intramuscular.

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# 7

# **Concepts And Technique Of Motor Points: Intra-muscular Stem Cell Injection**

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

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



Figure 1: A Neuromuscular Junction



Figure 2 : The Motor Unit

#### Concepts And Technique Of Motor Points: Intra-muscular Stem Cell Injection

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

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



*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.* 

Concepts And Technique Of Motor Points: Intra-muscular Stem Cell Injection



*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

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







## **Summary**

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. This is the point on the skin region where an innervated muscle is most accessible to percutaneous electrical excitation at the lowest intensity 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. 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. Intramuscular stem cells injection in motor points within the muscle. 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

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# SECTION B

# **Clinical Aspects**



# 8

## **Role Of Stem Cells In Spinal Cord Injury**

Spinal cord injury is a devastating event that occurs suddenly and whose consequences range from minimal symptomatic pain to a tragic quadriplegia. If cervical spinal damage is severe, quadriplegia results, whereas an injury to the thoracic or lumbar spine leads to paraplegia. Damage to the upper cervical levels (C2-C3) results in diaphragmatic palsy, which may lead to requirement of tracheostomy and ventilatory support. Apart from motor and sensory deficit, patients usually suffer from acute hyperesthesia or severe chronic pain, urinary and rectal dysfunction, and autonomic dystonia. The cause of a majority of the spinal cord injuries is either vehicular accidents, sports injuries, violence, or falls.

The physiopathology of spinal cord trauma is determined by two basic conditions: the trauma itself, involving cellular death or electrolyte, metabolite, and enzyme release, and the cascade of acute inflammation with swelling, ischemia, and reperfusion as a secondary neuronal injury.

The primary injury following spinal cord trauma rapidly progresses to secondary injury, leaving only a donut-like rim of mostly white matter at the trauma level. Hence, the outcome of spinal cord injury is quite poor .More than 60 percent of traumatic injuries in the United States, have been reported to be graded as ASIA A or "complete." Fewer than 3 percent of these individuals have shown recovery to the extent of walking. In fact, fewer than 10 percent regain enough sensory function to be reclassified as ASIA B, C, or D. Despite these statistics, 60 percent to 90 percent regain at least one motor level, a gain typically attributed to recovery of segmental nerve roots injured by traction injury.

The general understanding, as per various studies, is that most recovery occurs in the first six months and that; if recovery has not been observed in the first year to two, it will not occur thereafter. Most clinical studies report of substantial return of function (e.g., 1-2 ASIA grades) within one year from injury in patients ,with practically no reports of spontaneous recovery after 2 years. (1)



Fig 1: Sagittal T2W image through the dorsal spine reveals complete transection of the spinal cord due to fracture of D6 vertebral body which is displaced posteriorly resulting in gross kyphotic deformity.



Fig 2: Sagittal T2W image through the cervical spine reveals mal-alignment of the cervical spine with retro pulsed C6 vertebral body indenting on the cord with myelomalacic changes noted in the cervical cord.

The key element in management is prevention of secondary neurological damage occurring during transportation. A patient with suspected spinal injury should be immobilized (especially the spine) using either the cervical collar or the belts for thoracic and lumbar spine.

Dramatic improvements in quality of care, particularly in the neurosurgery field, have lead to improved initial recovery and reduced complications. Early surgical decompression and internal stabilization of the cervical spine, which avoids the need for external halo stabilization, have been important milestones in the early management of spinal cord injury, probably reducing the progression of secondary injuries. Internal stabilization allows a more rapid transition to rehabilitation and early mobilization, reducing complications associated with immobilization, such as deep vein thrombosis, trophic ulcers, etc.

### Classification of spinal cord injury

- A. Region wise:
  - i. Cranio Vertebral junction.
  - ii. Cervical.
  - iii. Thoracic.
  - iv. Lumbar.
- B. Type:
  - i. Fracture.
  - ii. Dislocation.
  - iii. Fracture dislocation.
- C. Complete / Incomplete.

The damage caused due to spinal injury can be divided into primary (which occurs at time of injury) and secondary (occurs after injury i.e. during transportation or during the stay in hospital).

### Investigations

- 1. Plain x-rays AP and lateral view.
- 2. MRI best diagnostic tool for spinal injury, as it shows the exact damage to the spinal cord and nerves and helps in deciding whether surgery is required, along with what type of surgery to be done.

Note: CT scan is not useful diagnostic tool, since structures cannot be visualized and should be done only if MRI is not available.

### Treatment

- 1. At the site of accident / clinic of the family physician :
  - a. Patient should be immobilized with the help of sand bags, plastic iv bottles along the neck/head and should be fastened to the stretcher.
  - b. Airway, breathing should be assessed and maintained . Regurgitation and aspiration should be managed accordingly.
  - c. Patient should be transported with no spinal movements and care should be taken while doing so, three persons should help in shifting / lifting the patient, they should lift the patient from the same side only.
  - d. For family physicians having to manage in their clinics due to transportation problem, 2gm of Methyl prednisolone sodium succinate (MPSS) in 200 ml of normal saline should be given over a period of half hour.
- 2. In the hospital:
  - a. Resuscitation and maintaining the airway with intubation / ventilator if required.
  - b. Heamodynamic state is maintained.
  - c. Methyl prednisolone sodium succinate (MPSS) -Should be started within 3 hrs of the injury and should be continued for next 24 hrs. If started within 3-8 hrs of injury then it should be given for 48 hrs . MPSS is given as a loading bolus dose of 30 mg/kg over ½ hr and a maintenance dose of 5.4 mg/kg/hr for the required period accordingly.
  - d. In patients with dislocation, traction needs to be applied.
  - e. If patient has no evidence of spinal cord compression on MRI and shows improvement on MPSS and traction, then there is no need for the surgery. However in patients with spinal cord compression or progressive neuro detereoration surgery must be considered.
  - f. Physiotherapy is given for rehabilitation.
- 3. Surgery: Basic aims of surgery are decompression of the neural elements, reduction of malalignment and restoration of spinal stability.
  - a. Anterior / Posterior
  - b. Decompression / Stabilization

- 4. Operative procedure in spinal injury:
  - a. Cranio Vertebral junction :
    - i. Anterior surgery Trans-oral excision of alantoid.
    - ii. Posterior surgery Cranio cervical decompression and/or stabilization.
  - b. Cervical spine :
    - i. Anterior Corpectomy /Disc excision with anterior stabilization.
    - ii. Posterior Decompressive Laminectomy.
  - c. Thoracic spine :
    - i. Trans thoracic Anterior Corpectomy with screws and rods/plate fixation.
    - ii. Posterior Decompressive Laminectomy and stabilization using pedicular screws and rods / plate fixation.
  - d. Lumbar spine :
    - i. Trans abdominal Anterior Corpectomy.
    - ii. Posterior Decompressive Laminectomy and stabilization using pedicular screws and rods /plate fixation.

Many patients require both anterior as well as posterior surgery so it is done as a single or in two stage procedure.

All the surgeries listed above are focused on the musculo skeletal system and none of them correct the neurological injury. The central nervous system (CNS) i.e. the brain and the spinal cord is the only tissue in the body which was considered to be incapable of regeneration, and hence any damage to it as irreversible. Despite the surgery, many patients are left with neurological deficits, which may recover to some extent with regular rehabilitation.

A combination of factors is responsible for the lack of neural regeneration and minimal functional recovery generally observed after spinal cord injury. The injury severes axons and the distal segment of the axon (which is isolated from the neuronal cell body) degenerates. The proximal axon segment typically survives but fails to regrow and re-innervate its targets. The lack of axonal regeneration is not primarily due to an inherent lack of axonal growth potential, but rather the presence of axonal growth inhibitors in the adult central nervous system. Myelin associated proteins and the glial scar, which forms at the injury site and in de-nervated axonal tracts, inhibit axonal growth.

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

McDonald et al. (2) first put forth the concept of use of stem cells for disorders other than haematopoietic. This has paved the way for a whole new area of regenerative medicine in neurological disorders.

Since 1998, this area has burgeoned and innumerable types of stem cells, with equally numerous routes of administering these have been extensively explored. This chapter is a compilation of all such explorations in clinical subjects, which have been

#### Role Of Stem Cells In Spinal Cord Injury

reported till date. However, before venturing into how stem cell therapy for spinal cord injury has evolved, it is imperative that we understand what is it that we are venturing out to achieve. This has been very simplistically explained by McDonald as follows: he puts forth that 1) it is not necessary to cure a nervous system injury, and also 2) a disproportionate return of function can result from a small degree of regeneration. It is now understood that substantial loss of spinal cord tissue, particularly gray matter, does not preclude near-normal long-tract function. He advocates a continuous crosstalk between the laboratory and clinic for reaching readily achievable goals that improve quality of life.

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

One of the earliest (Rabinovich et al, 2003) stem cell transplants for neurological disorders (spinal cord injury) reported was using minimally manipulated cells from fetal nervous and hemopoietic tissues (gestational age 16-22 weeks). These were implanted subarachnoidally into 15 patients (18-52 years old) suffering from traumatic spinal cord injury (SCI) at cervical or thoracic spine level. The duration of injury varied from 1 month to 6 years. Each patient underwent from one to four cell transplantations (CT) with various time intervals. In 11 of 15 cases, CT was combined with an operative partial disruption of a connective tissue cyst and with implantation into a spinal cord lesion of a spinal cord fragment together with olfactory ensheathing cells. Following cell tranplantation, six patients showed improvement in their neurological status from A to C grade of SCI, exhibiting incomplete restoration of both motor and sensory function. The status of other five CT-treated patients was reported to be SCI grade B and was characterized by appearance of contracting activity in some muscles and incomplete restoration of sensitivity. The remaining four patients did not exhibit any clinical improvements. No serious complications of CT were noted. The results suggested a clinical relevance of the CT-based approach to treating severe consequences of SCI. (3)

However, due to various ethical and medical concerns over embryonic and fetal stem cells, adult stem cells (bone marrow, olfactory ensheathing, etc.) have been tried extensively. Yoon Ha et al ,in 2004, carried out a comparison of a) transplantion of autologous bone marrow cells directly into the SCI sites and administered granulocyte macrophage colony stimulating factor (GM-CSF) and b)only administration of GM-CSF{n=1} . The patients were followed up between 4-6 months. Sensory and motor improvements were noticed in all three patients at varied time points(ranging from 13 days to 4 months) (4)

Féron et al., (2005) assessed the safety and feasibility of cultured autologous OEC's injected directly into the injured spinal cord and around it in three paraplegics, between 6 and 32 months post complete SCI. .No adverse effects were reported. (5) Further in 2008, the same group (Mackay-Sim et al.) published the outcome after 3 years of follow up. Except sensory improvement in one patient, no significant improvements (functionally or neurologically) were noticed. (6)

In a Chinese language article, Zhou et al,(2004) briefly reported on 70 cases following Bone-marrow stem cell (BMSC) transplantation. 37 of these were SCI patients; others had suffered mainly stroke or traumatic brain injury. The stem cells were either injected or implanted. Patients received various therapies post-treatment including physical therapy, hyperbaric oxygen therapy and acupuncture. The authors briefly reported that positive outcomes for the SCI patients were that sexual function improved in three patients and sensation and function improved in five cases. It is unknown; however, whether these were SCI patients. There were three cases of intra-cranial infection. This article lacked additional detail regarding the procedure and the baseline characteristics of the patients. (7)

Peripheral macrophages have been reported to synthesize nerve growth factor after peripheral nerve damage and eliminate myelin which inhibits neural regrowth. Knoller et al carried out a phase I study, in 2005, using incubated autologous macrophages. These were transplanted into patients' spinal cord within 14 days of injury. Out of the 8 patients treated in this study, 3 patients showed improvements of motor and sensory functions without any critical complications (8)

Park et al (2005) is one of the earliest reports of intrathecal autologous bone marrow cell transplantation (BMT) in conjunction with the administration of granulocyte macrophage-colony stimulating factor (GM-CSF). This therapy was carried out in six complete SCI patients. The follow-up periods were from 6 to 18 months, depending on the patients. Sensory and motor recovery was noticed between 3 weeks to 7 months. Four patients showed neurologic improvements in their American Spinal Injury Association Impairment Scale (AIS) grades (from A to C). One patient improved to AIS grade B from A and the last patient remained in AIS grade A. No immediate worsening of neurologic symptoms was found. Radiological changes on MRI were also noticed, though the implications of the same are not well understood. Serious complications increasing mortality and morbidity, however, were not found. (9)

Syková et al in two separate publications in the same year (2006) (10,11) have reported comparative results of autologous BMMC via either intravenous infusion versus intra-arterial infusion in patients of spinal cord injury. Both the papers reveal that patients receiving intra-arterial transplants show more improvements as compared to those receiving the intravenous transplants. Changes in ASIA scale as well as electrophysiological changes have been reported. No complications following transplants by either route were noted.

In a novel method, using combination of BM mesenchymal stem cells (MSC) and patient's autoimmune T cells, Moviglia et al (2006) demonstrated the regeneration phenomenon based on the controlled inflammatory activity at the injured site. They obtained successful results during treatment of two patients with chronic spinal cord injury. The therapeutic approach was based on the generation of controlled inflammatory activity at the injury site that induced a microenvironment for the subsequent administration of autologous, BM-driven transdifferentiated neural stem cells (NSC). Both the patients showed both motor and sensory recovery with no adverse effects. (12)

#### Role Of Stem Cells In Spinal Cord Injury

A series of publications exploring autologous BMSC transplantation either direct injection into the injury site or intrathecal delivery have, from Russia, Brazil, Mexico, Korea, China, India have come out in the last 3-4 years. These papers mainly address the safety aspects of autologous stem cells being delivered locally, so that the blood brain barrier can be bypassed, perhaps yielding more efficacious results. (10,11,13,14,15,16)

In this series, a report from Russia (Chernykh et al, 2007) reported neurological improvements in 66.7% of chronic SCI patients who underwent autologous BMSCs transplantation intravenously as well as at the site of injury. (13) On the other hand, Saberi et al. (2008) in a similar clinical trial carried out in 4 patients, found improvement in only 1 patient. (14).

Since, direct injection into the spinal cord is a complex process due to the formation of adhesions around the spinal cord as well as shrivelling of the cord post injury, Fukuki Saito et al, (2008) carried out a clinical trial which was aimed at treating a 35-year-old spinal cord injury patient by a novel method of injecting BMSCs into CSF through the lumbar puncture, and assessed the safety and efficacy of the procedure. Post transplantation, the patient did not experience any adverse effect. Whereas, definite improvements were observed in motor and sensory functions up to 6 months. (15)

In 2008, H. Deda et al used autologous bone marrow derived hematopoietic progenitor stem cells to treat 9 patients with chronic complete SCI. All the patients had American Spinal Injury Association (ASIA) Impairment Scale (ASIA) grade A. Post transplantation no patient experienced any complications. Three weeks after the operation all patients' movements and sensations were improved. To evaluate the patients, neurologic impairment scales (ASIA scores), pre- and post-operative Somato Sensorial Evoked Potential (SSEP) assessments and pre- and post-operative Magnetic Resonance Imaging (MRI) were used. All patients had ASIA grade B or C after the operation. Hence, concluded that BM-derived autologous stem cell therapy is effective and safe for the treatment of chronic SCI. (16)

Another interesting study, (Geffner et al, 2008) employed the strategy of administering bone marrow stem cells (BMSCs) via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenous. They studied 8 cases of SCI (four acute, four chronic) with approximately 2 years of follow-up that MRI illustrated morphological changes in the spinal cord of some of the patients following BMSCs administration. Comprehensive evaluations demonstrated improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring. Significant changes in bladder function were observed following BMSCs administration. No tumor formations, no cases of infection or increased pain were recorded. They reported that administration of BMSCs via multiple routes is safe and feasible for several SCI types with an important outcome of improved the quality of life noticed in most patients. (17)

In 2009, Cristante et al. reported the use of peripheral stem cell collected by apheresis in 39 patients of chronic SCI. Cells were delivered intra-arterially into the anterior spinal artery at or near the level of their SCI.SSEP evaluation after 30 months of cell transplantation showed improved latency in 66.7% of patients evaluation. Response rates similar in paraplegic and quadriplegic patients.(18)

Subbaiah et al in 2009 carried out transplantation of autologous cultured bone marrow derived stromal cells (BMSCs) in 5 cases of total transectional spinal cord injury. The report on the available data suggested that it was safe, efficacious and resulted in functional recovery in two patients. (19)

The year 2009 saw more reports of intrathecal transplantation of autologous stem cells appearing in the press, which studied not only the safety but also the efficacy of this route.

Pal et al. reported 30 patients with subacute or chronic SCI who received autologous BMSCs intrathecally, and only incomplete SCI patients (16.7%) were seen to have improved functionally without neurological or electrophysiological improvements (20). While another large-scale clinical trial in India (Kumar et al, 2009), consisting of 297 patients with chronic SCI, treated similarly, reported neurological improvements (32.6%) after a follow up of 3 months.(21)

Callera et al carried out a study wherein 10 patients received their own CD34+ cells labeled with nanoparticles via lumbarpuncture and 6 patients received magnetic beads without stem cells. On follow up the CSF was assessed for presence of cellular components. MRI done 20 and 35 days after transplantation showed that the magnetically labelled CD34+ cells were visible at the lesion site in 5 patients out of 10. These signals were not visible in the control group (22).

The advent of 2010 has seen the emergence of newer sources (cord blood) of adult stem cells, mesenchymal stem cells,(21) re-emergence of olfactory ensheathing cells(23) and a combination of stem cells with various drugs.(24)

T. E Ichim et al reported intrathecal administration of allogeneic umbilical cord blood ex-vivo expanded CD34 and umbilical cord matrix Mesenchymal Stem Cells, performed at 5 months, 8 months, and 14 months after spinal cord injury. Cell administration was found to be well tolerated with no adverse effects . Neuropathic pain subsided from intermittent 10/10 to once a week 3/10 VAS. Recovery of muscle, bowel and sexual function was noted, along with a decrease in ASIA score to "D". (25)

O.S Abdelaziz has presented a trial of 30 patients (5 females and 25 males) having chronic traumatic dorsal spinal cord injury with durations of at least 6 months in which 20 patients were administered autologous adult bone marrow mesenchymal stem cell through open surgical intraparenchymal and intralesional injection into the site of cord injury. The treatment was followed by monthly intrathecal injection of stem cells through lumbar or cisternal punctures. 10 other patients were not treated with stem cells and served as control cases. Clinical improvement was observed in 6 (30%) of 20 patients treated with stem cells transplantation.He reports that short duration of injury and small cord lesions correlated with good outcome. (26)

Lima et al. carried out a clinical trial in Portugal (2010) where in 20 patients (7 paraplegic and 13 tetraplegic) who sustained a traumatic SCI 18 to 189 months previously

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(mean = 49 months) underwent OECs transplantation. They found some neurological, functional, electrophysiological and urodynamic improvements in all the patients after OECs transplantation into the injured spinal cord (23)

A much publicised trial using differentiated embryonic stem cells has been initiated in the US recently. This FDA approved trial, being carried out by Geron Inc., has been the first of the ES cell based trials after the ban on ES cell research was overturned by President Obama. Details of this trial will be further mentioned in Appendix I.

At the NeuroGen Brain and Spine Institute, Mumbai out of 74 spinal cord injury patients who underwent intrathecal autologous bone marrow derived mononuclear cell transplantation, 12 patients shifted on ASIA scale by 2 grades showing improvements in muscle power and gait with assisted devices. On assessing them symptom-wise 48 patients showed improvement in sitting balance (static and dynamic),13 showed improvement in muscle strength, 4 showed complete recovery in bladder and bowel functions, 9 showed sensory recovery and 8 of them showed complete recovery from postural hypotension.(27)

## **Case Report**

### 1. Traumatic L1-L2 paraplegia

#### **Clinical presentation**

35 year old female had a vehicular accident 2 years ago, leading to L1-L2 compression fracture and paraplegia. The fracture was decompressed and fixed with pedicular screw and rod fixation. Neurologically, she was hypotonic and hyporeflexic. She had sensory loss below L2 level. She had grade 3 muscle power in bilateral hip and knee musculature, but Grade 0 muscle power in bilateral foot muscles. She had Grade 5 muscle power in left upper extremity, whereas right upper extremity could not be assessed because of non united and non healing fracture. She did intermittent catheterisation during night and emptied with increased intraabdominal pressure voluntarily, during the day. **On ASIA scale, her score was "A".** Functionally, she was dependent on the caregiver for all her activities of daily living (ADL). She was wheel chair bound for mobility as well as dependent for transfers. **On FIM scale, she scored 53.** 



Lateral X-ray of the lumbar spine reveals wedge compression of L1 vertebral body with fixation device in-situ

Sagittal T2W image through the dorso-lumbar spine reveal wedge compression fracture of L1 vertebral body with myelomalacic changes noted in the terminal cord and conus medullaris in a post-traumatic operated spine.

