Introduction

Stem cells and their unique properties: Stem cells are special cells which not only have the ability of self-renewal but can also be a lifelong source of specialised functional cells of different human organs. Development of a human embryo into a healthy new-born child is possible because of the unique ability of embryonic stem cells to form different tissues and organs. Most adult human tissues and organs also have stem cells that can produce their functional specialised cells as and when required. The self-renewal ability of stem cells ensures that stem cells are not depleted and enough stem cells remain to produce sufficient number of specialized cells of that organ during the long human lifespan, until aging starts affecting stem cells.

Stem cells in Regenerative Medicine and human diseases: When a disease or injury causes severe depletion of the functional cells of a human organ or system, the function of that organ or organ system is lost. In the natural healing process, some organs such as skin, blood, liver etc. can often regenerate its form and function by producing sufficient numbers of new functional cells from the stem cells present in them. However, specialized cells of some organs like the nerve cells in the brain, spinal cord, eyes and muscles have limited or no capacity to regenerate and restore full function. In the last two decades, medical science has undertaken extensive research to explore the potential of stem cells from the same organ or tissue type (homologous use) or from a different organ or tissue type (non-homologous use) to restore some lost bodily function. These stem cells may be from the same person (autologous source) or from another person (allogeneic source). Research to regenerate the form and function of a human organ or organ system from stem cells or tissue engineering is called 'Regenerative Medicine'.

Status of Stem cells in Regenerative Medicine and human diseases: Unfortunately, the promise of Regenerative Medicine in general, and stem cells in particular, is yet to be realized due to several technical, biological, ethical and medical challenges. To produce sufficient number of specialised cells for restoring a lost body function with just a small number of stem cells or by using stem cells from one organ to restore cells and function of a different organ (such as mesenchymal stem cells in bone marrow or fat tissue to restore nerve or muscle function) has proven to be far more difficult in humans than what was thought based on animal experiments. As a result, the inherent appeal of stem cells has remained largely unfulfilled in human diseases. The exception is however the use of "Haematopoietic Stem Cells" for reconstituting or regenerating the bone marrow in order to start producing blood and immune cells. Transplantation of enough number of "Haematopoietic Stem Cell" in a procedure called

Bone Marrow Transplantation or Haematopoietic Stem Cell Transplantation from the same person (autologous) or from another human donor (allogenic) is a recognized medical indication of stem cell use for benign and malignant life threatening haemato-lymphoid diseases or few immune related diseases. Haematopoetic stem cells are also progenitors for other cells like osteoclasts and have successfully used in osteopetrosis and some inborn errors of metabolism like Gaucher disease, mucopolysaccharidosis. Use of other types of stem cells and even the bone marrow derived stem cells to restore function of other organs remains experimental and is subject of ongoing controlled clinical trials. Not only the efficacy of these experimental stem cell use is uncertain, the process of taking out stem cells, culturing or growing them, storing them and putting them back can cause changes in these cells and sometimes serious side effects, including some reported cases of cancers.

Why Stem cells continue to be used for debilitating or incurable conditions outside controlled research studies: A large number of controlled prospective research studies (phase I, II and III clinical trials) investigating the safety and efficacy of stem cells for different diseases have been completed or are ongoing in Europe, USA, Korea and Japan. A small number of such research studies are also being conducted in other countries, including India. All developed countries have taken a very cautious and stringent regulatory approach regarding how different types of stem cells can be procured, processed, stored and used for preclinical or clinical research or as stem cell therapy outside research studies. Participants of regulated interventional research in any field, including stem cells, are made aware through a detailed written informed consent process about the experimental nature of the therapy, unproven efficacy and uncertainty regarding the benefits and risks of stem cells, the natural history of the disease, current standard therapy for that disease and any alternative treatments. It is the duty of the research sponsors to provide free of cost medical tests and treatments done as part of stem cell clinical trial and research, including the cost of procuring, storing and using stem cells. Circumventing the route of rigorous research studies to establish the safety and efficacy of a particular type of stem cells for a specific disease or aging condition, some unlicensed or even licenced and registered medical practitioners engage in unethical practices of selling unproven stem cell therapy as a magical remedy to desperate families with incurable and potentially fatal diseases with little or no hope of cure from other methods. Desperate patients from around the world including USA and Europe with stricter enforcement of regulations for stem cell use outside clinical trials get lured to stem cell clinics in South America, China, Russia and India. The US FDA and European Medical Agency has warned against this practice through several such advisories.