#### Clinical Improvement after stem cell therapy

Functionally she could independently roll on bed ,sit up from lying position, shift on the edge of the bed, stand up from wheel chair and transfer to the bed back .On MMT her knee flexors improved in strength from Grade 3 to 3++. **Her FIM score improved from 53 to 66** with independence seen in Upper body dressing , Lower body dressing, gait and stair case climbing.

She started to walk independently with the help of bilateral AFO and single elbow crutch and could climb stairs with one hand railing support. Her bilateral feet muscles started to show flicker of contraction , on attempting voluntary movement. **On ASIA scale she shifted from Grade A to B.** (*for pictures, refer to color plate.*)
# **Case Report 1**



Figure 1: Patient showing improved lower extremity strength and ability to climb stairs with assistance holding a railing and bil AFO and HKAFO.



Figure 2: Patient walking with a walker and bilateral AFO, showing improvement in her trunk stability and lower limb strength.

# **Case Report 2**



*Figure 3: Patient showing improvement in lower extremity strength and ability to walk with one person's assisistance.* 



*Figure 4 : Patient showing improvement in hand function, grip and pinch strength.* 

# 2. Traumatic C3-C4 Quadreiplegia.

#### **Clinical Presentation**

A 60 year old female suffered C3-C4 vertebrae fracture and quadriplegia, 6 years ago due to a fall in the bathroom. She was operated and spinal fixation was done for the same. Since then, she had been on continuous rehabilitation. She showed residual left sided weakness. More than right side. Neurologically, she was hypertonic with spasticity of Grade 2, according to modified Ashworth Scale, she was hyperreflexic. She had Grade 3 muscle power in right upper extremity and Grade 2 in left upper extremity. She had Grade 2 muscle power in right lower extremity and Grade 1 in left lower extremity, she had intact sensations except temperature and vibration sensation below C4 level neck. She did intermittent catheterisation every 3 hours. **On ASIA Scale she scored Grade B**. Functionally, she was dependent on a caregiver for all her ADL except eating.



Sagittal T1W image through the cervical spine reveals near complete transection of cord due to retro pulsed fractured fragment of C3/C4 vertebral body.

## Clinical Improvement after stem cell therapy

Functionally, her stamina improved tremendously. She started appreciating temperature sensation in right upper extremity. She also showed improved strength in right upper extremity with improved grasp and release of right hand. She could use her index finger and thumb for holding objects. Her gait improved, she could walk with one person's assistance and elbow crutches, with adequate foot clearance by left feet. She showed improved endurance and ability to sustain standing for half an hour continuously which was not possible before. She had also learnt to climb stairs with one railing support and a person's assistance. On ASIA scale she shifted from B to C. (for pictures, refer to color plate.)

# Summary

Spinal cord injury, generally leads to irreversible damage. This understanding has held forth for decades together. However, the discovery, that there are stem cell niches in the adult nervous system has lead to rethinking of this concept. Since the discovery by McDonald that stem cells can be used to generate neurons, the field of regenerative medicine has embraced the nervous system too. Exploring the options for repairing the spinal cord injury using various types of stem cells (embryonic, olfactory ensheathing, haematopoietic, mesenchymal, etc.) has become the life mission of various researchers and neurosurgeons. There have been encouraging results with many of these and more work is happening along these lines.

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# 9

# Role Of Stem Cells In Muscular Dystrophy

Muscular dystrophy (MD) refers to a group of hereditary muscle diseases that weaken the muscles which move the human body. Muscular dystrophies are characterized by defects in muscle proteins, and the death of muscle cells and tissue and progressive skeletal muscle weakness.

Most types of MD are multi-system disorders with manifestations in body systems including the heart, endocrine glands, skin, eyes, gastrointestinal and nervous systems. The condition may also lead to mood swings and learning difficulties. (1)

Muscular dystrophy is usually broadly classified into nine types including Duchenne, Becker, limb girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal and Emery-Dreifuss but there are more than 100 diseases with similarities to muscular dystrophy. (2)



Axial and Coronal T1W images reveal extensive fatty infiltration of the gluteus muscles bilaterally

#### Duchenne muscular dystrophy (DMD)

It is a severe recessive X-linked form of muscular dystrophy characterized by rapid progression of muscle degeneration, caused by a mutation in the dystrophin gene, located in humans on the X chromosome (Xp21). The dystrophin gene codes for the protein dystrophin, an important structural component within muscle tissue. Dystrophin provides structural stability to the dystroglycan complex (DGC), located on the cell membrane. The absence of dystrophin permits excess calcium to penetrate the sarcolemma (cell membrane). In a complex cascading process that involves several pathways and is not clearly understood, increased oxidative stress within the cell damages the sarcolemma, and eventually results in the death of the cell. Muscle fibers undergo necrosis and are ultimately replaced with adipose and connective tissue. (3)

#### Symptoms:

Frequent falls, fatigue, weakness of all antigravity muscles, Equinnus gait, Waddling gait (increased lumbar lordosis), swelling(pseudohypertrophy) of calf muscles, Muscle contractures of achilles tendon and hamstrings impair functionality because the muscle fibers shorten and fibrosis occurs in connective tissue.

#### Signs and tests:

Muscle wasting begins in the legs and pelvis, then progresses to the muscles of the shoulders and neck, followed by loss of arm muscles and respiratory muscles. Calf muscle enlargement (pseudohypertrophy) and a positive Gowers' sign is commonly observed. Cardiomyopathy (DCM) is common, but the development of congestive heart failure or arrhythmias (irregular heartbeats) is only occasional. Affected children usually tire more easily and have less overall strength than their peers.

#### **Becker muscular dystrophy**

It is also known as benign pseudohypertrophic muscular dystrophy which is an Xlinked recessive inherited disorder characterized by slowly progressive muscle weakness of limbs. (3)

#### Symptoms:

The pattern of symptom development resembles that of Duchenne muscular dystrophy, but with a later, and much slower rate of progression.

#### Signs:

Noticeable signs of muscular dystrophy also include the lack of pectoral and upper arm muscles, especially when the disease is unnoticed through the early teen years (some men are not diagnosed with BMD until they are in their thirties). Muscle wasting begins in the legs and pelvis (or core), then progresses to the muscles of the shoulders and neck, followed by loss of arm muscles and respiratory muscles. Calf muscle enlargement (pseudohypertrophy) is quite obvious. Cardiomyopathy may occur, but the development of congestive heart failure or arrhythmias (irregular heartbeats) is rare.

#### Limb-girdle muscular dystrophy (LGMD)

It is also known as Erb's muscular dystrophy which is an autosomal class of muscular dystrophy. The term "limb-girdle" is used to describe these disorders because the muscles most severely affected are generally those of the pelvic and shoulder girdle muscles. LGMDs transmitted by autosomal dominant inheritance are designated as LGMD type 1, and those transmitted by autosomal recessive inheritance are designated as LGMD type 2. The autosomal dominant forms tend to be less severe than the autosomal recessive. The dominant forms of LGMD can arise by a new mutation in the affected person. An autosomal dominant form (LGMD1A) has been mapped at 5q 22.3-31. (3)

#### Symptoms:

Muscle weakness, myoglobinuria, pain, myotonia, cardiomyopathy, elevated serum CK, and rippling muscles. The muscle weakness is generally symmetric, proximal, and slowly progressive.

#### Facioscapulohumeral muscular dystrophy (FSHMD, FSHD or FSH)

Also known as Landouzy-Dejerine, is a usually autosomal dominant inherited form of muscular dystrophy that initially affects the skeletal muscles of the face (facio), scapula (scapulo) and upper arms (humeral). More than 95% of FSHMD cases are known to occur due to deletion in FSHMD1A gene (4q35 deletion) on chromosome 4.(3)

#### Congenital muscular dystrophy (CMD)

It is the term used to describe muscular dystrophy that is present at birth. CMD describes a number of autosomal recessive diseases of muscle weakness and possible joint deformities, present at birth and slowly progressing. Life expectancies for affected individuals vary, although some forms of CMD do not affect life span at all. All such known dystrophies are genetically recessive and result from mutations in a variety of different genes, including those encoding the laminin 2 chain, fukutin-related protein. (3)

#### Diagnosis

Diagnosis of muscular dystrophy is based upon a combination of a characteristic clinical presentation, immunohistochemistry tests, muscle biopsy, etc. The level of Creatine kinase (CPK-MM) in the blood is found to be extremely high. An electromyography (EMG) shows myopathy. A muscle biopsy (immunohistochemistry or immunoblotting) or genetic test (blood test) confirms the absence of dystrophin. Genetic tests also demonstrates the various deletions depending on the type of MD such as that of sarcoglycan, dysferlin, etc

#### **Conventional treatment**

There is no known cure for muscular dystrophy, Treatment is generally aimed at controlling the onset of symptoms to maximize the quality of life, and include the following:

- 1. Corticosteroids such as prednisolone and deflazacort increase energy and strength and defer severity of some symptoms.
- 2. Mild, non-jarring physical activity such as swimming is encouraged. Inactivity (such as bed rest) can worsen the muscle disease.
- 3. Physical therapy is helpful to maintain muscle strength, flexibility, and function.
- 4. Orthopedic appliances (such as braces and wheelchairs) may improve mobility and the ability for self-care. Form-fitting removable leg braces that hold the ankle in place during sleep can defer the onset of contractures.
- 5. Appropriate respiratory support as the disease progresses is important. (1,2)

# Summary of current clinical evidence of the role of stem cells in Muscular Dystrophy

Cell therapy has evolved as one of the promising treatments for Muscular dystrophy. Encouraging and pioneering experiments in mouse models for various muscular dystrophies demonstrated that myoblasts could be transplanted into dystrophic muscle; these myoblasts repaired a small proportion of damaged myofibres. Subsequent work has been devoted to optimisation of this technique. In doing so, a number of adultderived stem cells have been isolated, including bone marrow-derived stem cells, bloodand muscle-derived CD133+ cells, muscle-derived stem cells (MDSC), side population (SP) cells and mesoangioblasts [4-9]. These cells have been characterized and used in animal transplantation experiments. Further research is ongoing, and is clearly necessary to make this therapy a viable treatment option for patients with muscular dystrophy. (10)

Huard et al (1992) transplanted myoblasts from immunocompatible donors into the muscles (tibialis anterior, biceps brachii, and/or extensor carpi radialis longus) of 4 Duchenne patients in the advanced stages of the disease. Although no immunosuppressive treatment was used, none of the patients showed any clinical signs of rejection such as fever, redness, and inflammation. One patient transiently produced antibodies against the donor myoblasts as determined by cytofluorometric analysis. This patient and 2 others were shown to form antibodies against their donor's myotubes. Muscle biopsies of the injected tibialis anterior of 4 patients revealed that 80%, 75%, 25%, and 0% of the muscle fibers, respectively and showed some degree of dystrophin immunostaining. The contralateral non-injected muscles of the latter 3 patients did not contain any dystrophin positive fibers, while that of the first patient showed dystrophin expression in 16% of the fibers examined. Myoblasts were also injected into the extensor carpi radialis longus or the biceps brachii of these patients. A few months subsequent to injection, one patient was shown to have a 143% increase of strength during static wrist extension. A double-blind strength-measuring protocol was not used. Furthermore, they noted that this change slowly decayed over time. The strength of 2 other patients was increased less remarkably (41% and 51%), while the strength of the fourth patient was unchanged.(11)

Gussoni et al (1992) aimed to demonstrate that the transplanted myoblasts persist

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#### Role Of Stem Cells In Muscular Dystrophy

and produce dystrophin in muscle fibres of DMD patients. To test this possibility, they transplanted normal myoblasts from a father or an unaffected sibling into the muscle of eight boys with DMD, and assessed their production of dystrophin. Three patients with deletions in the dystrophin gene expressed normal dystrophin transcripts in muscle biopsy specimens taken from the transplant site one month after myoblast injection. Using the polymerase chain reaction it was established that the dystrophin in the biopsies were derived from donor myoblast DNA.(12) In 1997, the same group demonstrated the fate of individual myoblasts after transplantation into muscles of DMD patients. Muscle biopsies from six patients with Duchenne muscular dystrophy (DMD) participating in a myoblast transplantation clinical trial were re-examined using a fluorescence in situ hybridization (FISH)-based method. Donor nuclei were detected in all biopsies analyzed, including nine where no donor myoblasts were previously thought to be present. In three patients, more than 10% of the original number of donor cells were calculated as present 6 months after implantation. Half of the detected donor nuclei were fused into host myofibers, and of these, nearly 50% produced dystrophin. Their findings demonstrated that although donor myoblasts persisted after injection, their microenvironment influenced whether they fuse and express dystrophin.(13) In 2002, they further reported the analysis of muscle biopsies from a DMD patient (DMD-BMT1) who received bone marrow transplantation at age 1 year for X-linked severe combined immune deficiency and who was diagnosed with DMD at age 12 years. Analysis of muscle biopsies from DMD-BMT1 revealed the presence of donor nuclei within a small number of muscle myofibers (0.5-0.9%). The majority of the myofibers produced a truncated, in-frame isoform of dystrophin lacking exons 44 and 45 (not wild-type). The presence of bone marrow-derived donor nuclei in the muscle of this patient documented the ability of exogenous human bone marrow cells to fuse into skeletal muscle and persist up to 13 years after transplantation. (14)

Karpati et al (1993) injected one biceps muscle of 8 patients with Duchenne muscular dystrophy at 55 sites with a total of 55 million viable, purified, and contamination-free normal myoblasts (myoblast transfer). The other biceps of each patient was injected with a placebo to serve as a control. The procedure was blinded to the patients, parents, and investigators. Myoblasts derived from a biopsy specimen of the fathers were cultured and purified under strict conditions and carefully screened for microbial contamination. All patients received cyclophosphamide for immunosuppression for 6 or 12 months. No serious complications were observed after myoblast transfer, indicating that the procedure is safe. The overall therapeutic efficiency of myoblast transfer was poor as judged by the results in maximal voluntary force generation, dystrophin content of the muscle, magnetic resonance imaging of the muscle. The authors concluded that an improved efficiency of the take of myoblasts might be achieved by using younger cells and injecting the myoblasts with a myonecrotic agent (to increase the prevalence of regeneration) and a basal laminal fenestrating agent.(15)

Mendell et al report injection of donor muscle cells once a month for six months to the biceps brachii muscles of one arm of each of 12 boys with Duchenne's muscular dystrophy. The opposite arms served as sham-injected controls. In each procedure 110 million cells donated by fathers or brothers were transferred. The patients were randomly assigned to receive either cyclosporine or placebo. Strength was measured by quantitative isometric muscle testing. Six months after the final myoblast transfer, the presence of dystrophin was assessed with the use of peptide antibodies specific to the deleted exons of the dystrophin gene. There was no significant difference in muscle strength between arms injected with myoblasts and sham-injected arms. In one patient, 10.3 percent of muscle fibers expressed donor-derived dystrophin after myoblast transfer. Three other patients also had a low level of donor dystrophin (< 1 percent); eight had none. The authors concluded that myoblasts transferred once a month for six months failed to improve strength in patients with Duchenne's muscular dystrophy.(16)

Tremblay et al (1993) studied the effects of myoblast transplantations without an immunosuppressive treatment on muscle strength, and the formation of dystrophinpositive fibers was studied in five young boys with Duchenne muscular dystrophy (DMD) using a triple blind design. Injections of myoblasts were made into one biceps brachii (BB), and the opposite BB, used as a control, was sham-injected; the experimenters and the patient were blind to the myoblast-injected side. At the same time, myoblasts were also injected in the left tibialis anterior (TA) of these patients. The strength developed during maximal static contractions of the elbow flexor and extensor muscles was measured with a Kin-Com dynamometer. No increase in static elbow flexion torque was measured at any time from 2 mo up to 18 mo after the transplantation. One month after the transplantation, the percentage of dystrophin-positive fibers in the myoblastinjected TA ranged from 0 to 36%, while it ranged from 0 to 4% on the control side. The expression of dystrophin in these fibers, however, was generally low, and most likely less than 10% of the normal level. In the biceps brachii of both sides 6 mo after the transplantation, less than 1.5% of dystrophin-positive fibers were detected. The injections also triggered a humoral immune response of the host. Antibodies were capable of fixing the complement, and of lysing the newly formed myotubes. One of the antigens recognized by this immune response is possibly dystrophin. These results strongly suggested that myoblast transplantations, as well as gene therapy for DMD, cannot be done without immunosuppression.(17)

Neumeyer et al (1998) evaluated myoblast implantation therapy in three subjects with Becker muscular dystrophy who received 60 million myoblasts in one tibialis anterior (TA) muscle 2 months after beginning cyclosporine immunosuppression (5 to 10 mg/kg) that continued for 1 year. Strength of the implanted and control TA muscles was measured before and after treatment using a gauge to record TA contraction force. The protocol controlled for the effects of cyclosporine and myoblast injections. In this pilot study, myoblast implantation did not improve strength of the implanted TA muscles.(18) Vilquin et al (2003) evaluated the feasibility of this concept, they explored and compared the growth and differentiation characteristics of myoblasts prepared from phenotypically unaffected muscles of five FSHD patients and 10 control donors. According to a clinically approved procedure, 109 cells of a high degree of purity were obtained within 16-23 days. More than 80% of these cells were myoblasts, as

demonstrated by labeling of the muscle markers CD56 and desmin. FSHD myoblasts presented a doubling time equivalent to that of control cells; they kept high proliferation ability and did not show early telomere shortening. In vitro, these cells were able to differentiate and to express muscle-specific antigens. In vivo, they participated to muscle structures when injected into immunodeficient mice. These data suggest that myoblasts expanded from unaffected FSHD muscles may be suitable tools in view of autologous cell transplantation clinical trials.(19)

Tremblay et al (2003) carried out a study on monozygotic twin girls, both carriers of Duchenne muscular dystrophy, only one a severe symptomatic carrier and the other asymptomatic due to opposite lyonization. Myoblast clones were obtained from a muscle biopsy of the asymptomatic carrier. PCR amplification showed that most (94%) of these clones produced normal dystrophin mRNA. Roughly 704 million myoblasts were produced from 119 clones. These myoblasts were transplanted into the extensor carpi radialis (ECR) and in the biceps of one arm of the manifesting carrier while the other arm acted as the control. The strength of the patient was evaluated in a series of preand post-tests and a biopsy was obtained about 1 yr after the transplantation. The myoblast injections produced a significant force gain (12%-31%) in wrist extension but no force gain for elbow flexion. Muscle biopsies on the injected and control muscles obtained 1 yr after the injections showed only a small increase in the number of dystrophin positive fibers and the presence of numerous small type II fibers. The small beneficial effect of this transplantation cannot be attributed to immune problems, the donor and the recipient being identical twins, but may be due to a low level of spontaneous muscle regeneration.(20)

Skuk et al (2004) carried out a trial on three Duchenne muscular dystrophy (DMD) patients who received injections of myogenic cells obtained from skeletal muscle biopsies of normal donors. The cells (30 x106) were injected in 1 cm3 of the tibialis anterior by 25 parallel injections. They performed similar patterns of saline injections in the contralateral muscles as controls. The patients received tacrolimus for immunosuppression. Muscle biopsies were performed at the injected sites 4 weeks later. They observed dystrophinpositive myofibers in the cell-grafted sites amounting to 9 (patient 1), 6.8 (patient 2), and 11% (patient 3). Since patients 1 and 2 had identified dystrophin-gene deletions these results were obtained using monoclonal antibodies specific to epitopes coded by the deleted exons. Donor dystrophin was absent in the control sites. Patient 3 had exon duplication and thus specific donor-dystrophin detection was not possible. However, there were four-fold more dystrophin-positive myofibers in the cell-grafted than in the control site. Donor-dystrophin transcripts were detected by RT-PCR (using primers reacting with a sequence in the deleted exons) only in the cell-grafted sites in patients 1 and 2. Dystrophin transcripts were more abundant in the cell-grafted than in the control site in patient 3. They concluded that significant dystrophin expression could be obtained in the skeletal muscles of DMD patients following specific conditions of cell delivery and immunosuppression.(21)

Though transplantation of myoblasts can enable transient delivery of dystrophin and improve the strength of injected dystrophic muscle, this approach was seen to have various limitations, including immune rejection, poor cellular survival rates, and the limited spread of the injected cells. It was thought that isolation of muscle cells that could overcome these limitations would enhance the success of myoblast transplantation significantly. The efficiency of cell transplantation could potentially be improved through the use of stem cells, which display unique features, including (1) self-renewal with production of progeny, (2) appearance early in development and persistence throughout life, and (3) long-term proliferation and multipotency. The development of muscle stem cells for use in transplantation as treatment for patients with muscle disorders was thought to be an attractive proposition in the early 2000s.

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

Zhang et al (2005) studied the feasibility of treatment of Duchenne muscular dystrophy (DMD) with umbilical cord stem cell transplantation. HLA matching was conducted for a 11-year-old DMD boy with family history was underwent umbilical cord blood stem cell transplantation and a sample of umbilical cord stem cells with 5 matched HLA sites was found in the cord blood bank with 27.32 x 10(8) nucleated cells, about 2.6 times that of the treatment dosage for him. After pretreatment with busulfan 14 mg/kg.d, cyclophosphamide 50 mg/kg.d, and rabbit anti-human thymocyte globulin 10 mg/kg.d, the allogeneic cord blood stem cells were transplanted intravenously. Cyclosporin A, methylprednisolone and MMF were used after the transplantation so as to prevent graft versus host reaction. Prostaglandin E1 was used to prevent Budd-Chiari syndrome, and ganciclovir was used to prevent cytomegalovirus infection. At the same time, Gran, granulocytic cell stimulating factor, and gammaglobulin were also used. Biochemistry test, including serum creatine kinase (CK), was conducted. Evidence of reconstruction of blood making, including conversion of blood type, was observed. PCR-STR analysis was used to observe the status of implantation of the donor umbilical cord blood stem cells.(1) 12 days after transplantation, the white blood cells (WBC) of peripheral blood were  $0.5 \times 10(9)/L$ , 14 days after, the numbers of WBC and neutrophils were  $1.0 \times 10(9)/L$  and  $0.6 \times 10(9)/L$  respectively. In 37 days, granulocytic cell stimulating factor was no more used, the peripheral blood WBC fluctuated around 3.34 approximately 12.2 x 10(9)/L. In the 27th day, the number of blood platelets was more than 20 x 10(9)/L and hemoglobin rose to 88 g/L. On the 24th day red blood cells transfusion was stopped. (2) In the 42nd day, the blood type of the patient transformed from type A before transplantation to type AB (the blood type of transplanted stem cells is type B). (3) PCR-STR test of the peripheral blood made 17, 26, and 42 days after transplantation showed that the gene type of the patient was mixed mosaic: The ratio of donor gradually increased from 40% approximately 45% to 55% approximately 65%. (4) In the 38th day I degrees GVHD appeared. (5) serum CK level declined from 6000 U/L to 600 approximately 2200 U/L. (6) In the 42nd day, physical examination revealed obviously improvement in walking, turning the body over, and standing up. This was the first case of prospective clinical transplantation on DMD by allogeneic cord blood

stem cell. Umbilical cord stem cell transplantation helps re-build blood-making function, and improve locomotive function with a mild GVHD reaction. The genotype of rebuilt blood is mosaic but the ratio of gene mosaic gradually turn from recipient gene type > donor gene type to recipient gene type < donor gene type. The serum CK level decreases significantly after transplantation, which may slow down the necrosis of muscle cell. The study concluded that the DMD patients could be benefited by stem cell transplantation.(22)

Torrente et al (2007) tested the safety of autologous transplantation of musclederived CD133+ cells in eight boys with Duchenne muscular dystrophy in a 7-month, double-blind phase I clinical trial. Stem cell safety was tested by measuring muscle strength and evaluating muscle structures with MRI and histological analysis. Timed cardiac and pulmonary function tests were secondary outcome measures. No local or systemic side effects were observed in all treated DMD patients. Treated patients had an increased ratio of capillary per muscle fibers with a switch from slow to fast myosinpositive myofibers. (23)

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). A total of 82 cases were treated by the double transplantations of BMSC and CB-MSC. They were diagnosed by clinical manifestations, CK, LDH, genetic analysis, electromyography, MRI and pathologic examination of biopsied muscle specimens from July 2007 to July 2008. Control group was self-made at before and after treatment and cases were followed up for 3 - 12 months. Eighty-two patients underwent the double transplantations of bone mesenchymal stem cell (BMSC) and human umbilical cord blood MSC (CB-MSC). (1) BMSC: 80 - 150 ml bone marrow sample was collected through a puncture at bilateral posterior superior iliac spine. Ficoll density gradient centrifuge was employed to separate individual monocyte for induced differentiation. (2) CB-MSC: 80 - 160 ml umbilical cord blood was harvested and processed likewise as above. (3) Stem cell transplantation: Both BMSC and CB-MSC were collected and prepared into 1 x 10(8)/ml and  $1 \times 10(7)$ /ml cell suspension respectively. They were transplanted in divided dose into the extremity muscle and vein. The clinical and laboratory parameters were monitored at 3, 6, 9 and 12 months. It was found that 31 cases (37.8%) obtained a remarkable efficacy, 37 cases (45.1%) were effective and 14 cases (17.1%) had no change. Total effective rate was 82.9%. Seventy patients (85.4%) felt limbs warmly, appetite improved, gained weight, had better appetite and action were nimble. Activity of daily living scale (ADL) in 72 patients (87.8%) increased as compared with pre-treatment (P < 0.01). LDH decreased at post-treatment [(475 + - 223) u/L vs (410 + - 216) u/L, P < 0.05, t = 6.650]. Creatine kinase [(2952 +/- 2259) u/L vs (2841 +/- 2092) u/L, P = 0.223, t = 1.094] and creatine [(26 +/- 12) micromol/L vs (25 +/- 11) micromol/L, P = 0.306, t = 1.029] decreased slightly. Adherence to therapy among children and no adverse reaction was reported during the course of treatment. The authors concluded that the double transplantation of BMSC and CB-MSC was convenient, safe and effective in the treatment of progressive muscular dystrophy and could be considered as a new therapy of PMD.

MSC represents a possible tool of cellular therapeutics for PMD. (24)

Looking at these recent upcoming publications, the trend in muscular dystrophy appears to be towards adult stem cells, which are finding their niche in the overall development of stem cell therapy for a whole range of diseases.

At the NeuroGen Brain & Spine Institute, Mumbai out of 72 muscular dystrophy patients who underwent intrathecal autologous bone marrow derived mononuclear cell transplantation, 41 were suffering from Duchene Muscular Dystrophy type, 17 had Limb Girdle Muscular Dystrophy, 11 had Congenital Muscular Dystrophy, 2 had Becker's Muscular Dystrophy and 1 had Fascio Scapulohumeral Dystrophy. The mean follow up of 6 months showed that out of 72, 32 showed improved trunk strength, 30 improved in their lower extremity strength with 11 of them showing gait improvement and 20 improved in upper extremity strength.

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# **Case Report**

## 1. Duchenne's Muscular Dystrophy

#### **Clinical presentation**

18 year old male, known case of DMD since the age of 1 year. He was diagnosed because of family history and increased CPK. He then started developing bilateral lower limb weakness with difficulty in walking and used to walk on toes. He could walk till 15 years of age. Meanwhile upper extremity weakness also developed with difficulty in overhead activities.Neurologically, he was hypotonic and hyporeflexic. He had all sensations intact. He had grade 1 strength in bilateral upper limb and lower limb proximally and grade 3 distally in all 4 limbs. On examination, he had right sided Scoliosis. He was cachexic with poor chest expansion and history of repeated Lower Respiratory Tract Infection. On investigation, increased CPK, EMG and MRI Musculoskeletal system confirmed diagnosis of DMD.Functionally, he was dependent for ADL and wheelchair bound for mobility. **On FIM he scored 63**. On Brooke & Vignos Scale he scored 6 for upper extremities & 10 for lower extremities.



X-Ray Spine showing severe scoliosis

#### **Clinical Improvement after stem cell therapy:**

Functionally he reported that his grip strength had improved significantly. His neck control and movements had become better than before. His sitting balance and sitting tolerance had improved. His posture became more erect. He could stand on balance board with push knee splints for 15-20 minutes. Also the caretaker felt that lifting and transferring him became easier and while standing he could balance his trunk better. On Manual Muscle Testing, hip knee & shoulder girdle muscles have increased in strength from grade 0 to grade 1++.

Radiological improvement: On MRI repeated after  $3\frac{1}{2}$  months (31/8/2010) following changes were seen when compared with the previous one (3/5/10) :





Figure 1: Patient showing improved neck holding and ability to hold neck erect while standing.

**Case Report 2** 



Figure 1: Patient showing ability to stand independently in the parallel bars with HKAFO and balance.

#### Role Of Stem Cells In Muscular Dystrophy

Improvement in the degree of fatty infiltration with a minimal possible muscle regeneration was reported in the vastus medialis, vastus lateralis and semi tendinosus muscle in the thigh. Similar improvements were also noted in the tibialis anterior, medial and lateral head of gastrocnemius muscle in the leg. In the arm, improvement was noted in the long and lateral head of triceps muscle and biceps brachii muscle. The above clinical improvements and changes seen on MRI suggest regeneration of muscles following Stem Cell Therapy.



Pre and Post stem cell therapy Axial T1W images through the thigh muscles reveals improvement in the grade of fatty infiltration with minimal possible muscle regeneration in the vastus medials and lateralis



*Pre(A) and Post stem cell (B) therapy Axial T1W images through the hamstring muscle reveals improvement in the grade of fatty infiltration with minimal possible muscle regeneration in the semtendinosus* 

## 2. Duchenne's Muscular Dystrophy

#### **Clinical Presentation:**

11year old male, known case of DMD since 2005. Symptoms noticed were bilateral lower limb weakness and difficulty in getting up from floor, climbing stairs and walking. Gradually, weakness progressed. He could manage to walk till November 2009. Neurologically, he was hypotonic and hyporeflexic. He had all sensations intact. He had grade 2+ strength in bilateral lower limb proximally and grade 3 distally. He had grade 3 strength in bilateral upper limb proximally and grade 3++ distally. On examination, he had (i) Bilateral pseudohypertrophy of calf, (ii) Bilateral Genu Valgum and (iii) Bilateral T.E.V. On Investigation Genetic testing revealed: Deletions in Exon 8 through 11 within dystrophin gene.MRI Musculoskeletal system (upper limbs & lower limbs) revealed marked fatty infiltration with volume loss of predominantly the pelvic girdle muscles involving the glutei, posterior thigh muscles, adductors and vasti. There was relative sparing of the sartorius, the gracilis, adductor longus and semitendinosis muscles. There was partial fatty infiltration of the leg muscles with sparing of the extensor compartment (tibialis anterior extensor hallucis longus and extensor digitorum longus)seen. There was partial fatty infiltration involving the proximal fibers of the biceps.Rest of the arm and forearm muscles were spared. EMG study showed an excess of 'myopathic' potentials at all sites of examination. Increased CPK, EMG, MRI



Axial and Coronal T1W images reveal moderate fatty infiltration of the medial head of gastrocnemius(red arrow) with moth-eaten appearance noted in the lateral head (green arrow) and soleus(blue arrow). The tibialis anterior muscle (white) appears to be relatively spared

musculoskeletal system and genetic tests confirm diagnosis of DMD. Functionally, he needed assistance for all his ADL. He was studying, but was wheelchair bound for mobility. On FIM he scored 85. On Brooke & Vignos Scale he scored 4 for upper extremity and 9 for lower extremity.

#### Clinical Improvements seen after stem cell therapy

Functionally, his stamina to exercise had improved. He reported that his leg muscles had increased strength, mainly knees (L >> R) as they did not buckle now easily, as before.

He could walk about 80 steps with the help of walker and splints, which he had stopped in November 2009. His knee and ankle appeared to be more straighter than before. He had started showing toning of muscle with weight loss. Mother also reported that his calf muscles had become softer now, as compared to before. His trunk control had improved. He could now sit on edge of cot, bend and pick up objects with improved reach outs without losing balance. He could smoothly perform overhead activities with weights as well, which were difficult for him earlier. He could walk independently, after putting on calipers without any assistive device, for about 15-20 steps at a time. His reflexes and reaction time to commands had improved. His FIM Score improved from 85 to 90.On Brooke & Vignos Scale his upper extremity shifted from 4 to 3 & lower extremity improved from 9 to 7.

On Manual Muscle Testing following changes were seen:Hip girdle muscle improved from grade 1+ to 2++ bilaterally, Knee extensors improved from grade 2 to 3 -, Shoulder girdle improved from garde 3 to 3++, with trunk muscle (abdominals & back extensors) from grade grade 2 to 3++.

Role Of Stem Cells In Muscular Dystrophy



Six members of the same family afflicted with Duchenne Muscular Dystrophy.



An awareness symposium held for hundreds of families afflicted with Muscular Dystrophy at Coimbatore.

# Summary

Muscular dystrophy (MD) refers to a group of hereditary muscle diseases that lead to progressive weakening of the muscles. Muscular dystrophies are characterized by defects in muscle proteins, and the death of muscle cells and tissue and progressive skeletal muscle weakness. Muscular dystrophy is usually broadly classified into nine types including Duchenne, Becker, limb girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal and Emery-Dreifuss but there are more than 100 diseases with similarities to muscular dystrophy. Cell therapy has evolved as one of the promising treatments for Muscular dystrophy.Types of cell therapy explored ranges from myoblasts to mesangioblasts to adult haematopoietic and mesenchymal stem cells. Various studies have established safety of these cells and efficacy appears to be promising in improving the quality of life.The final frontier for definitve therapy would of course be gene therapy.

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

# 10

# **Role Of Stem Cells In Multiple Sclerosis**

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating disease of the Central Nervous System (CNS). It affects largely young adults between the ages of 20 & 40, & is often referred to as the "great crippler of young adults". It was Dr. Jean Charcot in 1868 who defined the disease by its clinical & pathological characteristic: paralysis & the cardinal symptoms of intention tremor, scanning speech & nystagmus later termed Charcot's Traid. Using autopsy studies he identified areas of hardened plaques & termed the disease sclerosis in plaques.

Sensory Symptoms:	Hypoesthesia, numbness, Paraesthesia
Motor Symptoms :	Weakness or Paralysis
	Fatigue
	Spaticity
	Incoordination
	Intentional Tremor
	Impaired Balance
	Gait Disturbances.
Pain:	Dysesthesias
	Optic or trigeminal Neuritis
	Lhermitte's Sign :((Tingling in spine with limbs on neck flexion.)

## **Common Symptoms In Multiple Sclerosis: (1)**

	Chronic Pain.
Visual Symptoms:	Blurred or double vision
	Diminished acuity or loss of vision
	Scotoma
	Nystagmus.
Bladder Symptoms:	Urinary Urgency, Frequency
	Nocturia
	Incontinence
	Urinary Hesitancy, Dribbling
Bowel Symptoms:	Constipation
	Diarrhoea
	Incontinence
Speech & Swallowing:	Dysarthria
	Diminshed verbal Fluency
	Dysphonia
	Dysphagia
Cognitive Symptoms:	Memory or recall problems
	Decreased attention , concentration
	Diminished abstract reasoning
	Diminished problem solving, judgment.
	Diminished speed of information processing.
	Diminished visual -Spatial abilities.
Emotional Symptoms:	Depression
	Pseudo bulbar Affect
	Anxiety
Cardiovascular Dysautonomia	Very rare.

# Pathophysiology:

In patients with MS, the immune response triggers the production of T-Lymphocytes, macrophages, & immunoglobulins (antibodies). In turn, a command or trigger protein, the antigen is activated, producing autoimmune cytotoxic effects within the CNS.

#### Role Of Stem Cells In Multiple Sclerosis

The blood brain barrier fails & myelin sensitized T - Lymphocyte cells enter & attacks the myelin sheath that surrounds the nerve. Myelin serves as an insulator, speeding up the conduction along nerve fibres from one node of ranvier to another termed saltatory conduction. It also serves to conserve energy for the nerve because depolarization occurs only at the nodes. Disruption of myelin sheath produces active demyelination, slowing neural transmission & causing nerves to fatigue rapidly. With severe disruption, conduction block occurs with resulting disruption of function.



Sagittal T2W image reveals multiple hyperintense lesion perpendicular to the calloso-septal surface (Dawson's fingers) consistent with Multiple Sclerosis

## **Definitions & Terminology Used to Describe Categories of MS:**

- 1. Relapsing Remitting MS (RRMS): Characterized by relapses with either full recovery or some remaining neurological signs / symptoms & residual deficit upon recovery; the period between relapses are characterized by lack of disease progression.
- 2. Primary Progressive MS (PPMS): Characterized by disease progression from onset without plateaus or remissions or with occasional plateaus & temporary minor improvements.
- 3. Secondary Progressive MS (SPMS): Characterized by initial relapsing remitting course, followed by progression at a variable rate that may also include occasional relapses and minor remissions.
- 4. Progressive Relapsing MS (PRMS): Characterized by progressive disease from onset but without clear acute relapses that may or may not have some recovery or remission commonly seen in people who develop the disease after 40 years of age.
- 5. Benign MS: Characterized by mild disease in which patients remain fully functional in all neurological systems 15 years after disease onset.
- 6. Malignant MS (Marburg's variant ): Characterized by rapid progression leading to significant disability or death within a relatively short time after onset.

Local inflammation, edema & infiltrates around the acute lesion can cause a mass effect (abnormally high pressure), further interfering with conductivity of the nerve fibre. Conceivably, this inflammation (which gradually subsides) may in part, account for the pattern of fluctuations in function that characterize this disease. During the early stages of MS, oligodendrocytes (myelin producing cells) survive the initial insult & can produce remyelination. This process is often incomplete & as the disease becomes more chronic, stalls altogether. Eventually, the oligodendrocytes become involved & myelin repair cannot occur. Demyelinated areas eventually become filled with fibrous astrocytes & undergo gliosis. At this stage , the axon itself becomes interrupted & undergoes retrograde degeneration (dying axonopathy). In advanced cases there are both acute & chronic lesions of varying size scattered throughout the CNS (brain, brainstem, cerebellum & spinal cord). They primarily affect white matter early, with lesions of gray matter evident in more advanced disease. There are certain areas of predilection, such as optic nerves , periventricular white matter, spinal cord (corticospinal tracts, posterior white columns) & cerebellar peduncles. (2)

The standard therapies available for MS are use of Interferons, Glatiramer acetate(copolymer), intravenous immunoglobulin, Cytotoxic immunosuppresants: such as cyclophosphamide, azathioprine, cyclosporine, mitoxantrone, etc. Cladribine, Linomide etc.

For acute exacerbations Methylprednisolone, Oral steroids and Plasma exchange are practiced.

Neurorehabilitation helps to maintain /preserve status quo and also reduce spasticity and prevent contractures.

Available treatments for MS are not curative. They are able to reduce inflammation in the CNS and delay the advance of the disease, but disease control is frequently unsatisfactory. The use of stem cells in the treatment of MS is currently, to a large extent, based on the immunosuppressor and immunomodulatory effects of autologous haematopoietic stem cell transplantation (AHSCT), which may favour the immunological balance.(3) Furthermore, the multifocal nature of MS makes the injection of stem cells into each affected site impracticable, which means that the cells need to be attracted to the pathological areas. The intravenous administration of stem cells may be an alternative in MS and other neuroinflammatory conditions, in which there is permeability of the hematoencephalic blood brain barrier in the inflammatory areas. Moreover, the discovery that stem cells are capable of reaching the CNS and of transdifferentiating or acquiring oligodendrocyte and possibly neuronal properties, suggests that they may be able to act in remyelination and neuron repair.

# Summary of current clinical evidence of the role of stem cells in Multiple Sclerosis

Based on the above pathophysiology, intense immunosuppression followed by autologous haematopoietic stem-cell transplantation has been assessed over the past few years as a possible new therapeutic strategy in severe forms of multiple sclerosis. Pioneering studies began in 1995, and since then (till 2008), more than 400 patients worldwide had been treated with this procedure. Small uncontrolled studies showed that about 60-70% of treated cases did not progress in the follow-up period of at least 3 years. Transplant-related mortality, which was 5-6% in the first reported series, had reduced in the past 5 years to 1-2%. Relapses dramatically decreased and inflammatory MRI activity was almost completely suppressed. Patients with severe, rapidly worsening

#### Role Of Stem Cells In Multiple Sclerosis

multiple sclerosis who are unresponsive to approved therapies could be candidates for this treatment, but its clinical efficacy has still to be shown in large, prospective, controlled studies. (4) The mechanism by which AHSCT affects the course of MS is not known. The immediate effect appears to be the eradication of auto-reactive clones. Patients treated with AHSCT show a low T CD4+ cell count (5) and, as a consequence, the inflammatory process is reduced and there is a possible clinical improvement. Besides this immediate effect, the infusion of stem cells after high doses of immunosuppression appears to give rise to a "new" tolerance system (6) Another hypothesis to explain the improvement of patients is the capacity of stem cells to transdifferentiate into glial cells and neuronal precursors, contributing to the repair of damaged nervous tissue. Thus, AHSCT can lead to prolonged periods of stabilization of MS or change the degree of severity in the course of the disease.