https://www.fda.gov/consumers/consumer-updates/fda-warns-about-stem-cell-therapies

https://www.fda.gov/news-events/press-announcements/statement-stem-cell-clinic-permanent-injunction-and-fdas-ongoing-efforts-protect-patients-risks

https://www.fda.gov/news-events/press-announcements/federal-court-issues-decision-holding-us-stem-cell-clinics-and-owner-adulterated-and-misbranded-stem

Is Stem cell research permitted or encouraged by the governmental agencies?

The unethical and unregulated use of stem cells as, often promoted as a magical remedy is not allowed by the government in the developed world and many Low and Middle Income Countries (LMIC) including India. However, considering the incurable nature of many diseases, and the acknowledged potential of stem cells, most countries, including India, encourage and fund scientific, ethical and regulated research in the field of stem cells. The purpose of such research is to obtain safety and efficacy data with the use of a particular type of stem cell in a particular condition. To provide guidance and to facilitate human research in stem cells, while curbing exploitation of vulnerable patients, the Indian government through the Indian Council of Medical Research (ICMR) has come out with successive National Guidelines in this field since 2007. The most recent National Guidelines for Stem Cell Research with inputs from all stakeholders including various government agencies and regulators, patients, medical and scientific experts and the industry, was released in 2017. These guidelines are revised at regular intervals to incorporate any new evidence for the safety or efficacy of stem cells.
https://www.icmr.nic.in/sites/default/files/guidelines/Guidelines_for_stem_cell_research_2017.pdf

Need for National Guidelines for evidence-based use of Stem cells as a routine or standard treatment option: In many countries including India, there is a lack of clarity among patients, and to some extent among the medical community, whether stem cell therapy can be considered as a standard treatment option for a specific medical condition or should remain as an unproven experimental approach. There are several reports of increasing use of stem cells therapy for a wide range of diseases, often with little or no scientific evidence of efficacy or cure. Unethical promotions with false claims and misleading advertisements have been widely used to promote unscientific stem cell therapy. Several instances of public exploitation and grievances from members of the public have been received by the ICMR and other government agencies from aggrieved patients describing how they were lured into unproven stem cell therapies. Often the complainants demanded actions to be taken by the regulatory agencies and professional bodies to curb such practices. With this background, the Govt. of India has entrusted the ICMR to frame guidelines on stem cell therapy.

In order to develop a scientific and unbiased guideline for evidence based use of stem cell as a routine or standard treatment option in India, the ICMR has solicited opinion from expert clinicians, professional medical societies and through its website from any clinician or member of public to submit level I or level II scientific evidence for clinical efficacy of stem cells in any

disease indications with reference for such evidence from peer reviewed Pubmed indexed medical and scientific journals.

https://icmr.nic.in/content/icmr-inviting-level-i-or-level-ii-scientific-evidence-and-grade-or-b-recommendation-use-stem

A critical review of the comments and evidence provided by medical experts and their professional societies or any member of the public and the scientific literature was done to draft guidelines and statements for evidence-based use of stem cell therapy.

Statements have been prepared for individual diseases or groups of diseases or conditions on the "EVIDENCE BASED STATUS FOR THE USE OF STEM CELLS IN (Disease condition)". In these statements the first section is for the public and patients using layman terms while the second section is for doctors, scientists and allied healthcare professionals providing major research studies in the scientific literature, scientific level of evidence and a summary recommendation based on the current scientific evidence.

International Society for Stem Cell Research (ISSCR)

The International Society for Stem Cell Research (https://www.isscr.org/) is the leading professional organization of stem cell scientists and represents over 4,000 members in 67 countries including India. Like ICMR in India, FDA in USA, EMA in Europe, this international society also felt the urgent need to address the growing public concern regarding the unscientific or unethical use of stem cell therapy. The ISSCR has also issued a statement on reporting false marketing claims and adverse events from clinics offering unapproved stem cell therapies.

https://www.closerlookatstemcells.org/patient-resources/how-to-report-false-marketing-claims-and-adverse-events-from-clinics-offering-unapproved-stem-cell-therapies/.