Fassas et al, in 1997 presented a phase I/II pilot study comprising 15 patients with progressive MS who were treated with BEAM followed by autologous blood SCT and antithymocyte globulin (ATG).. Allergy (93%) and infections (87%) were the principal toxic complications. Mild, transient, neurotoxicity was observed in six patients in the immediate post-transplant period. The median follow-up time is 6 months (6-18). Durable neurologic improvements have been detected on both the EDSS (7/15) and SNRS (15/15) systems. One patient worsened at 3 months and two had relapsed. (7) Though initial studies by this group were encouraging in aspects of safety and non aggravation of the disability, their next two publication in 2000 and 2002 showed variable results. (8,9) Their subsequent study (Fassas et al in 2000,n=24)revealed that patients did manifest with some early neurotoxicity such as fever and infection(aspergillosis) and late complication constituting autoimmune thyroiditis in one patient. Improvements or stabilization was seen in 78% (18/23; 78%) while 5 continued to progress. Out of the 18 who had stabilized, 9 continued to maintain the stable condition while the other 9 relapsed or slowly continued to progresses. The probability of progression-free survival (compared to entry status) at 3 years was reported to be 92% for patients with secondary progressive disease and 39% for the primary progressive type. So the conclusion, regarding safety and efficacy appeared not so convincing, though they claimed that these results were better than those achieved by any other treatment of progressive multiple sclerosis. (9) Further ahead in 2002, in a multicenter study (20 centre) of 85 patients with progressive MS who were treated with autologous HSCT (bone marrow or peripheral blood) in 20 centres and which was reported to the autoimmune disease working party of the European Group for Blood and Marrow Transplantation (EBMT)., they found that three patients experienced transient neurological complications during the mobilization phase. In a median follow-up of 16 [3-59] months, they reported 7 deaths, 5 due to toxicity and infectious complications, 2 with neurological deterioration. Neurological deterioration during transplant was observed in 22 patients (either transient or progressive); Neurological improvement was seen in 18 (21 %) patients. Confirmed progression-free survival was 74 % (varying as per type of MS) at 3 years. MRI data showed activity in 8% cases after the transplantation. Though the progression free survival did appear to be positive, the mortality and complications far outweigh the benefits. (8)

Burt et al in 1998 carried out a clinical trial of 10 patients with autoimmune diseases out of which 6 patients had rapidly progressing MS who underwent autologous hematopoietic stem cell transplantation. AHSCs collected from bone marrow or mobilized from peripheral blood underwent enriching for CD34+ count and were reinfused after either a myelosuppressive or myeloablative regimen. Regimen-related non-hematopoietic toxicity was minimal. All patients improved and/or had stabilization of disease with a follow-up of 5 to 17 months (median, 11 months). The patients showed improvement in the Scripps NRS functional scale .No changes were seen on imaging. Hence they concluded that intense immunosuppressive conditioning and autologous T-cell-depleted hematopoietic transplantation was a safe treatment for these patients with severe autoimmune disease. (10)

Two studies published in 2000, Openshaw et al (11) and Kozák et al (12) with 5 and 11 subjects respectively reported varying safety and stalling of progression outcome in progressive MS. Openshaw et al. claimed mortality in 2 patients, progression in 1 and stability in 2 patients was reported while Kozák et al reported better results in disease stabilization and improvement (7/8) with deterioration in only one patient. The latter also did not observe any adverse effects or mortality.

Carreras et al in 2003 reported complications during ASCT such as engraftment syndrome, which developed in three patients, CMV reactivation in one, and neurologic deterioration in two patients coinciding with high-fever in a phase II trial (n=15) (13)In the same year, Nash et al (n=26) report death due to Epstein-Barr virus (EBV)-related post-transplantation lymphoproliferative disorder apart from mild to irreversible deterioration in neurological condition in 2 patients. They also reported an engraftment syndrome characterized by non infectious fever with or without rash developed in 13 of the first 18 patients ,sometimes associated in some cases with transient worsening of neurologic symptoms. This study has put forth important clinical issues in the use of HDIT and stem cell transplantation for MS along with emphasizing the need for modifications of the initial approaches of heavy immunosuppression to reduce treatment risks. (14)

Fagius et al in 2009 reported an overall reduction in relapse rate (15) while E. Krasulová et al (2010) in his 10 year trial of 26 patients showed a favourable outcome in young patients with relapsing remitting MS. Patients were evaluated at baseline and every six months post ASCT for adverse events and clinical outcome. Follow-up period was 11-132 months (median 66). Progression-free survival was calculated using the Kaplan- Meier method. At 3 and 6 years of follow-up 70.8% and 29.2% of patients respectively were free of progression. Patients with relapsing multiple sclerosis course, disease duration <5 years and age <35 years had a more favourable outcome. There was no death within 100 days after ASCT. The authors concluded that ASCT represented a viable and effective treatment option for aggressive multiple sclerosis. (16)

Saiz et al and G. Mancardi et al, (2005) studied 14 and 3 patients, respectively, with severe multiple sclerosis treated with AHSCT and found dramatic alteration/stalling of disease process over 2-3 years. (17,18).In other studies as well the authors found that

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HSCT is able to induce a prolonged clinical stabilization in severe progressive MS patients following failure of conventional treatment, resulting in both sustained treatment-free periods and quality of life improvement. (19,20)

Shevchenko et al in 2008 presented results of a prospective clinical study of safety and efficacy of HDCT+ auto-HSCT in MS patients. One hundred and nine patients with various types of MS were included in this study. The patients underwent early, conventional, or salvage/late transplantation. The transplantation procedure was well tolerated by MS patients, with no transplant-related deaths at all. The efficacy analysis was performed in 79 patients. Forty-two achieved an objective improvement of neurological symptoms (defined as a ?0.5 point decrease in EDSS score as compared to the baseline and confirmed over 6 months), and 37 patients had disease stabilization (steady EDSS level as compared to the baseline and confirmed over 6 months). Quality of life (QoL) was assessed in 44 patients. Thirty-nine patients exhibited a QoL response 1 year after transplantation. This study provides ample evidence in support of HDCT+ auto-HSCT efficacy in MS patients. The results obtained demonstrated that transplantation appears to be effective in patients with various types of MS. (21)

Novik et al in 2010 studied the clinical outcomes in patients with different MS types (n=156) after HDIT+ASCT. No transplant-related deaths were noted. The efficacy analysis was performed in 101 patients at 6 months post transplant: 48 patients (48%) achieved an objective improvement of neurological symptoms; 52 (51%) disease stabilisation; 1 patient (1%) progressed. At long-term follow-up (median, 27.5 months) clinical response was classified as improvement in 41 (62%); stabilization in 20 (30%); progression in 5 (8%) patients. No active, new or enlarging lesions were registered in patients without disease progression. The authors concluded that HDIT+ASCT was a safe and effective treatment for MS. (22)

A huge observational study by the EBMT Working Party on Autoimmune Disease, collating data from 549 centres of 900 patients(345 MS patients) treated over 10 years revealed that the 100-day transplant-related mortality was 2% for MS. Five years after HSCT, the progression-free survival was 45% (95% CI: 38-52%) for MS. This data confirmed that autologous HSCT was still a valid therapeutic option for patients with an autoimmune disease that is progressing despite standard therapy. (23)

Reston et al in 2010 carried out a systematic review of eight case series to evaluate the safety and efficacy of autologous hematopoietic cell transplantation in patients with progressive multiple sclerosis (MS) refractory to conventional medical treatment. Data from different studies were statistically combined using meta-analysis. An additional six studies were included for a summary of mortality and morbidity. For secondary progressive MS, immunoablative therapy with autologous bone marrow/peripheral blood stem cell transplantation was associated with higher progression-free survival (up to 3 years following treatment) when using intermediate-intensity conditioning regimens compared with high-intensity conditioning regimens. Treatment-related mortality was about 2.7%. Patients with secondary progression-free survival following autologous stem cell transplantation with intermediate-intensity conditioning regimens than with high-intensity conditioning regimens. (24)

Amongst reports of high dose suppressive protocols, are interspersed studies which have tried modified protocols using non-myeloablative approaches. Novik et al, in 2009, reported clinical and patient-reported outcomes of HDCT + auto-HSCT with a n?nm??l??bl?tiv? approach in a 35 year old male patient affected by secondary progressive MS. The patient recorded a post transplant drop in EDSS score by 3 months posttransplant, which sustained for 18 months along with imaging showing a reduction in number and size of lesions with a suppression of activity in the earlier active lesions. The authors demonstrated that HDCT + auto-HSCT might be considered as an effective treatment for MS patients with high disease activity and relatively low disability rate. (25)In another study (2009) using nonmyeloablative AHSCT (n=21), Burt et al showed that 17 of 21 patients (81%) had improved and five patients (24%) relapsed but achieved remission after further immunosuppression. After a mean of 37 months (range 24-48 months), all patients were free from progression (no deterioration in EDSS score), and 16 were free of relapses. Significant improvements were noted in neurological disability, as determined by EDSS score, neurological rating scale, paced auditory serial addition test ,25-foot walk and quality of life. (26) Side effects reported were diarrhoea due to Clostridium difficile, dermatomal zoster and a late immune thrombocytopenic purpura.

Rice et al in 2010 carried out a phase I study to assess the safety and feasibility of intravenous, autologous bone marrow (BM) cell therapy, without immunosuppressive preconditioning, in six patients with relapsing-progressive multiple sclerosis (MS). Cells were harvested, filtered and infused intravenously in a day-case procedure that was well tolerated by patients and was not associated with any serious adverse events. Over a period of 12 months after the therapy, clinical disability scores showed either no change (Extended Disability Status Score, EDSS) or improvement (MS impact scale-29, MSIS-29), and MMEPs showed neurophysiological improvement. MRI scans did not show any significant changes over a post-therapy period of 3 months. (27)

More localized route of administration of autologous stem cells was tried in MS patients. The aim was to assess safety and improve efficacy, in tune with addressing the neurological disability rather the disease itself. Hence, no chemotherapy was required. Bonab et al in 2007 reported a pilot study of ten patients who were intrathecally administered culture expanded MSCs. During 13 to 26 months of follow up (mean: 19 months), the EDSS of one patient improved from 5 to 2.5 score. Four patients showed no change in EDSS. Five patients' EDSS increased from 0.5 to 2.5. In the functional system assessment, six patients showed some degree of improvement in their sensory, pyramidal, and cerebellar functions. One showed no difference in clinical assessment and three deteriorated. The result of MRI assessment after 12 months was as following: seven patients with no difference, two showed an extra plaque, and one patient showed decrease in the number of plaques. Their study emphasized on the feasibility of autologous MSC for treatment of MS patients. (28)

In other similar study, Yamout et al (2010) explored the safety and therapeutic

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benefit of intrathecal injection of ex-vivo expanded autologous bone marrow derived mesenchymal stem cells (BM-MSCs) in 10 patients with advanced multiple sclerosis (MS). Patients were assessed at 3, 6 and 12 months. Assessment at 3-6 months revealed Expanded Disability Scale Score (EDSS) improvement in 5/7, stabilization in 1/7, and worsening in 1/7 patients. MRI at 3 months revealed new or enlarging lesions in 5/7 and Gadolinium (Gd+) enhancing lesions in 3/7 patients. Vision and low contrast sensitivity testing at 3 months showed improvement in 5/6 and worsening in 1/6 patients. Early results showed hints of clinical but not radiological efficacy and evidence of safety with no serious adverse events. (29)

Karussis et al in 2010, carried out a phase I/II open-safety clinical trial to evaluate the feasibility, safety, and immunological effects of intrathecal and intravenous administration of autologous mesenchymal stem cells (MSCs) (also called mesenchymal stromal cells) in patients with multiple sclerosis (n=15) and amyotrophic lateral sclerosis (ALS).After culture, a mean (SD) of  $63.2 \times 106$  MSCs were injected intrathecally and intravenously. Follow-up duration was mean ?25 months. No major adverse effects were reported during follow-up. The mean (SD) EDSS score improved from 6.7 (1.0) to 5.9 (1.6). The authors concluded that transplantation of MSCs in patients with MS and ALS was a clinically feasible and relatively safe procedure and induced immediate immunomodulatory effects. (30)

An interesting use of endometrial regenerative cells containing mesenchymal cell like population administered intrathecally and intravenously has been reported by Zhong et al recently (2009). The case with the longest follow up, of more than one year, revealed no immunological reactions or treatment associated adverse effects. With the caveat of a small sample size and limited number of injections, they reported that ERC may be administered intravenously and intrathecally without immediate immunological reactions or ectopic tissue formation. The preliminary data suggest feasibility of clinical ERC administration and supported further studies with this novel stem cell type. (31)

NeuroGen Brain & Spine Institute conducted a study on 22 multiple Sclerosis patients who underwent intrathecal autologous bone marrow derived mononuclear cell transplantation. The mean follow up of 6 months showed that 11 patients shifted on EDSS Scale showing objective Neurological Improvement. On assessing them symptom-wise 17 patients showed reduction in spasticity,9 showed improved upper extremity and trunk coordination,, 6 improved in speech clarity and 8 showed increased in muscle strength.(32)

# **Case report**

## 1. Multiple Sclerosis

#### **Clinical presentation:**

35 year old female, a known case of multiple sclerosis since 7 years, presented with chief complaints of difficulty in writing, slipping of footwear, intentional tremors and coordination. Neurologically, she had hypertonia, with no sensory problems. She had nystagmus with squint. Power in muscles of all 4 limbs was Grade 4. Urinary status showed incontinence with urgency of micturition. She had spastic Ataxic Gait with poor balance. On Investigation: MRI of brain was suggestive of demyelination in cerebral periventricular, white matter, brain stem and cerebral hemispheres. However EMG and NCV studies were normal.



Sagittal FLAIR image reveals multiple hyperintense lesion perpendicular to the calloso-septal surface (Dawson's fingers) consistent with Multiple Sclerosis.

#### **Clinical Improvement after stem cell therapy:**

Functionally while shifting, she could keep her feet down and shift with minimal support thereby showing improvement in static and dynamic trunk balance. She could stand without much shaking thus showing increase in standing tolerance & reduced incoordination. Her upper extremity coordination improved and she was able to pick up different objects from different directions without losing balance. Her speech improved significantly, and dysarthria reduced. Relatives could now understand the telephonic conversation much better and clearer. Neurologically, her nystagmus got totally resolved Incoordination had reduced, with improved hand control and ease in feeding, brushing, cutting vegetables. Her urine control became better, in terms of reduced urgency and frequency. Her fine motor coordination skills improved & she could write easily on the black board.

# **Case Report 1**



*Figure 1: Patient showing improved standing balance and ability to walk independently in the parallel bars.* 



*Figure 2: Patient showing an attempt to write as her upper extremity incoordination had reduced.* 

# **Case Report 2**



Figure 3: Patient showing improved lower extremity strength and coordination, so as to be able to perfom stair case climbing with assistance.



Figure 4: Patient showing improved upper limb coordination in performing finger-nose-finger movements rapidly.

#### 2. Multiple Sclerosis

#### **Clinical Presentation:**

40 year old male, a known case of multiple sclerosis gives history of loss of balance and limping noticed in bilateral lower limbs since 2002. Neurologically, he was hypertonic with spasticity of Grade 1 according to Modified Ashworth Scale and hyper reflexic. He had muscle power of Grade 3++ in bilateral upper limb & lower limb. He complained of urgency of urination. Psychologically, he was very depressed and had fear of progression of disease. Functionally, he could ambulate with a walker/assistive device, but used wheel chair, because of fear of falling. He complained of difficulty in writing, buttoning and fine activities of upper limbs, but otherwise was independent in all his activities of daily living (ADL). He is a Management teacher by profession.



Axial FLAIR image reveal hyperintense lesions in the bilateral corona radiata with smaller morphology lesion in the left frontal sub cortical white matter

#### **Clinical Improvement after stem cell therapy:**

Neurologically, patient reported that his hand & upper body tremors had reduced to almost negligible after stem cell transplant . He also reported reduction in urgency of urinations & he could hold upto 3- 4 hrs) He also reported that his fatigue levels had reduced significantly..

He could walk independently without support for atleast 20 -25 steps. He also reported that his lower limbs strength has improved & functionally he could climb stairs with very little assistance. He could get up independently from bed, which was not possible before. Mother reported that he is much more mobile than before & his voice tone quality had improved.
### Summary

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating disease of the Central Nervous System (CNS). & the cardinal symptoms of intention tremor, scanning speech & nystagmus later termed Charcot's Traid. Based on the above pathophysiology, intense immunosuppression followed by autologous haematopoietic stem-cell transplantation(AHSCT) has been assessed over the past few years as a possible new therapeutic strategy in severe forms of multiple sclerosis with varying safety and efficacy.More recently, intrathecal autologous stem cell transplantation, obviating the need for chemotherapy, is being tried and found to be a feasible option for addressing the disability caused by the MS.

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## 11

## **Stem Cell Transplantation For Cerebral Palsy**

Cerebral palsy is a term used to describe a broad spectrum of motor disability which is non progressive and is caused by damage to brain at or around birth. It is a disorder which develops due to damage to CNS and this damage can take place before, during or immediately after the birth of the child. However the child may appear to worsen if not given proper intervention not because of an increase in lesion but just because the damaged brain is not able to cope up with the physical demand of the growing body and the increasing demand of the environment surrounding the child. (1-3)

### **Clinical Symptoms:**

It is characterized by muscle spasticity, muscle weakness, uncontrolled movements, impaired mobility, speech impairment and/or challenges in eating, dressing, bathing,



Axial FLAIR images reveal diffuse hyperintensities in the bilateral corona radiata and periventricular white matter consistent with periventricular leukomalacia

etc depending on the area of the brain affected.(Table:1) Movement dysfunction is often accompanied by seizures, visual impairment, hearing loss, osteoporosis, learning disabilities, and behavior problems. Risk factors for cerebral palsy include prenatal anemia, improper nutrition, infections, x-rays, premature delivery. Hypoxia and ischemia are also major risk factors prenatally and during delivery

ANATOMICAL AFFECTION	MRI FINDINGS	CLINCAL FINDINGS
Bilateral white matter necrosis,descending motor fibres,optic,acoustic radiations	Periventricular leucomalacia	Spastic diplegic, quadriplegic, visual & cognitive defects.
Focal, multifocal ischaemic brain necrosis	Specific vascular infarction, typically left MCA	Hemiplegic, seizures.
Basal ganglia neuronal injury	Status marmoratus	Choreoathetosis or mixed picture.
Selective neuronal necrosis	Lateral geniculate, thalamus, basal ganglia	Mental retardation, seizures
Parasaggital cerebral injury	Bilateral medial & posterior portions of cortex	Upper extremity more severely affected than lower.

## Symptoms seen in the CP correlating to the area of brain damaged seen on a MRI (4-6)

### **Classification of Cerebral Palsy: (7)**

### **Cerebral Palsy is classified as follows:**

- A) Topographical Classification:
  - a. Quadriplegic: Involvement of four limbs. Wherein, arms are affected more than the leg. It is also called double hemiplegia.
  - b. Diplegia: Involvement of four limbs with legs more affected than hands.
  - c. Paraplegia: Involvement of both legs only.
  - d. Triplegia: Involvement of three limbs.
  - e. Hemiplegia: Involvement of one side of body.
  - f. Monoplegia:One limbs affected.
- B) According to types:
  - a. Spastic
  - b. Athetoid.

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- c. Ataxic.
- d. Mixed.

The assessment tools used to evaluate neurologic impairment are: (8)

- 1) Modified ashworth scale for tone assessment,
- GMFCS E&R Scale (Gross motor function classification system expanded & revised),
- 3) FIMS Scoring (Functional independence measure score),
- 4) FMS (Functional mobility scale).

### **Conventional Therapies:**

### 1. Rehabilitation:

Rehabilitation is aimed at improving infant caregiver interaction, giving family support, supplying resources, parental education and promoting motor and developmental skills. It focuses on development of skills necessary for performance of ADL. These include play self care activities such as dressing grooming and feeding and fine motor tasks such as drawing and writing. Rehabilitation also addresses cognitive and perceptual disabilities especially in visual motor area and adaptation of equipment and seating to allow better upper extremity use and to promote functional independence. Neurodevelopmental Therapy and Sensory integration is a specialized techniques used to achieve these goals. The common principles in these therapies include development of sequence learning, normalization of tone, training of normal movement patterns, inhibition of abnormal patterns and prevention of deformity. In general since a child learns motor control in a cephalocaudal direction, therapist will work in the same direction, first trying to establish trunk control and then working towards control of lower extremity. (9)

### 2. Hyperbaric Oxygen Therapy:

There is currently no known medical treatment for CP other than supportive care, usually provided by a multidisciplinary team in most pediatric centers. There is growing interest in the use of hyperbaric oxygen therapy (HBO2) for children with CP.

During HBO2, the partial pressure of oxygen (pO2) in the blood is greatly increased by exposing the child to elevated ambient pressures while breathing 100% oxygen. Treatment regimens differ but generally treatments involve pressurization to 1.5 to 2 atmospheres for 60 to 90 minutes. The child must breath 100% oxygen. In a multiplace chamber (hyperbaric chamber in which several patients can be treated simultaneously), this is administered through an enclosed vinyl diving hood with neck seals. Justifications for HBO2 in CP vary but generally rely on a claim that high tissue oxygen levels can improve the function of damaged cells within the central nervous system. (10-14)

#### 3. Botulinum Toxin A Injection

Botulinum Toxin A injection cause a focal dose dependent reversible chemodenervation of the muscle. Patient who could benefit most from this treatment is one who is hypertonic either spastic or dystonic whose abnormal muscle tone is interfering with function or who is expected to develop joint contracture with growth because of this abnormal tone. Generally patients younger than 4 years without fixed contracture respond most favourably with Botulinum Toxin. (15)

Conventional therapies available for cerebral palsy, as in the case of other neurological injuries, offer limited scope for improvement. Also, as compared with other neurological injuries happening in adult life, the complexity of hypoxic injuries perinatally pose a greater problem. This is due to the fact that apart from injury to a very immature nervous sytem, this injury also affects the innate growth capacity or resources (the neuronal progenitors or stem cell niches in the growing brain) of the human brain. Hence, cell transplants offers a external supporting matrix for promoting angiogenesis and regeneration in the developing brain. Studies on mammalian models of brain injury have provided evidence for this rationale. Mueller et al. (2005) examined whether human neural stem cells (hNSCs) replace lost cells in a newborn mouse model of brain damage. Mice received brain parenchymal or intraventricular injections of hNSCs derived from embryonic germ (EG) cells. The stem cells migrated away from the injection site and were found at sites of injury within the striatum, hippocampus, thalamus and white matter tracts and at remote locations in the brain. Subsets of grafted cells expressed neuronal and glial cell markers. hNSCs restored partially the complement of striatal neurons in brain-damaged mice. They concluded that human EG cell-derived NSCs can engraft successfully into injured newborn brain, where they can survive and disseminate into the lesioned areas, differentiate into neuronal and glial cells and replace lost neurons. (16)

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

While the use of stem cell therapy is promising, currently, there are no controlled trials in humans with cerebral palsy. However, studies in animals with experimentally induced strokes or traumatic injuries have indicated that benefit is possible. The potential to do these transplants via injection into the vasculature rather than directly into the brain increases the likelihood of timely human studies. As a result, variables appropriate to human experiments with intravascular injection of cells, such as cell type, timing of the transplant and effect on function, need to be systematically performed in animal models with hypoxic-ischaemic (HI) injury, with the hope of rapidly translating these experiments to human. (17)

The Department of Neurology, All India Institute of Medical Sciences initiated the project on intra-arterial infusion of autologous bone marrow stem cells in patients with static encephalopathy including cerebral palsy way back in 2005 to test the hypothesis that intra-arterial infusion of autologous bone-marrow derived stem cells in patients with nonprogressive (static) encephalopathy, with special reference to cerebral palsy, and hypoxic-ischemic encephalopathy is feasible, safe and improves neurological functional outcome. (18)The results or data of this study are currently not available for review.

#### Stem Cell Transplantation For Cerebral Palsy

Around the time that centres in India started working on the concept of regeneration for CP, a Russian study using fetal stem cells was published.Seledtsov et al (2005) presented the results of a controlled study of cell therapy in 30 patients with severe forms of cerebral palsy. A cell suspension from immature nervous and hemopoietic tissues was injected into the subarachnoidal space of a recipient through a spinal puncture. Immune sensitization to donor antigens (detected by suppression of lymphocyte migration) was noted in few patients. One year after treatment activity of the major psychomotor functions in treated patients considerably surpassed the normal. No delayed complications of cell therapy were noted. Their findings suggested that cell therapy was an effective, safe, and immunologically justified method of therapy for patients with cerebral palsy. (19)

Another data available from the centre of immunotherapy who had subjected 125 severely brain injured patients with cerebral palsy to a stem cell transplantation therapy via a lumbar puncture (subarachnoidally) showed apparent neurological improvement in 85% of cell grafted cerebral palsy patients.(20)

However, upcoming work seems to focussed more on the cord blood as a source of stem cells. A Mexican study on the safety and efficacy of stem/progenitor cells from umbilical cord blood was that stem/progenitor cells from umbilical cord blood presented data based on parental observations and completed questionnaires concerning the responses of cerebral palsy-stricken children to treatment with umbilical cord stem cells was launched during 2004. As part of this Mexican study, eight children (3-12 years of age) diagnosed with cerebral palsy underwent transplants with 1.5 million stem/ progenitor cells (CD34+ and CD133+) that had been purified and ex-panded from the American Association of Blood Banks (AABB)-certified human umbilical cord blood. According to parent tendered observational re-ports, none of the children had graft versus host reactions. Eight out of eight children showed some improvement in mobility and/or cognitive function. Six children (75%) were rated as improving in muscle tone, hip movement, leg movement, rolling to the side, balancing while sitting and balancing while standing by the end of the six month follow up. (21)

At the XCell-Centre (Germany), De Munter et al carried out a study on 116 cerebral palsy patients who underwent adult autologous stem cells derived from the bone marrow. The mean age of the patients was 11.5 years; 50% was below 7.0 years; gender distribution was equal. The stem cells were injected intrathecally by lumbar puncture. 66.4% patients reported improvements. Improvements mainly included increased motor functioning with decreased spasticity, resulting in improved sitting, standing, walking and posture stability, as well as an improvement in mental functions resulting in better communication. Improvement of speech was mentioned by 28 patients, and a major reduction or even absence of epileptic seizures during the recorded post-treatment period was reported by 11 patients. The authors concluded that autologous Stem Cell transplantation was not only a safe, but also a potentially clinically effective therapy for CP patients. (22)

An ongoing pilot study at the Duke University, North Carolina, US, is aimed at

evaluating a quick response to cerebral palsy in infants. Infants that show signs of CP in the first two weeks after birth, and whose parents have consented to cord blood drawing, will have their stem cells infused into them.

Another FDA-approved clinical trial, based on Duke pilot model, is being conducted at the Medical College of Georgia, Atlanta. The aim, again, is to determine whether an infusion of stem cells from umbilical cord blood can improve the quality of life for children with cerebral palsy. The study will include 40 children age 2-12 whose parents have stored cord blood at the Cord Blood Registry in Tucson, Arizona. (23)

Data from these ongoing trials is expected to put more light on the effect of stem cells in CP and is expected to be quite promising.

At the NeuroGen Brain and Spine Institute, Mumbai out of 16 cerebral palsy patients who underwent bone marrow derived autologous stem cell transplantation, 6 showed improvement in oromotor functions like speech, swallowing and neck holding, 8 of them showed reduction in seizure frequency and 10 showed normalisation of tone.(24)

### **Case Report**

### 1. Cerebral Palsy :

#### **Clinical presentation :**

3 year 5 month old female, known case of CP born of a Full Term - C section delivery, gives a history of delayed milestones, sucking and swallowing difficulty since birth. She has no history of seizures or any other birth injury.

On evaluation, neurologically she was hypertonic and hyperreflexic with grade 1 spasticity in bilateral lower limb according to Modified Ashworth Scale. She had all sensations intact including bladder and bowel functions. She had grade 3++ strength in bilateral upper extremity but grade 3 voluntary control in bilateral lower extremity. Her milestone development was as follows: could perform rolling independently, couldn't crawl and walk,could sit up without support, had palmar grasp. She also had good voluntary control in bilateral upper extremity and spastic pattern movements in bilateral lower extremity. She was well preserved andresponded to all commands. She had a nasal twang while speaking and she could swallow only semisolid food. Her vision and hearing was normal. Functionally, she was dependent on her mother for most activities of daily living (ADL) and mobility. But when given assistance, she could walk with equinnus gait. Her MRI Brain, EMG, NCV were normal.



Axial T1W image through the basal ganglia reveals mild prominence of the cortical sulci.

### Clinical Improvement after stem cell therapy

Her dynamic sitting balance had improved significantly. She could perform reach outs in sitting, kneel standing position independently and then she could do overhead bilateral clapping and balance in that position, indicating improved trunk control.She could get up from supine position by herself.Her hand opening had improved significantly and she was able to hold objects with open hand.Her neck control had improved, as compared to before. She could stand once made to do so on standing

### **Case Report 1**



Figure 1: Patient showing improved hip and knee stability with sustenance of half kneeling position independently.



Figure 2: Patient showing enough balance to stand independently and attempting to walk.



*Figure 3: Patient showing reduced athetoid movements and standing steady with bilateral push knee splints.* 

*Figure 4: Patient showing improved neck holding while setting and ease in upper extremity exercises.* 

### **Case Report 2**

frame, wherein she could bend, touch the floor and pick up objects and come up erect. After the therapy she started attempting to walk a few steps(with support) with her soles touching the ground. Earlier, she would walk with support, but on her toes. According to her parents, she could stand independently with AFO for few seconds.While walking, she had started bearing weight on both her legs (with AFO).

### 2. Dystonic Cerebral Palsy :

### **Clinical presentation:**

14 year old male, known case of cerebral palsy, had a history of twin delivery and developed fungal infection and jaundice 4-5 days after birth.He was admitted in the NICU for 40 days. Neurologically, he was dystonic.He had intact sensation with normal bladder and bowel control. Cognitively, he was normal and very co-operative. Due to severe dystonic movements, he was unable to hold his head. Attitude of left upper extremity was of elbow flexion,wrist flexion and finger extension.There was no purposeful activity possible with the left hand.Positive supporting reaction in bilateral lower extremity was poor,therefore, he was unable to stand without any knee support.He was dependent on his mother for all hisactivities of daily living (ADL). On evaluation using **Fahn-Marsden Dystonia Scales he scored 95 out of 120** with severe dystonia seen in speech, swallowing, neck arm and trunk area. **On Dystonia Disability Scale, he scored 26 out of 29** with major areas of handicap being handwriting, self feeding, personal hygiene, dressing and walking. MRI brain and EEG were found to be normal.



Axial T1W image grossly shows normal brain morphology through the basal ganglia reveals mild prominence of the cortical sulci.

### Clinical Improvement after stem cell therapy:

Functionally, he started to assist in bathing, when put on chair, puts water, rinses hair after shampoo. Mother reports that earlier it was very difficult to bathe him. Now eae in the activity is experienced with contribution from the patients side. After SCT, he could wear a splint and stand for 10-15 mins. He could operate a remote control while sitting and could operate a laptop which was not possible before, due to dystonia. Mother

reported that rolling was better, controlled and independent after the therapy.His speech had improved significantly as he could speak longer sentences.Neurologically, dystonic movements especially in left upper extremity and bilateral lower extremity spasticity had reduced. His hand function improved with increased grip strength. His grip strength and release has mainly improved in his left hand. Before the therapy, he could not open his left hand which always remained fisted with no voluntary hand opening. He could also straighten his left elbow after the therapy, which was not possible earlier due to spasticity.On reassessment, there was improvement on the **Frahn Marsden Dystonia Scales, he shifted from 95 to 30.5 out of 120** thereby showing reduced dystonic movements in neck, arms, trunk and speech improvement. **On Dystonia Disability Scale, he shifted from 26 to 20 out of 29** with major areas of improvement being self feeding, hygiene, dressing and initiation of mobility in standing.

### Summary

Cerebral palsy is a term used to describe a broad spectrum of motor disability which is non progressive and is caused by damage to brain at or around birth. It is characterized by 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. Conventional therapies available for cerebral palsy, as in the case of other neurological injuries, offer limited scope for improvement. cell transplants offers a external supporting matrix for promoting angiogenesis and regeneration in the developing brain.Fetal stem cells and adult haematopoietic stem cells have been found to safe and feasible options for treating CP.However, more recently, the focus has shifted to cord blood sources.

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## 12

### **Stem Cell Transplantation In Stroke**

Stroke is defined as sudden neurological deficit caused by focal vascular lesion in the brain. The vascular lesion could be either hemmorhagic or thromboembolic phenomena (leading to ischaemia) involving the blood vessels supplying various parts of the brain .The extent of neurological involvement may range from mild motor deficit to gross involvement of various function namely sensorimotor, perceptual, emotional, behavioural, memory intelligence, speech and language function.

### **Etiology:**

Different mechanisms have been found to cause vascular insufficiency to the brain resulting in stroke. However, the most common causes are:

- 1. **Thrombus:** This is mainly due to the presence of atherosclerotic plaque in the cerebral arteries as a result of severe platelet adhesion, fibrinous coagulation and decreased fibrinolysis activity.
- 2. **Emboli:** These are free flowing bodies in the cerebral blood stream in the form of dislodged thrombus, fats, air, tissue particle, etc which gets trapped at any one point along their course, frequently at the bifurcation of the arteries, and cause occlusion to the cerebral occlusion.
- 3. **Hemorrhage :** It occurs due to the rupture of the blood vessels in the brain. Following hemorrhage tissue death occurs due to both ischemia and mechanical injury to the brain substance as a result of compression by the clot. Hemorrhage usually occurs either due to hypertension , arteriovenous malformation or even due to trauma.

There are some risk factors that can predispose to stroke. The common ones are diabetes, high blood pressure and cardiac disorders. Modifiable risk factors are cigarette smoking, obesity, sedentary lifestyle and excessive alcohol intake.

### Pathophysiology of Cerebral Ischemia and consequent infarction

The two pathophysiological changes leading to cerebral infarction are loss in the supply of oxygen and glucose due to vascular occlusion, and various changes in cellular metabolism consequent as a result of collapse of energy producing processes with disintegration of cell membrane. Complete occlusion to brain substance causes severe damage to it with a zone of infarction, which is however found to be smaller than the actual area supplied by the involved artery. The margin of this infarcted zone consists of cells that are alive but metabolically less active. These surrounding areas are termed as ischemic penumbra. These areas are nourished by meningeal collaterals.

The necrotic tissue swells rapidly mainly due to excessive intercellular water content. Also, lack of oxygen is another factor that could contribute to swelling. This vascular lesion to the brain causes release of neurotransmitters like glutamate and aspartate by the ischemic cells, which excites neurons and produces an intracellular influx of Na and Ca leading to irreversible cell damage. Thus recent research attempts at blocking this action of gluatamic and aspartate on nearby cells, which will reduce the secondary involvement of surrounding viable cells.

Cerebral edema begins within few minutes and reaches a maximum by about 4 days, however it mostly disappears by 3 weeks. This edema can increase the intracranial pressure and can even cause contralateral and caudal shift of brain structure.(1)

### **Neurovascular Syndromes:**

Cerebral Blood flow (CBF) is controlled by a number of autoregulatory mechanisms (cerebral) that modulate a constant rate of blood flow through the brain. These mechanisms provide homeostatic balance, counteracting fluctuations in systolic blood pressure while maintaining a normal flow of 50 to 60 ml / 100 gm of brain tissue per minute. The brain has high energy requirements and very little metabolic reserves. Thus ,it requires a continuous rich perfusion of blood to deliver oxygen and glucose to the tissues. Cerebral blood flow represents approximately 17 % of available cardiac output. (1,2)

Depending on the areas of the brain and the arteries affected clinical symptoms vary.

- A) Occlusion of proximal Middle Cerebral Artery (MCA)
  - It produces extensive neurological damage with significant cerebral edema and list of clinical manifestations called MCA Syndrome: (3)

Structures involved	Neurological deficit
Motor area of face, arm, and fibres descending from leg area to enter the coronal radiata	Paralysis of contralateral face, arm and leg
Somatosensory area of face, arm and face leg	Sensory impairment over contralateral and leg
Motor (Broca's) area on dominant hemisphere	Motor speech disorder

Stem Cell Transplantation In Stroke

Central language area and parieto- occipital cortex of the dominant hemisphere	Central aphasia, word deafness, anomia, jargon speech, alexia, agraphia, acalculia, and finger agnosia
Non-dominant parietal lobe	Perceptual disorder like unilateral neglect, anosognosial unawareness of hemiplegic side, apraxia, and spatial disorganization
	Homonymous hemianopia and loss of conjugate gaze to the opposite side
Parietal lobe	Ataxia of contralateral limbs
Bilateral frontal lobe	Brun's ataxia or apraxia of gait
Supramarginal gyrus or inferior parietal lobe	Loss or impairment of optokinetic nystagmus
Posterior limb of internal capsule and adjacent corona radiata	Pure motor hemiplegia without sensory and visual involvement

# B) Clinical Manifestations of Anterior Cerebral Artery Occlusion are as follows: (3)

Structure involved	Neurological deficit
Motor leg area and involvement of motor arm area	Paralysis of opposite foot and involvement of opposite arm
Sensory area of foot and arm	Cortical sensory loss over foot and arm
Bilateral involvement of posteromedial part of superior frontal gyrus	Urinary incontinence
Medial surface of posterior frontal lobe	Contralateral grasp reflex, sucking reflex and gegenhalten (paratonic rigidity), frontal tremor
Severe frontal lobe infarction	Memory loss and behavioral impairments
Supplementary motor area of dominant hemisphere	Aphasia
Corpus callosum	Apraxia and agraphia
Bilateral motor area of leg	Cerebral hemiplegia

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## C) Clinical Manifestations of Posterior Cerebral Artery Occlusion are as follows: (3)

Structure involved	Neurological deficit
Thalamus	Hemianesthesia (contralateral sensory loss) or thalamic sensory syndromes (unpleasant hemibody sensation with spontaneous pain)
Occipital cortex	Homonymous hemianopia, visual agnosia, prosopagnosia
Bilateral occipital cortex involvement	Cortical blindness
Temporal lobe ischaemia	Amnesic syndrome with memory defect
Midbrain	Skew deviation, athetoid posturing, postural tremor, hemiballismus)
Cerebral peduncle	Contralateral hemiplegia
Motor tract between red and	Decerebrate attacks vestibular nuclei

### **D)** Lacunar Syndromes:

Lacunar syndromes are caused by small vessel disease deep in the cerebral white matter (penetrating artery disease). Lacunar syndromes are consistent with specific anatomic sites.

Structure Involved	Neurological deficit
Posterior limb of internal capsule, pons & pyramid	Pure motor lacunar stroke
Venterolateral thalamus or thalamocortical projections	Pure sensory lacunar stroke
Base of pons , genu of anterior limb or the internal capsule.	Dysarthria / Clumsy hand syndromes
Pons , genu of internal capsule, coronal radiate or cerebellum.	Ataxic Hemiparesis
Junction of internal capsule & thalamus	Sensory / Motor Stroke
Putamen , global pallidus, subthalamic nucleus.	Dystonia Movements

#### **Conventional therapies:**

An ischemic stroke is caused by a thrombus (blood clot) occluding blood flow to an artery supplying the brain. Definitive therapy is aimed at removing the blockage by breaking the clot down (thrombolysis), or by removing it mechanically (thrombectomy). The more rapidly blood flow is restored to the brain, the fewer brain cells die. (4)

Other medical therapies are aimed at minimizing clot enlargement or preventing new clots from forming. To this end, treatment with medications such as aspirin, clopidogrel and dipyridamole may be given to prevent platelets from aggregating. (5)

Stroke rehabilitation should be started as quickly as possible and can last anywhere from a few days to over a year. 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. However, patients have been known to continue to improve for years, regaining and strengthening abilities like writing, walking, running, and talking. Daily rehabilitation exercises should continue to be part of the stroke patient's routine. Complete recovery is unusual but not impossible and most patients will improve to some extent: proper diet and exercise are known to help the brain to recover.