In parallel with the ICMR initiative and public advertisement inviting comments and evidence for stem cell use from public and medical professionals, the ISSCR has also come out with factsheets on current status of stem cell use. The ISSCR document highlights that other than Hematopoietic stem cell (also called Bone Marrow) transplant for certain haematological or immune system disorder, the "list of diseases for which stem cell treatments have been proven to be beneficial and/or have obtained regulatory approval for use is still very short" and that "some bone, skin and corneal (eye) injuries and diseases can be treated by grafting or implanting tissues in which stem cells are essential for the healing process".The ISSCR cautions that "However, clinics around the world continue to provide unproven stem cell treatments and often market them as cures for a variety of diseases and conditions without sound scientific

evidence or regulatory approval. These so-called treatments have, in some cases, caused patients great harm physically, and at great expense financially".
https://www.isscr.org/professional-resources/scientific-professional-resources/disease-fact-sheets
https://www.isscr.org/scientific-clinical-resources/disease-fact-sheetshttps://www.closerlookatstemcells.org/2020/01/14/truths-around-stem-cell-treatments/

The ISSCR concise factsheets provide the current state of stem cell science for specific diseases, including background on the disease, rationale for using cell-based therapies, evidence for specific approaches and current status of the field with respect to clinical trials. A total of 11 conditions have been covered so far.

1. Age-related macular degeneration
2. Amyotrophic lateral sclerosis
3. Chronic obstructive pulmonary disease
4. Diabetes
5. Huntington's disease
6. Liver disease
7. Multiple sclerosis
8. Myocardial infarction / Heart failure
9. Osteoarthritis
10. Parkinson's disease
11. Paediatric leukodystrophies

Evidence Based Status of Use of Stem Cells in Duchenne Muscular Dystrophy

A. Information for public and patients

What is Duchenne Muscular Dystrophy (DMD)?

Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration and weakness due to the alterations of a protein called *dystrophin* that helps keep muscle cells intact. DMD occurs because of a change in the DMD gene that results in the decrease of the dystrophin protein in the muscle cells. As a result of this the muscle becomes frail and does not work efficiently. DMD is one of four conditions known as dystrophinopathies. The other three diseases that belong to this group are Becker Muscular dystrophy (BMD, a mild form of DMD); an intermediate clinical presentation between DMD and BMD; and DMD-associated dilated cardiomyopathy (heart-disease) with little or no clinical skeletal, or voluntary, muscle disease. DMD symptom onset is in early childhood, usually between ages 2 and 3. The disease primarily affects boys, but in rare cases it can affect girls. The children have difficulty in climbing stairs, jumping and running. They develop a waddling walk and can have prominent calf muscles.

What is the standard treatment for DMD?

Patients with Duchenne Muscular Dystrophy are advised supportive physical therapies along with steroids as the standard of care. The two options for steroids are prednisolone or deflazacort, as indicated. They increase muscular strength and retard the progression of disease. They also reduce the need for scoliosis surgery, improve lung and cardiac function. Other drugs that are used for older patients include beta-blockers and angiotensin-converting enzyme inhibitors to delay DMD cardiomyopathy.

Recently precision therapy using the mutation type is FDA approved. Exon skipping with ASO (antisense oligonucleotides) bind to a specific mRNA to allow exon skipping and formation of a normal short dystrophin protein. This restores the reading frame of the protein. There is some expression of the shortened but functional dystrophin protein. Exon skipping related to exons 51and 53 are currently approved for treatment of patients amenable to this therapy. Ataleuren, a small molecule for treatment of a specific type of DMD mutation is used under conditional approval in Europe. It is important to follow up with a doctor who has experience in managing patients with DMD

Have stem cells been used in DMD?

Along with this management, few studies have tested the use of various forms of stem cells to treat patients with DMD and repair the function of damaged muscle. Cell therapy approaches

aim to provide new cells to patients to compensate for their deteriorated cells in the targeted tissue. Cells can be from a donor or from the patient himself. Both approaches have advantages and disadvantages. While cells from the donor are mutation free; an immune reaction is a risk. Autologous cells have lower risk of causing an immune reaction; however, the cells would need to be repaired prior to reinjection. However most of these studies have one or more flaws and do not support the use of stem cells over the current standard of care treatment for patients with DMD.