Stem cell therapy offers hope for stroke patients, especially for those who have missed the narrow 3- hour window for administration of tissue plasminogen activator. Borlongan and Hess (2006) provided preclinical evidence that neuroteratocarcinoma (NT2N) cells, a clonal cell line, considered to be neural progenitor cells, significantly attenuated motor and cognitive deficits when transplanted to adult rats 4 weeks after middle cerebral artery occlusion. (6) It is possible that transplanted cells secrete trophic factors that help to maintain marginally surviving cells or otherwise enhance the local environment sufficiently to improve function. Transplantation might also conceivably produce a host reaction that could include sprouting of new axons and synapse formation. It remains uncertain which type of cell would be most appropriate for transplantation into stroke patients. Various cell types (e.g., porcine foetal cells, embryonic stem cells, and immortalized neuronal cells and bone marrow stromal cells) are being investigated.

Recent experimental studies raised the possibility of using mesenchymal stem cells (MSCs) as stroke therapy. There is increasing evidence that MSCs promote functional recovery in animal models of ischemic stroke. In specific culture conditions, human MSCs can differentiate into cells that express markers of neuronal progenitor cells and can engraft and migrate along paths that resemble those of neuronal progenitor cells. MSCs are eminently suitable for human trials because these cells can be obtained readily from bone marrow under local anesthesia, are easily expanded by culture, and potentially could be delivered to injured brain tissue without the need for invasive stereotaxic operations. Moreover, the use of patients' own bone marrow cells should circumvent the problems of host immunity and graft versus-host disease.(7)

The outcome of stem cell transplantation depends on the type of stroke lesions as well as the timing of the transplantation. Not all stroke lesions may be acquiescent to cell transplantation. Cortical lesions are more accessible to transplantation as compared to white matter infarcts. (8)

## Summary of Current Clinical Evidence of The Role Of Stem Cells In Stroke

The few clinical studies of cell transplant for stroke performed to date are limited by the few patients treated, varying cell types and minimal controls. The results, however, provide preliminary insights into clinical questions that may be difficult to approach with animal studies and may help guide design of future trials of cell therapy.

Three of the 4 completed studies included patients with stroke involving the basal ganglia. For instance, Kondziolka et al (2000) who carried out transplantation of cultured neuronal cells derived from an immortalized tumor cell line (LBS cells) in 12 patients with basal ganglia stroke and fixed motor deficits. Serial evaluations (12 to 18 months) of these patients showed no adverse cell-related serologic or imaging-defined effects. They reported that total European Stroke Scale score improved in six patients (3 to 10 points), with a mean improvement 2.9 points in all patients (p = 0.046). Six of 11 PET scans at 6 months, also showed improved fluorodeoxyglucose uptake at the implant site. The authors thus reported that neuronal transplantation was feasible in patients with motor infarction. (9) They further carried out an observer-blinded phase 2 trial of 18 patients where in they tested the usefulness of human neuron transplantation followed by participation in a 2-month stroke rehabilitation program compared with rehabilitation alone in patients with substantial fixed motor deficits associated with a basal ganglia stroke. Patients were randomized at two centers to receive either 5 or 10 million implanted cells in 25 sites (seven patients per group) followed by participation in a stroke rehabilitation program, or to serve as a nonsurgical control group (rehabilitation only; four patients). The surgical techniques used were the same at both centers. All patients underwent extensive pre- and postoperative motor testing and imaging. The primary efficacy measure was a change in the European Stroke Scale (ESS) motor score at 6 months. Secondary outcomes included Fugl-Meyer, Action Research Arm Test, and Stroke Impact Scale scores, as well as the results of other motor tests. Nine strokes were ischemic in origin and nine were hemorrhagic. Serial evaluations (maximum duration 24 months) demonstrated no cell-related adverse serological or imaging-defined effects. One patient suffered a single seizure, another had a syncopal event, and in another there was burr-hole drainage of an asymptomatic chronic subdural hematoma. Four of seven patients who received 5 million cells (mean improvement 6.9 points) and two of seven who received 10 million cells had improved ESS scores at 6 months; however, there was no significant change in the ESS motor score in patients who received cell implants (p = 0.756) compared with control or baseline values (p = 0.06). Compared with baseline, wrist movement and hand movement scores recorded on the Fugl-Meyer Stroke Assessment instrument were not improved (p = 0.06). The Action Research Arm Test gross hand-movement scores improved compared with control (p = 0.017) and baseline (p = 0.001) values. On the Stroke Impact Scale, the 6-month daily activities score changed compared with baseline (p = 0.045) but not control (p = 0.056) scores, and the Everyday Memory test score improved in comparison with baseline (p = 0.004)

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values. Although a measurable improvement was noted in some patients and this translated into improved activities of daily living in some patients as well, this study did not find evidence of a significant benefit in motor function as determined by the primary outcome measure. (10)

Meltzer et al (2001) used positron emission tomography (PET) with [18F]fluorodeoxyglucose (FDG) to map the metabolic brain response to neuronal cell implantation in the first human neuroimplantation trial for stroke. Twelve patients (nine men, three women; mean age +/- standard deviation, 60.8+/-8.3 yr) with chronic basal ganglia infarction and persistent motor deficit underwent FDG PET within 1 week before and 6 and 12 months after stereotactic implantation of human neuronal cells. Serial neurological evaluations during a 52-week postoperative period included the National Institutes of Health stroke scale and the European stroke scale. Alterations in glucose metabolic activity in the stroke and surrounding tissue at 6 and 12 months after implantation correlated positively with motor performance measures. (11)

Stilley et al (2004) also reported change in cognitive function after neuronal cell transplantation as a treatment for basal ganglia stroke. Nine subjects (two controls, seven transplants), all over 2 years post stroke, completed a comprehensive neuropsychological test battery prior to and 6 months after treatment. Four transplanted subjects who had strokes in the nondominant hemisphere showed marked improvement on the Rey Complex Figure, a test of visuospatial/constructional ability and nonverbal memory. (12)

The basal ganglia, does appears to provide a convenient target for stereotactic implantation of cells because a relatively small infarct in this area may cause significant and measurable motor deficits. The circumscribed area of infarction can be treated with small infusion volumes at several locations in one or more passes through the area. However, cell type and the mechanism of repair would ultimately decide the site of implantation.

However, all the trials till 2005 were carried out using tumor cell lines, which had the potential to cause serious side effects. Hence, in the later half of this decade, other sources of stem cells began to be explored.

Savitz et al (2005) presented the first report on the transplantation of non-tumor cells in ischemic stroke patients. They aimed to study the safety and feasibility of fetal porcine transplantation in 5 patients with basal ganglia infarcts and stable neurological deficits. To prevent rejection, cells were pretreated with an anti-MHC1 antibody and no immunosuppressive drugs were given to the patients. The first 3 patients had no adverse cell, procedure, or imaging-defined effects. The fourth patient had temporary worsening of motor deficits 3 weeks after transplantation, and the fifth patient developed seizures 1 week after transplantation. MRI in both patients demonstrated areas of enhancement remote from the transplant site, which resolved on subsequent imaging. Two patients showed improvement in speech, language, and/or motor impairments over several months and persisted at 4 years. The study was terminated by the FDA after the inclusion of 5 patients,due to occurrence of adverse events in two patients.(13)

Rabinovich et al (2005) carried out a study wherein, the cell suspension consisting of cells from immature nervous and hemopoietic tissues was subarachnoidally transplanted to 10 patients with brain stroke. Clinical effects of varying degrees were attained in all patients. Six months after cell therapy functional activity significantly increased in contrast to clinically comparable control group. No serious complications of cell therapy were observed. They concluded that cell therapy was a more or less safe method of treatment, which could be effectively used in the treatment of brain stroke consequences. (14)

Though fetal stem cells used in these two studies pose less chances of tumorogenicity, but they carry the burden of ethical issues with them. Hence, researchers resorted to the more safer option of adult haematopoietic stem cells and mesenchymal stem cells

Bang et al. (2005) carried out a randomized clinical trial using autologous mesenchymal stem cells. Thirty patients with cerebral infarcts within the middle cerebral arterial territory and with severe neurological deficits were randomly allocated into one of two treatment groups: the MSC group (n = 5) received intravenous infusion of 1 x 108 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. Neuroimaging was performed serially in five patients from each group. Outcomes improved in MSC-treated patients compared with the control patients: the Barthel index (p = 0.011, 0.017, and 0.115 at 3, 6, and 12 months, respectively) and modified Rankin score (p = 0.076, 0.171, and 0.286 at 3, 6, and 12 months, respectively) of the MSC group improved consistently during the follow-up period. Serial evaluations showed no adverse cell-related, serological, or imaging-defined effects. They concluded that the intravenous infusion of autologous MSCs appeared to be feasible and a safe therapy that may improve functional recovery. (15)

Though, in animal studies, bone marrow stromal cells are seen to localize to areas of brain injury, but whether this results in survival of functioning cells after human stroke is unclear. It is not yet clear whether sufficient numbers of cells can be delivered intravenously to achieve clinical effectiveness in patients with extensive areas of infarction in the territory of the major cerebral arteries.

A simple study of understanding the effect of just mobilizing the autologous bone marrow stem cells was carried out by Shyu et al (2006). They explored the therapeutic potential of G-CSF therapy in ischemic stroke in a phase I study. The assessment of functional score at 12 months revealed significant improvement in fluorodeoxyglucose in the cortical areas surrounding the ischemic core in G-CSF patients compared with control patients over and above improvement in motor scale score. This effect could either be due to the increased number of stem cells in the peripheral blood(and probably in the local nervous system area) or due to possible effect of the G-CSF itself on the on neurons, it is likely that earlier the treatment, the more potent the neuroprotective effects. It appears that G-CSF may hold as an important therapeutic probability of stroke management in the future. (16)

Stem Cell Transplantation In Stroke

Carlos et al (2009) transplanted the bone marrow stem cells (BMSC) stereotactically into the perilesional area in five patients bearing sequels of stroke, to evaluate the safety of the procedure and tolerance to the transplanted cells. The cells were implanted in several points along tracts in the perilesional region. No important adverse events derived from surgery or transplants were observed during the one year follow-up period. Few patients also showed neurological improvements. (17)

Lee et al (2010) conducted a study to evaluate the long-term safety and efficacy of intra venous MSCs transplantation in a larger population. They performed an openlabel, observer-blinded clinical trial of 85 patients with severe middle cerebral artery territory infarct. 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. Of the 52 patients who were finally included in this study, 16 were the MSC group and 36 were the control group. Four (25%) patients in the MSC group and 21 (58.3%) in the control group died during the follow-up period, and the cumulative surviving portion at 260 weeks was 0.72 in the MSC group and 0.34 in the control group (log-rank; p = .058). Significant side effects were not observed following MSC treatment. The occurrence of comorbidities including seizures and recurrent vascular episodes did not differ between groups. When compared with the control group, the follow-up modified Rankin Scale (mRS) score was decreased, whereas the number of patients with a mRS of 0-3 increased in the MSC group (p =.046). Clinical improvement in the MSC group was associated with serum levels of stromal cell-derived factor-1 and the degree of involvement of the subventricular region of the lateral ventricle. The authors concluded that intravenous autologous MSCs transplantation was safe for stroke patients during long-term follow-up. (18)

The Xcell-centre, Germany has put up data of 60 stroke patients (on their website) who underwent bone marrow derived autologous stem cell transplantation. The cells were injected intrathecally by lumbar puncture. Mean follow uptime was 9 months. Over 40% of the patients reported clinical improvement with both decreased spasticity and paresis, resulting in improved walking (42%), stability (40%), and motor skills (development proximal > distal). Aphasia improved in 39% and hemianopia in 20%. (19)

NeuroGen Brain and Spine Institute presented a study of 11 stroke patients who underwent bone marrow derived autologous stem cell transplantation The mean follow up of 6 months showed that out of 11, 6 showed reduction in spasticity assessed on Modified Ashworth Scale. 6 showed improvement in speech and gait while 4 improved in hand function and 3 showed improved cognition.(20)

### **Case Report**

### 1. Hemorrhagic Stroke

### **Clinical presentation:**

60 year old female presented with a history of haemorhagic infarct in November 2008 which led to right side hemiplegia. MRI revealed left thalamic bleed with intraventricular extension. She had been treated for the same and she had a VP shunting in place. Neurologically, she was hypertonic with spasticity of Grade 1 (According to Modified Ashworth Scale), she was hyperreflexic with reduced sensations over right half of the body. She had muscle power of Grade 2 in right upper extremity and lower extremity with Grade 5 in left side. She had impaired bladder and bowel sensation and was on indwelling catheter. She also suffered from osteoarthritis. She had right elbow flexion contracture of about 35 degree and bilateral knee flexion contractures of 20 degree. She was cognitively impaired with affection of orientation in time, place and person. Functionally, she was dependent on a caregiver for all her activities of daily living (ADL). **On FIM she scored 39.** 



Axial T2W images reveal nearly well defined iso-hyperintense focus in the left corona radiata with effacement of the body of the ipsilateral lateral ventricle.

### Clinical Improvement after stem cell therapy:

Earlier, before the therapy there was total neglect of right upper extremity. According to her son, she became aware of right upper limb after the transplant.Neurologically, tightness/spasticity and clawing of right hand had reduced with improved hand opening. She could stand up independently from a chair and could maintain standing for a minute which was not possible before. She could lift her right upper extremity, move it and also open her fist, which was not possible before. She could cut vegetables, peel peas, pick up coins, and marbles with her right hand. **On FIM she improved from 39 to 84**. Emotionally, she responded better with reduction seen in depression and crying

**Case Report 1** 



*Figure 1: Patient showing reduction in spasticity of the hand and ability to open her fist.* 



Figure 2: Patient showing improved right upper limb and trunk strength and ability to stand up from the chair independently.

### **Case Report 2**



*Figure 3: Patient attempting to come to quadruped position during therapy sessions.* 



*Figure 4: Patient showing improved trunk strength and ability to perform bridging independently.* 

spells. Cognitively she showed improved attention span and she could accurately tell the time, days of the week, months of the year, colours and actively participate in conversations. She could independently transfer from chair to bed and vice versa. On bed, she could shift and roll independently. In lying position, she could lift her right lower extremity.

### 2. Right sided Hemiplegia with Cerebellar Atrophy (Left MCA Infarct)

### **Clinical presentation:**

34 Year old male presented with a history of left MCA infarct in March 2006, leading to right sided hyemiplegia with loss of speech. He was operated for decompression craniotomy following which he had neurological recovery with residual right hand spasticity and aphasia. Later, he had convulsions in 2007 and developed eptoin toxicity and was unconscious for about 2-3 months. He had residual right sided hemipleiga with aphasia and left sided incoordination. Neurologically, he was hypertonic with Grade 3 spasticity in right side upper extremity and lower extremity according to Modified Ashworth Scale. He was hyperreflexic. On examination, he had good voluntary control in right hip and shoulder, elbow and poor control at wrist and ankle on right side with near normal left side strength. He had left sided incoordination. He had speech abnormality with aphasia. He had good bowel and bladder control. Functionally, he was dependent on wife for most ADL and wheelchair bound for mobility. On FIM he scored 48. MRI brain revealed marked diffuse cerebellar atrophy along with a large left MCA territory gliotic infarct with foci of hemosiderin staining and exvacuo dilatation of adjacent lateral ventricle which represented sequelae of prior vascular insult. MRA brain and neck revealed severe attenuation of left MCA with paucity of its cortical branches, with normal EEG report.



Axial T2W images reveal encephalomalacic changes of the left frontal convexity with ex-vacuo dilatation of the frontal and occipital horn of the ipsilateral lateral ventricle.

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### **Clinical Improvement after stem cell therapy:**

Functionally, he was able to roll and come to quadruped position independently, which he could not do before. He could stand with the help of splints and walker alone for 10-15 minutes. His standing and walking balance had also improved. He was also able to move his ankle voluntarily. His head and neck control had improved along with improvement in left hand coordination. **His FIM score had increased from 48 to 52** with independence in upper body dressing. His vocalization and voice quality became louder and clearer after therapy.

### Summary

Stroke is defined as sudden neurological deficit caused by focal vascular lesion in the brain. The vascular lesion could be either haemorhagic or thromboembolic phenomena (leading to ischaemia) involving the blood vessels supplying various parts of the brain. Stem cell therapy offers hope for stroke patients, especially for those who have missed the narrow 3hour window for administration of tissue plasminogen activator. Transplanted cells secrete trophic factors that help to maintain marginally surviving cells or otherwise enhance the local environment sufficiently to improve function. Transplantation might also conceivably produce a host reaction that could include sprouting of new axons and synapse formation. It remains uncertain which type of cell would be most appropriate for transplantation into stroke patients. Various cell types (e.g., porcine foetal cells, embryonic stem cells, and immortalized neuronal cells and bone marrow stromal cells) are being investigated. Improvement are generally noticed in spasticity and paresis, resulting in improved walking, stability, and motor skills (development proximal > distal), aphasia and hemianopia.

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### Role Of Stem Cells In Motor Neuron Disease

MND is also known as Charcot's disease & Lou Gehrig's disease. It is one of the most devastating types of neurological disorder, with no known cause. MND manifests in different forms like

- 1. Amyotrophic lateral sclerosis (ALS)
- 2. Progressive muscular atrophy (PMA)
- 3. Progressive bulbar palsy (PBP)
- 4. Primary lateral sclerosis (PLS)

ALS can be defined as a neurodegenerative disorder characterized by progressive muscular paralysis reflecting degeneration of motor neurons in the primary motor cortex, brainstem and spinal cord. "Amyotrophy" refers to the atrophy of muscle fibers, which are de-nervated as their corresponding anterior horn cells degenerate, leading to weakness of affected muscles and visible fasciculations. "Lateral sclerosis" refers to hardening of the anterior and lateral corticospinal tracts as motor neurons in these areas degenerate and are replaced by gliosis. (1)

The syndrome of progressive muscular atrophy (PMA) accounts for 5-10% of patients with MND, and indicates a pure lower motor neurone syndrome without accompanying upper motor neurone signs

Primary lateral sclerosis is a clinically progressive pure upper motor syndrome that cannot be attributed to another disease process.

Progressive Bulbar Palsy is a condition with involvement of motor nuclei in the lower brain stem. (2)

Subtype	Nervous System Pathology
Amyotrophic Lateral Sclerosis	Degeneration of the corticospinal tracts, neurons in the motor cortex & brain stem & anterior horn cells in the spinal cord.
Primary Lateral Sclerosis	Degeneration of upper motor neurons
Progressive Bulbar Palsy	Degeneration of motor neurons of cranial nerves IX to XII
Progressive muscular atrophy	Loss or chromatolysis of motor neurons of the spinal cord & brainstem.

(Reference: Rowland, LP:Diverse forms of Motor neuron disease.Adv Neurol 36:1, 1982.)

### **Clinical Features:**

The features of ALS were first clearly described as a clinico-pathological entity by Jean Martin Charcot in 1869

Approximately two thirds of patients with typical ALS have a spinal form of the disease (classical 'Charcot ALS'). They present with symptoms related to focal muscle weakness where the symptoms may start either distally or proximally in the upper limbs and lower limbs. Rarely, patients may notice focal muscle wasting before onset of weakness, and some patients may present with a spastic paraparesis. Patients may have noticed fasciculations (noticed as involuntary muscle twitching) or cramps preceding the onset of weakness or wasting for some months (or years), but rarely are these the presenting symptoms. The weakness is usually of insidious onset, and patients may notice that symptoms are exacerbated by cold weather. Although it is usually asymmetrical at onset, the other limbs develop weakness and wasting sooner or later, and most patients go on to develop bulbar symptoms and eventually respiratory symptoms.

Patients with bulbar onset ALS usually present with dysarthria of speech. Rarely, patients may present with dysphagia for solid or liquids before noticing speech disturbances. Limbs symptoms can develop almost simultaneously with bulbar symptoms and in the vast majority of cases will occur within 1-2 years. Almost all patients with bulbar symptoms develop sialorrhoea (excessive drooling) due to difficulty swallowing saliva and mild UMN type bilateral facial weakness which affects the lower part of the face. 'Pseudobulbar' symptoms such as emotional lability and excessive yawning are seen in a significant number of cases.

Type of Impairment / Location	Clinical Manifestation of Pathology
Impairments related to LMN pathology	Muscle weakness, hyporeflexia, hypotonicity,atrophy,muscle cramps, fasciculations.

Common Impairments Associated with MND (3)
Role Of Stem Cells In Motor Neuron Disease

Impairments related to UMN pathology	Spasticity, Pathological reflexes, hyperreflexia, muscle weakness.
Impairments related to bulbar pathology	Dysphagia, dysarthria, sialorrhea, peudobulbar affect.
Respiratory Impairments	Exertional dyspnea, nocturnal respiratory difficulty, orthopnea, hypoventi lation.
Other Impairments	Dementia, Cognitive Impairments
Rare Impairments	Ocular Palsy, bowel & bladder dysfunction.
Indirect & Composite Impairments	Fatigue,weight loss,cachexia,decreased ROM,pain,gait disturbance,depression, anxiety, deconditioning, balance & postural control impairments

On examining the cranial nerves, the jaw jerk may be brisk, especially in bulbaronset disease. An upper motor neurone type facial weakness affects the lower half of the face causing difficulty with lip seal and blowing cheeks, but often varying degrees of UMN and LMN facial weakness coexist. The gag reflex is preserved and is often brisk while the soft palate may be weak. Patients develop fasciculations and wasting of the tongue, and tongue movements are slowed due to spasticity. Sensory examination is almost always unremarkable. Respiratory failure and other pulmonary complications are the usual cause of death in ALS.

# Summary of Revised El Escorial Research Diagnostic Criteria for ALS (Brooks et al., 2000)

The diagnosis of ALS requires:

- 1. Evidence of LMN degeneration by clinical, electrophysiological or neuropathological examination;
- 2 Evidence of UMN degeneration by clinical examination, and
- 3 Progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination,

Together with the absence of:

- 1. Electrophysiological and pathological evidence of other disease that might explain the signs of LMN and/or UMN degeneration, and
- 2. Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs UMN signs: Clonus, Babinski sign, absent abdominal skin reflexes, hypertonia, loss of dexterity.

denervation. Regions reflect neuronal pools: bulbar, cervical, thoracic and lumbosacral.

#### Pathogenesis of motor neuron degeneration in MND

Most ALS cases are sporadic but 5-10% of cases are familial, and of these 20% have a mutation of the SOD1 gene and about 2-5% have mutations of the TARDBP (TDP-43) gene. Two percent of apparently sporadic patients have SOD1 mutations, and TARDBP mutations also occur in sporadic cases. The diagnosis is based on clinical history, examination, electromyography, and exclusion of 'ALS-mimics' (e.g. cervical spondylotic myelopathies, multifocal motor neuropathy, Kennedy's disease) by appropriate investigations. The pathological hallmarks comprise loss of motor neurons with intraneuronal ubiquitin-immunoreactive inclusions in upper motor neurons and TDP-43 immunoreactive inclusions in degenerating lower motor neurons. Signs of upper motor neurone and lower motor neurone damage not explained by any other disease process are suggestive of ALS. (4,5)

#### **Conventional therapies:**

The management of ALS/MND has considerably changed over the past two decades, with an emphasis on coordinated multidisciplinary care between specialist, community based therapists and palliative care teams. Advanced directives on end of life care, respiratory and nutritional management during late stages of life are focused on.

Riluzole is the only approved drug that has been shown to have a modest effect on prolonging life in ALS patients. The mechanism of action of riluzole is not entirely certain but is thought to include interference with N-methyl-D-aspartate (NMDA) receptor mediated responses, stabilisation of the inactivated state of voltage-dependent sodium channels, inhibition of glutamate release from pre-synaptic terminals, and increasing of extracellular glutamate uptake. (6)

Despite improved understanding of the mechanisms underlying ALS, in clinical practice the management of ALS remains essentially supportive and focused on symptom relief. However, over the past few years stem cell research has expanded greatly as a tool for developing potential new therapies for treating incurable neurodegenerative diseases.

A stem-cell therapy could restore or preserve the function of both upper and lower motor neurons, and new neurons could become integrated into existing neural circuitries.

# Summary of current clinical evidence of the role of stem cells in Motor Neuron Disease

Recent studies have indicated that it is possible to generate motor neurons in culture from stem cells that include ESCs and NSCs (7) germ cells delivered into the cerebrospinal fluid of rats with motor neuron injury can migrate into the spinal cord and induce motor recovery, probably through neuroprotection. It is unrealistic to expect that the transplantation of stem cells or stem cell-derived motor neurons in ALS patients in a clinical setting would replace lost neurons, integrate into existing neural circuitry, and

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restore motor function. Rather, preventing cell death in host motor neurons via provision of neurotrophic factors by transplanted stem cells or stem cell-derived motor neurons is a more realistic and achievable approach. The recent breakthroughs in stem cell research might nevertheless provide possibilities for neural implantation and cell replacement therapy for patients with ALS.

Additionally, the studies suggested that successful stem cell therapy for ALS likely would require that the cells be combined with other drugs or treatments, such as antioxidants and/or trophic molecules.

One of the earliest studies in ALS was done by Mazzini et al (2007), an Italian group who, reported their experience with autologous bone marrow mesenchymal stem cells in nine patients with ALS. The patients received intraspinal injections of autologous MSCs at the thoracic level and were monitored for 4 years. No significant acute or late side effects were recorded. No modification of the spinal cord volume or other signs of abnormal cell proliferation were observed. Four patients showed a significant slowing down of the linear decline of the forced vital capacity and of the ALS-FRS score. Their results demonstrate that MSCs represent a good chance for stem cell cell-based therapy in ALS and that intraspinal injection of MSCs is safe also in the long term. (8) A new phase 1 study was carried out by the same group to verify this data and was reported in 2009, wherein ten ALS patients were enrolled and regularly monitored before and after transplantation by clinical, psychological, neuroradiological and neurophysiological assessments. There was no immediate or delayed transplant-related toxicity. Clinical, laboratory, and radiographic evaluations of the patients showed no serious transplantrelated adverse events. Magnetic resonance images (MRI) showed no structural changes (including tumor formation) in either the brain or the spinal cord. The authors concluded MSC transplantation into the spinal cord of ALS patients was safe. (9)

The above study was performed following a rationale that bone marrow (BM)derived cells might supply motor neurons and other cells with a cellular milieu more conducive to survival in ALS. Though the source of stem cells appears logical, it was found that direct injection of stem cells in ALS is problematic because of the large expanse of the neuraxis that would need to be injected. Hence, it was suggested that transiently increasing the number of circulating hematopoietic stem cells might be a useful therapeutic approach. However, agents stimulating the activation and mobilization of hematopoietic stem cells may have adverse effects such as activation of microglial cells.(10)

Following this line of thought, Cashman et al (2008) carried out a pilot trial of the collection and reinfusion of granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) in ALS patients and found no adverse effects, paving the way for a properly powered therapeutic trial with an optimized regimen of G-CSF. (10)

Around the same period, Tarella et al carried out a multicentre study with an aim to evaluate and characterize the feasibility and safety of bone marrow-derived cell (BMC) mobilization following repeated courses of granulocyte-colony stimulating factor (G-CSF) in patients with amyotrophic lateral sclerosis (ALS). Between January 2006 and March 2007, 26 ALS patients entered a multicenter trial that included four courses of BMC mobilization at 3-month intervals. In each course, G-CSF (5 microg/kg b.i.d.) was administered for four consecutive days; 18% mannitol was also given. Mobilization was monitored by flow cytometry analysis of circulating CD34+ cells and by in vitro colony assay for clonogenic progenitors. Co-expression by CD34+ cells of CD133, CD90, CD184, CD117 and CD31 was also assessed. Only Twenty patients completed the four-course schedule. There were two severe adverse events: one prolactinoma and one deep vein thrombosis. Circulating CD34+ cells monitored during the G-CSF courses were markedly increased. Circulating clonogenic progenitor levels paralleled CD34+ cell levels. Most mobilized CD34+ cells co-expressed stem cell markers, with a significant increase in CD133 co-expression.

The authors concluded that it was feasible to deliver repeated courses of G-CSF to mobilize a substantial number of CD34(+) cells in patients with ALS; mobilized BMC included immature cells with potential clinical usefulness. (11)

On similar lines, few more studies have been published, in which mobilization of autologous bone marrow derived stem cells has been shown to be safe and which appears to slow the decline in the deterioration of ALS patients.

Zhang et al investigated the safety and efficacy of the granulocyte colony stimulating factor (G-CSF) in 13 patients with amyotrophic lateral sclerosis (ALS). Five-day administration of 2 microg/kg once a day was followed by a six-month observation period. The primary and secondary endpoints were the changes of ALS functional rating scale (ALSFRS) and the compound muscle action potential (CMAP) amplitude, respectively. It was found that the declines of ALSFRS and CMAP amplitude after G-CSF administration were significantly less than those measured prior to the treatment. The results suggested G-CSF is safe in ALS patients, and may affect the rate of motor decline. (12)

Beatrice et al (2010) proposed to use cell subsets induced by G-CSF to slow down disease progression in patients with amyotrophic lateral sclerosis (ALS). 39 Patients with definite or probable ALS were assigned in a double-blind manner to receive G-CSF (17 patients) or placebo (18 patients) every three months for a year. G-CSF was effective in mobilizing CD34+ to blood. The outcome measures used showed no statistically significant benefit, although there was a trend of slowing disease progression following two G-CSF treatments, as shown by lower slopes of ALSFRS-R and QoL in the first six treatment months. The treatment had no major side-effects. The authors concluded that G-CSF administration in ALS patients caused successful mobilization of autologous bone marrow cells, but was not effective in slowing down disease deterioration. (13)

Some researchers, in the meantime, hypothesized that autologous stem cells may not be effective in ALS, since the genetic make-up of those cells would be similar. Even if integration into the CNS occurs, these cells again would be a subject of degeneration as the original motor neurons. Hence, Appel et al (2008), carried out a small clinical trial of allogeneic hematopoietic stem cell (from HLA-matched siblings) transplantation in six patients. Two patients died, one progressed, two experienced a slowing of progression, and one patient had an unexpectedly stable course. Although in the central nervous system, 17% to 25% of total DNA was donor-derived, in the motor cortex, less than 1% was donor-derived DNA. Unusually high numbers of CD68+ cells were found in the CNS, suggesting a neuroinflammation induced by chemokine signaling. Their study demonstrated that peripheral cells derived from donor hematopoietic stem cells can enter the human CNS primarily at sites of motoneuron pathology and engraft as immunomodulatory cells. Although unmodified hematopoietic stem cells did not benefit these sporadic amyotrophic lateral sclerosis patients, such cells may provide a cellular vehicle for future CNS gene therapy. (14)

Other routes of transplantation are also being tried in the last few years, such as direct transplantation and intrathecal routes with reasonable safety.

Deda et al (2009) carried out a clinical trial with thirteen patients with sporadic amyotrophic lateral sclerosis (SALS) who underwent bone marrow (BM)-derived hematopoietic progenitor stem cell transplantation. Patients with bulbar involvement and severe loss of movement were selected. The aim was to put the stem cells into the end of the brain stem and at the beginning of the spinal cord because the blood-brain barrier is intact in ALS and this region was the most affected part in the patients. Under general anesthesia, a total laminectomy was performed at the C1-C2 level. Stem cells were injected to the anterior part of the spinal cord. During the follow-up of 1 year after stem cell implantation, nine patients became much better compared with their preoperative status, confirmed by electroneuromyography (ENMG). One patient was stable without any decline or improvement in his status. Three patients died 1.5, 2 and 9 months, respectively, after stem cell therapy as a result of lung infection and myocardial infarction (MI). The results showed that stem cell therapy was a safe, effective and promising treatment for ALS patients. (15)

Karussis et al (2010) carried out a phase I/II open-safety clinical trial, which included 15 patients with multiple sclerosis (MS) and 19 patients with amyotrophic lateral sclerosis (ALS), to assess the feasibility, safety, and immunologic activity of autologous mesenchymal stem cells (MSCs) given intrathecally and intravenously. Patients with ALS had a mean ALS Functional Rating Scale (ALSFRS) score of  $20.8 \pm 8.0$ . No major adverse effects were reported during the 25-month follow-up. During the first 6 months of observation, the ALS group had a stable mean ALSFRS score. At 24 hours after MSC transplantation, immunologic testing showed an increase in the proportion of CD4+/CD25+ regulatory T cells, a decrease in lymphocytic proliferative responses, and expression of CD40+, CD83+, CD86+, and HLA-DR on myeloid dendritic cells. (16)

CD133(+) stem cells are known to have the capacity to differentiate into neural lineages. Martinez et al (2009) carried out a study including 10 patients with confirmed ALS to analyze the safety of autologous transplantation of CD133(+) stem cells into the frontal motor cortex. Bone marrow was stimulated with filgrastim (G-CSF) given

subcutaneously daily for 3 days. Peripheral blood mononuclear cells were obtained after admission by leukapheresis. The cell suspension was conjugated with anti-human CD133 superparamagnetic microbeads, and linked cells were isolated in a magnetic field. The isolated cells (2.5-7.5x10(5)) were resuspended in 300 microL of the patient's cerebrospinal fluid, and implanted in motor cortexes using a Hamilton syringe. Patients were followed up for a period of 1 year. The survival of treated patients was statistically higher (P=0.01) than untreated control patients. Delay in progression was recorded along with improvement in quality of life. The authors concluded that the treatment provided was safe and well-tolerated by ALS patients. (17)

Embryonic or neurogenic stem cell research will require extensive basic research to establish preclinical feasibility studies in animal models before any human studies can be performed. A number of investigators have expressed serious concern about this type of stem cell research in human patients before the necessary basic research is done (19)

The Phase I trial to evaluate the safety of Neuralstem's spinal cord stem cells in the treatment of ALS, the first FDA-approved ALS stem cell trial, has been underway since January, 2010. The trial will consist of up to 18 ALS patients, who will be examined at regular intervals post-surgery, with final review of the data to come six months after the last patient is treated. While the trial is primarily evaluating the safety of the cells and procedure, it will also seek some secondary efficacy endpoints including attenuation of motor function loss, maintenance of respiratory capacity, and stabilization of patients along the ALS functional rating scale.

In all, some progress on the safety aspects of adult stem cells has been studied in ALS/motor neurone disease. More needs to be done for other sources of stem cells as well as efficacy of the same.

At the NeuroGen Brain & Spine Institute, Mumbai out of 47 motor neuron disease patients who underwent intrathecal autologous bone marrow derived mononuclear cell transplantation, 14 showed some minor improvements whereas 33 patients kept on deteriorating with symptoms of early fatigue, weakness, muscle wasting and bulbar symptoms, which progressed with the natural course of the disease. Out of the 14 patients who improved, symptoms which showed results were improved neck holding, speech, swallowing, reduction in fasciculations and a halt in the progression of muscle weakness. (18)

# **Case Report**

#### 1. Motor Neuron Disease

#### **Clinical presentation:**

51 year old male, a known case of MND since July 2008, gives history of difficulty in speech and swallowing. Gradually, upper extremity weakness also began which spread to lower extremity. He soon developed neck drop. Neurologically, he was hypertonic and hyperreflexic in bilateral lower extremities and upper extremities with spasticity of Grade 4 in lower extremities and Grade 1 in upper extremities according to Modified Ashworth Scale. He had all sensations intact. He had Grade 0 muscle power in all the limbs and poor neck control. He had severe dysarthria with neurogenic dysphagia with oral and pharyngeal involvement. He had abdominothoracic type of breathing and used accessory muscles for respiration. Functionally, he was dependent on the caregiver for all his ADLs and wheelchair bound for mobility. **On FIM he scored 25.** On ALS Functional rating scale he scored 6

#### **Clinical Improvement after stem cell therapy:**

Neurologically, his oromotor functions had improved, he could eat & swallow rice and dal which he could not do before. His speech was clearer as compared to before. He could drink liquids easily and swallow tablets, which he could not do before. Spasticity in both his lower extremities reduced from Grade 4 to grade 3 (according to Modified Ashworth Scale). Functionally, turning in bed had become easier and effort required by caretaker was less. He was able to turn his leg on his own. **On ALS Functional Rating Scale, he improved from 6 to 8.** 

Muscles in both the upper extremities started showing improvement in strength. On Manual Muscle Testing, bilateral shoulder girdle and elbow muscles changed from Grade 0 to 2 and Grade 0 to 1, respectively. Also, strength in bilateral quadriceps improved from grade 0 to 2.

The improvements following stem cell therapy were sustained only for a brief period .The patient suffered recurrent chest infection following silent aspirations (due to his weak swallowing reflexes). He finally succumbed to chest infections leading to respiratory distress.

#### 2. Motor Neuron Disease

#### **Clinical presentation:**

40 years old female, a known case of MND, reported of sudden onset of swallowing difficulty and frequent episodes of choking while drinking water since November 2009, which gradually increased in frequency. So, PEG was implanted in December 2009, for feeding. Meanwhile, speech started getting affected with decreased clarity and volume. Soon, she developed weakness in facial muscles with difficulty in closing both the eyes,

# **Case Report 1**



Figure 1: Patient showing sustained standing on the tilt table with bilateral push knee splints and performing upper limb activities with controlled neck hiolding.



*Figure 2: Patient showing improved neck holding and ability to sit on wheel chair with erect neck.* 

# Case Report 2



*Figure 3: Patient showing improved orbicularis oculi muscle strength and voluntary closure of her eyes.* 



*Figure 4: Patient showing improved lip movements and pouting of lips with assistance.* 

#### Role Of Stem Cells In Motor Neuron Disease

whistling and blowing cheeks. Three months later, she presented with complaints of breathing difficulty, mainly in supine position. Neurologically, she had hyperreflexia. All her sensations were intact, except vibration, temperature and touch, which was decreased in upper half of the face. She had near normal muscle power in all 4 limbs with weakness in neck musculature. She had mainly facial muscle weakness and respiratory muscle involvement. On investigation, EMG confirmed diagnosis of ALS. On speech evaluation, she had mild dysarthria. **On ALS Functionally Rating Scale, she scored 26.** Functionally, she was independent in all ADL and mobile but needed PEG feeding and was on liquid diet. **On FIM, she scores 115.** 

#### **Clinical Improvement after stem cell therapy:**

Functionally, there was improvement in stamina and she could lie supine without feeling breathless. Speech clarity and volume had improved. She could eat her meals orally, without choking and had stopped having meals through PEG completely after the therapy. She also reported improved strength in facial muscles with reduced wrinkles on forehead. She could close her mouth, puff her cheeks and purse her lips which was not possible before the therapy. Her drooling of saliva had also reduced significantly & she could swallow it easily.On Manual Muscle Testing, neck muscles improved in strength from grade 3 to 4 and facial muscles improved from Grade 0 to 3. On ALS Functionally Rating Scale, she improved from 26 to 30. On FIM, she improved from 115 to 120.

# Summary

Motor neuron disease is also known as Charcot's disease & Lou Gehrig's disease. It is one of the most devastating and progressive types of neurological disorder, with no known cause. MND manifests in different forms like Amyotrophic lateral sclerosis (ALS),Progressive muscular atrophy (PMA), Progressive bulbar palsy (PBP) and Primary lateral sclerosis (PLS). Despite improved understanding of the mechanisms underlying ALS, in clinical practice the management of ALS remains essentially supportive and focused on symptom relief. However, over the past few years stem cell research has expanded greatly as a tool for developing potential new therapies for treating incurable neurodegenerative diseases. Stem cell therapy could assist symptomatic relief by preventing cell death in host motor neurons via provision of neurotrophic factors by transplanted stem cells. Recent studies also suggest that successful stem cell therapy for ALS likely would require that the cells be combined with other drugs or treatments, such as antioxidants and/or trophic molecules.

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# SECTION C

# **Important Related Aspects**



"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

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# 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.

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- 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".

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- 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

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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 and T1 injuries or incomplete injuries may have clawing or hyperextension of metacarpophalagneal joints which is due to stronger pull of finger extensors over finger flexors. Thus splints to block metacarpophalagneal joints and promote weak instrinsic muscle function.
  - Cost, time and material should be considered when deciding between custom made and prefabricated.
  - Education regarding splint wearing schedule, skin checks and splint care should be emphasized. (13)

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

be addressed in areas of sexual dysfunction, alternative behaviours, precautions and other related areas.

- Coordinated effort between client, significant other, psychologist and urologist can help with treatment of sexual dysfunction.
- Options like surgical implantation of a penile prosthesis, vacuum erection devices, intracorporeal injection therapy and use of lubricants can be used to treat sexual dysfunction.(15)

#### Psychological Aspect in Spinal Cord Injury

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

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

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

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

#### 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)

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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)

# Importance of Neurorehabilitation - Concept of NRRT

# 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)

- 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 taskoriented 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.

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.

# Importance of Neurorehabilitation - Concept of NRRT



*Figure 1: Exercises showing trunk strengthening activity for a quadriplegic patient.* 



*Figure 2: Exercises showing strengthening of intrinsics of foot.* 



Figure 3: Trunk strengthening activity on swiss ball.



Figure 4: Exercises showing weight shifts in quadruped position.



Figure 5: Swiss ball activity showing trunk strengthening and dynamic sitting balance activity.



*Figure 6: Suspension therapy to strengthen the lower extremities.*


*Figure 7: Recreational activity, on standing board, to strengthen upper extremity for muscular dystrophy patients.* 



*Figure 8: Balance board and reach out activities to train dynamic standing balance.* 



*Figure 9: Initiation of gait training with the help of walker.* 



*Figure 11: Gait training with bilateral push knee splints.* 



*Figure 10: Activities to improve hand function and training of sitting balance.* 



Figure 12: Over head activities on standing board, to strengthen upper extremity and improve hand function.

Importance of Neurorehabilitation – Concept of NRRT

**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.

# Summary

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. The philosophic foundation of Rehabilitation team is to promote purposeful activity thereby preventing dysfunction and eliciting maximum adaptation.

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# Complications

Cell replacement therapy is an exciting research area and t 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 the 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.

Complications in stem cell therapy could be because of the stem cells per se and due to the surgical procedure involved in the therapy.

# Complications due to 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 deligent research will be required before using various human ES cell lines for cell transplantation as a therapeutic option for patients with degenerative disease.

However, in the literature reviewed, so far, for treatment of neurological diseases using autologous adult stem cells, we have not come across any reported complication, such as tumorogenecity, on subsequent use of these cells. None of the published human case reports have reported any major adverse events. Also, no cases of deterioration of the neurological conditions has been attributed to the stem cell transplantation per se so far.

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

#### Complications of the Implantation procedure

Whilst there are no cases reported on any complications due to the stem cells perse, there are many possible complications that can occur due to the procedure of injecting or implanting the stem cells. These complications , generally, are those of the surgical procedure.For instance, following are common side effects of the baone marrow aspiration and the intrathecal injection procedure:

(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.

### Complications

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 of 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 and intrarterial injections, have possibilities of side effects or complications, specific to the respective procedures.

# Summary

Though,ES cells and fetal stem cells may provide potential treatment options for degenerative diseases in the future, their unlimited self-renewal and high differentiation potential poses the risk of tumor induction after engraftment. Hence, adult stem cells are preferred, since spontaneous malignant transformation of adult stem cells in neurological disorders have not been reported yet.Howeever, caution and diligent recording still needs to be continued to pick up side effects and complications of stem cells, so that they can be used safely.

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# **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 guidelines for stem cell research more than two years ago.



### Regulation of Stem Cell Therapy

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

#### Regulation of Stem Cell Therapy

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. However, 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

### Regulation of Stem Cell Therapy

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.

# Summary

A host of ethical and moral issues are associated with the evolving stem cell field, 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. A overview of the regulatory procedures in the global players shows that these vary from non existent 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.

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# **Ethics**

Consensus on the potential of stem cell therapy to address various incurable, debilitating disorders is unanimous. Stem Cell research and therapy is the 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

### Ethics

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 EScells 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 EScell 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 cellreplacement 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

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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 interexperimental 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

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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

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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 must recognize that small improvements that do not mean anything to us can mean a lot to a patient with severe physical limitations. That at the end of the day all ethics, moral, values principles, laws and regulations have just one purpose. The well being of the common man.

What has unfortunately happened in the field of stem cell therapy is that the regulations we have made to protect ourselves are now limiting us and tying us up. These regulatory chains need to be unshackled. Physicians need to be free to use whatever modality of treatment they believe is in the patients best interests. However the other side of the argument is that these are helpless patients and they are likely to be exploited by physicians offering stem cell therapy. We must

however note that there are black sheep in every profession. That those who don't have values and principles are doing all manner of unprincipled and unethical practices with conventional treatments also. On the other 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. Ethics

# Summary

The ethics in stem cell research basically revolves around embryonic stem cell research which been hounded by objections and restrictions due to the source of its procurement, by various religious bodies of the world.Apart from religious issues, embryonic stem cell use involves aspects realed to side effects, such as teratoma formation and rejection of allogenic tissue.Adult stem cells, so far have been subject of very little controversy.Only ethical aspects regarding its proper, controlled use for various disorders needs to be worked out.

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# **Future Directions**

There are two basic aspects of the way stem cell therapy is being done at present that are likely to change in future, as it becomes an integral part of medical practice. Presently stem cell therapy is (i) Being done for chronic cases and late stage diseases with relatively advanced and irreversible neurological deficits and (ii) Stem cell therapy is being done as a stand alone procedure.

In the future both these are likely to change in that(a) It is more likely to be done in the acute setting and in the earlier stages of the injury / disease and (b) It is likely to get amalgamated with the more conventional medical and surgical treatments and so be offered in conjunction with other therapies.

Work being done at the Department of Neurosurgery of the LTMG Hospital & LTM Medical college in Mumbai highlights the above. The department is embarking on two phase I and II prospective randomized controlled trials both of which are being done in the acute setting in conjunction with the conventional treatments.

- (i) In the study on acute MCA infarcts, stem cell therapy is being combined with a surgical bypass procedure and being compared to the best medical treatment.
- (ii) In the study on acute spinal cord injury, conventional decompression and stabilization is being combined with intraspinal stem cell transplantation and this is being compared to surgical decompression and stabilization alone.

A brief summary of the protocols of the 2 trials are as follows:

#### Study 1: Acute MCA Infarct Trial

This will be a single centered Phase I/ phase II randomized, open label, controlled

trial to evaluate the safety and efficacy of antilogous bone marrow derived stem cell transplant and superficial temporal artery to middle cerebral artery (STA-MCA) Bypass surgery in patients with acute ischemic middle cerebral artery (MCA) territory stroke.

Primary objective is to evaluate the safety and secondary objective is to evaluate and compare the efficacy of autologous bone marrow stem cell transplant and superficial temporal artery (STA) middle cerebral artery (MCA) bypass in patients with acute ischemic middle cerebral artery (MCA) territory stroke.

There are four arms of the trial-A) Best medical treatment (BMT) [according to AHA guidelines], B) BMT + Superficial temporal artery to middle cerebral artery (STA-MCA) bypass surgery, C) BMT + Autologous bone marrow stem cell transplant intravenously and D) BMT + STA-MCA bypass surgery with autologous bone marrow stem cell transplant intraarterially into the cerebral artery.

The hypothesis is that the functional improvements measured with Barthel index in patients with acute ischemic MCA territory stroke will be more than that with best medical treatment alone in groups receiving stem cell treatment IV along with best medical treatment and in groups receiving STA MCA bypass surgery along with best medical treatment and hence also in a combination of these two therapies. The last two modalities (STA MCA Bypass & stem cell treatment) could offer a synergistic effect which would be expected to cause additional improvement more than the either of two alone.

Sample size is 11 patients per arm (n=44) [p=0.01, power=95% and considering 20% dropout rate].

Inclusion criteria include - a) Male or female of 18 - 70 years, b) acute (< 7 days) ischemic MCA stroke as determined by clinical history & examination and demonstrated by Perfusion Computed Tomography brain, Computed Tomography Angiography of brain & Positron Emission Tomography- Computed Tomography (PET-CT) scan of the brain, c) signs and symptoms of MCA territory stroke with NIHSS score of > 15 at presentation and who does not improve on NIHSS score > 5 during evaluation for eligibility for inclusion, d) Cerebral angiography showing impaired collaterals and either- (i) Stenosis or occlusion of the ipsilateral middle cerebral artery, or (ii) Stenosis of the ipsilateral internal carotid artery at or above the C2 vertebral body (i.e. inaccessible to carotid endarterectomy), e) PET-CT Scan of Brain showing impaired cerebral blood flow in these patients with stenosis / occlusion of the internal carotid or MCA and decreased cerebral reserve.

Exclusion criteria include a) received thrombolytic treatment for stroke, b) nonatherosclerotic conditions causing or likely to cause cerebral dysfunction (fibromuscular dysplasia, arteritis, blood dyscrasia, c) severe head injury with skull fracture / EDH / SDH / Intracranial hemorrhage, d) previous neurosurgery/ craniotomy, e) any other acute or chronic neurological disease besides acute ischemic MCA stroke.

Parameters of study include- A)Clinical- Barthel Index, NIH Stroke Scale, Modified Rankin Score, B)Investigations- MRI - Perfusion / Diffusion, DSA (Cerebral angiography), PET-CT SCAN. These parameters will be recorded/evaluated during enrollment, at 3 months and 6 months post intervention. Future Directions

# Study 1: Management of acute MCA infarct : STA-MCA Bypass combined with stem cell therapy (Fig. 1 to 6)



Figure 1:



Figure 2



Figure 3

Figure 4



Figure 5



# Study 2: Management of acute dorso lumbar spine trauma : Pedicle screw and rod fixation combined with stem cell therapy.







#### Future Directions

#### Study 2:- Acute Dorsolumbar Spinal Cord Injury Trial:-

A single centered Phase I/ phaseII randomized, open label, controlled trial to evaluate the safety and efficacy of autologous bone marrow derived stem cell transplant in patients with acute dorsolumbar spinal cord injury with fracture dislocation and compression with complete paraplegia that require surgical decompression and stabilization. Aim is to provide a better quality of life to patients suffering from acute dorsolumbar spinal cord injury with fracture dislocation and compression with complete paraplegia.

Primary objective is to evaluate the safety and secondary objective is to evaluate the efficacy of autologous bone marrow stem cell transplant in patients with acute dorsolumbar spinal cord injury with fracture dislocation and compression with complete paraplegia who require surgical decompression and stabilization.

There are two arms of the trial- A) Standard Surgical Treatment (decompression and stabilization, B) Standard Surgical Treatment + Autologous bone marrow stem cell transplant.

The hypothesis is that the clinical improvement measured with American Spinal Injury Association (ASIA) Impairment Scale in patients with acute dorsolumbar spinal cord injury with fracture dislocation and compression with complete paraplegia that require surgical decompression and stabilization will be more in patients receiving a combination of Standard surgical treatment and autologous bone marrow derived stem cell transplant as compared to patients receiving standard surgical treatment alone.

Sample size is 20 patients per arm (n=40).

Inclusion criteria include-a) Male or female of 18 - 70 years, b) acute (< 48 hours) dorsolumbar spinal cord injury with fracture dislocation and compression with complete paraplegia (Grade=0 power in both lower limbs) that require surgical decompression and stabilization as determined by clinical history, examination and MRI of spine, performed for clinical reasons.

Exclusion criteria include- a) Any other acute or chronic neurological disease besides acute dorsolumbar spinal cord injury with fracture dislocation and compression with complete paraplegia like multiple sclerosis, stroke etc., b) Penetrating spine injuries, c) Associated head injury, d) Previous spine injury.

Parameters of study include- A) Clinical- American Spinal Injury Association (ASIA) Impairment Scale, B) Investigations- MRI Scan of spine and Tractography. These parameters will be recorded/ evaluated during enrollment, at 3 months and 6 months post intervention.

#### Case report:

A case report of how stem cell transplantation will ,in future ,get integrated into the acute management methods of acute neurotrauma:-

In which is the first case of its kind, a patient with a dorsal spinal bullet injury and a resultant paraplegia ,in the acute setting , underwent the following : [1] Removal of the bullet from the spine, [2] Surgical decompression via laminectomy and corpectomy and [3] Stabilization with titanium pedicular screws and rods. Despite a near complete cord transaction and a complete spinal cord injury following this combination, he showed a improvement both in his sensations and in his limb power. A brief summary is as follows:-

A 45-year-old male presented with history of single penetrating gunshot injury to the left flank. Immediately after the injury , he developed bilateral flaccid paralysis of the lower extremities, sensory loss below waist and bowel and bladder incontinence. There was no other associated injury. On local examination, there was an entry wound at left dorsolumbar region approximately 4 cm from the midline with no exit wound. Neurological examination revealed sensory level at L1, sensory loss below L1, paraplegia with grade 0 power in both lower limbs. Computed tomography (CT) scan revealed fracture left laminae of L1-L2 vertebra with multiple small fragments of bone in spinal canal at L1-L2 level. Bullet was seen lodged in left half of L2 vertebral body. This was suggestive of bullet traversing from left side through L1 spinous process and spinal canal and getting lodged in right half of L2 vertebral body. Surgical decompression with bullet removal and spine stabilization, and autologous bone marrow stem cell transplant in to the spinal cord was done simultaneously. L2 laminectomy was done and dural tear with ragged dural edges, with cerebrospinal fluid leak was found. The bone fragments in the canal were removed. After opening the dura, transaction of nerve roots at that level with severely contused cord and anterior dural tear were also noted. After retraction of the thecal sac, bullet was seen lodged in the L2 vertebral body which was removed. The stem cells, which had been obtained from the patients bone marrow , were directly injected into the spinal cord 1 cm above and below the damaged cord bilaterally, entering through the dorsal root entry zone (DREZ). Then dural edges were defined and repaired with local facial patch. As posterior element (spinous process, lamina, facet joint) and L2 body anteriorly were fractured, spine was stabilized with transpedicular titanium screw & rod. The patient improved after the operation. At 5 months of follow up the patient has improved from ASIA scale A to C.

This case highlights the beneficial clinical effects of the combination of spinal decompression and stabilization (conventional therapy) with stem cell therapy (new therapy) done in the acute setting. This case also indicates what the future of stem cell therapy will be. We would therefore like to end this book with the same statement that we started the book with. Yes, "Stem cell therapy is an idea whose time has come."

# Summary

As Stem Cell Therapy gets more established, its real future lies it is being amalgamated with the more conventional treatment forms. Also, it is more likely to be used as the initial part of therapy in the acute stages rather than for chronic and late stage diseases. An example of such mergers are the two clinical trials on Acute MCA infact and Acute Dorsolumbar spinal cord injury that are being initiated at the Department of Neurosurgery of the LTMG Hospital in Mumbai.

# Appendix

# Appendix I

# **Major Ongoing Clinical Trials**

## 1. Geron Corp. Menlo Park, California

First FDA approved Human Clinical Trial of Embryonic Stem Cell-Based Therapy. The study will evaluate the safety of cells derived from human embryonic stem cells (hESC). The trial will transplant oligodendroglial precursor cells (OPC's) derived from an early (hESC) line into spinal cord injury patients.

#### 2. Neuralstem, Inc, Emory University, USA

The Phase I trial to evaluate the safety of Neuralstem's spinal cord stem cells in the treatment of ALS, the first FDA-approved ALS stem cell trial, has been underway since January, 2010. The trial will ultimately consist of up to 18 ALS patients, who will be examined at regular intervals post-surgery, with final review of the data to come six months after the last patient is treated. While the trial is primarily evaluating the safety of the cells and procedure, it will also seek some secondary efficacy endpoints including attenuation of motor function loss, maintenance of respiratory capacity, and stabilization of patients along the ALS functional rating scale.

#### 3. Miami Project to Cure Paralysis, Miami, USA

The Miami Project to Cure Paralysis is a research center dedicated to research in the field of paralysis and spinal cord injury, with the eventual object of finding a cure for paralyzing injuries. Based at the Leonard M. Miller School of Medicine of the University of Miami, it is considered a world leader in neurological injury research. One of The Miami Project's most anticipated human clinical trial initiatives is Autologous Schwann Cells for acute and chronic spinal cord injury. The trial is yet to gain approval from the Food and Drug Administration (FDA) to begin a Phase 1 trial.

### 4. TCA Cellular Therapy, LLC Covington, LA.

They are carrying out a Phase I trial to assess the safety of intrathecal infusion of

autologous bone marrow derived mesenchymal stem cells for SCI. A couple of thousand adult stem cells have been extracted from the patient's own bone marrow, Mesenchymal Stem Cells have been separated, purified, multiplied to millions and will be infused into the patients and its safety will be assessed

5. StemCells Inc.

StemCells, Inc has received authorization from Swissmedic, the Swiss regulatory agency for therapeutic products, to initiate a Phase I/II clinical trial in Switzerland of the Company's proprietary HuCNS-SC(R) product (purified human neural stem cells) in chronic spinal cord injury. The trial is designed to assess both safety and preliminary efficacy in patients with varying degrees of paralysis who are 3 to 12 months post-injury.

#### 6. Duke University, USA

They are carrying out a randomized study of Autologous Umbilical Cord Blood reinfusion in children with Cerebral Palsy. The purpose of this study is to determine the efficacy of a single intravenous infusion of autologous umbilical cord blood (UCB) for the treatment of pediatric patients with spastic cerebral palsy.

### 7. Medical College of Georgia, USA

The researchers are conducting the first FDA-approved clinical trial to determine whether an infusion of stem cells from umbilical cord blood can improve the quality of life for children with cerebral palsy. The study will include 40 children age 2-12 whose parents have stored cord blood at the Cord Blood Registry in Tucson, Ariz.

# Appendix II

# **Regenerative Medicine Journals**

- 1. Cellular Reprogramming (formerly Cloning and Stem Cells) Editor-in-Chief, Professor Sir Ian Wilmut jane.taylor@ed.ac.uk http://mc.manuscriptcentral.com/cellreprogramming
- 2. Stem Cell Research & Therapy Editors-in-Chief Rocky Tuan, Timothy O'Brien http://stemcellres.com/
- 3. Cell stem cell Editor-in chief Emilie Marcus emarcus@cell.com / stemcell@cell.com

# 4. Stem cell reviews and report. Editor-in-Chief: Kursad Turksen kturksen@ohri.ca. http://www.springer.com/life+sciences/cell+biology/journal/12015

5. Stem cells Editor-in-Chief Miodrag Stojkovi http://www.stemcells.com

- 6. Current protocols in stem cell biology Editor- in- Chief Andrew Elefanty http://cda.currentprotocols.com/WileyCDA/CPTitle/isbn-0470528311
- 7. Current stem cell research & therapy http://bentham-editorial.org/ samina@benthamscience.org www.benthampublishingservices.com
- 8. Journal of hematotherapy & stem cell research Editor- in-Chief Denis English www.liebertonline.com/toc/scd.2/8/4
- 9. Nature reports. stem cells Editor- in-Chief Monya Baker www.nature.com/stemcells/
- **10.** The open stem cell journal Editor- in-Chief Bruce A. Bunnell toscj@benthamopen.org

## **11.** Stem cell research Editor- in-Chief A. Elefanty

Andrew.Elefanty@med.monash.edu.au

# **12. Stem cells International** Editors

Nadire N. Ali Anthony Atala sci@sage-hindawi.com

# 13. Stem cells and development

Editor- in-Chief Graham C. Parker gparker@med.wayne.edu http://www.liebertpub.com/products/eboard.aspx?pid=125

## **14.** Journal of tissue engineering and regenerative medicine Editor- in-Chief Rui L. Reis

cs-journals@wiley.com

## **15.** The open tissue engineering and regenerative medicine journal Editor- in-Chief Cesario V. Borlongan totermj@benthamopen.org

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**16. Regenerative medicine** Editor- in-Chief Chris Mason info@futuremedicine.com

## 17. Bone marrow transplantation Editor- in-Chief Linda Casey bmtran@imperial.ac.uk http://www.nature.com

# 18. Cell transplantation Editor- in-Chief Paul R. Sanberg Camillo Ricordi celltransplantation@gmail.com http://www.cognizantcommunication.com/filecabinet/Cell/ct.htm

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In 1995, he worked at the Karolinska hospital in Stockholm Sweden, where Neural transplantation was done for the first time in the world and in 1998 worked at the University of Colorado health sciences center in Denver, USA where the world's first randomized trial for fetal cell transplantation was done for Parkinson's Disease. Its his life's mission and passion to bring about regeneration within the nervous system. He setup the stem cell and genetic research laboratory at the LTMG hospital which was the first of its type in Mumbai. He is a Neurosurgeon, Medical teacher and Scientist attempting to combine the best of science, medicine and humanity to alleviate the suffering of patients with neurological disorders. He is a staunch believer that stem cell therapy can relieve a lot of human suffering of neurological patients and makes every attempt to popularize this new approach amongst the medical community. This book is one such attempt for the same purpose. He can be reached at **alok276@gmail.com or Ph: +91 22 25283706, +91 9820046663.** 

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Price : ₹ 1500 ISBN : 81-86876-06-5