The muscles in the human body are of various types eg trunk muscle, limbs muscle. Their origin and programming is different as is the type of muscle fibre they are composed of. To derive each kind from a stem cell in the correct position is currently not achieved. Making a mature skeletal muscle from the stem cell using current methods is limited.

We are aware that many patients with DMD in India are offered and have undergone stem cell therapy for DMD. ICMR with expert inputs from various specialists in this field, has reviewed extensively and discussed existing scientific and medical literature related to stem cell therapy in DMD. Consensus statements from various professional societies world over have also been reviewed and they also do not recommend use of stem cells in DMD without genetically engineering them to correct the gene defect. The latter are also in clinical trials and not the standard of care world over.

Recommendations (2021): Based on the review of available scientific evidence, stem cell therapy should NOT be offered as a standard or routine therapy to patients with Duchenne Muscular Dystrophy (DMD). These guidelines will be periodically reviewed for any new evidence showing benefit or harm with the use of stem cells for Duchenne Muscular Dystrophy.

CAUTIONARY NOTE

Use of any type of stem cell in DMD should be restricted to clinical trials that have necessary approval from regulatory authorities in India. These trials should follow the National Guidelines on Stem Cell Research and patients in these trials should be closely monitored for the possibility of any harm with use of stem cells. As per the ICMR National Bioethics guidelines 2017, trial participants should have read and signed the informed consent form which explains them existing standard of care, alternative therapies, possible benefits as well as harm due to experimental treatments like stem cell therapy. Participants should not be made to pay for any expenses incurred beyond routine clinical care and which are research related including tests, investigations and any interventions (such as stem cells). This is applicable to all participants, including those in comparator/control groups. Participants in a

clinical trial should be provided compensation in the event of any harm or permanent injury or death due to the use of experimental stem cell therapy.

B. Information for Medical / Scientific / Allied Health Professional

Duchenne Muscular Dystrophy occurs due to a mutation in the dystrophin gene. The disorder causes progressive muscular damage and degeneration occurs in people with DMD, resulting in muscular weakness, associated motor delays, loss of ambulation, respiratory impairment, and cardiomyopathy. In 2018 DMD care considerations, were published which were supported by the US Centers for Disease Control and Prevention (CDC) with involvement of the TREAT-NMD network for neuromuscular diseases, the Muscular Dystrophy Association, and Parent Project Muscular Dystrophy
(Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac,bone health, and orthopaedic managementDiagnosis and management of Duchenne muscular dystrophy, part 3: primary care, emergency management, psychosocial care, and transitions of care across the lifespan)

The details of some of the studies of the use of stem cells in DMD are given below:

Duchenne Muscular Dystrophy	
S.No.	**Review of Literature** **Critique / Applicability of the study results**
i.	Source:Wharton jelly-derived MSCs (WJ-MSCs) RoA:four doses of intramuscular and four doses of intra-arterial Dai A, Baspinar O, Yeşilyurt A, Sun E, Aydemir Çİ, Öztel ON, Capkan DU, Pinarli F, Agar A, Karaöz E. Efficacy of stem cell therapy in ambulatory and nonambulatory children with Duchenne muscular dystrophy - Phase I-II. DegenerNeurolNeuromuscul Dis. 2018 Oct 26;8:63-77. 4 ambulatory and 5 non-ambulatory children. Well define criteria.Only one patient (patient number 3) has shown remarkable new myoblastic signal activityquantitative analysis of muscle strength, did not show a significant difference though EMG showed increase amplitude. Improved quality of life in first year but progressive decline thereafter. Small sample size. Follow up with possible requirement of additional doses not available.In the analysis, no comparison between ambulatory and non-ambulatory patients.

ii.	Source: Umbilical cord derived mesenchymal stem cells (UCMSCs); RoA: Intravenous and intramuscular Rajput BS, Chakrabarti SK, Dongare VS, Ramirez CM, Deb KD. Human Umbilical Cord Mesenchymal Stem Cells in the Treatment of Duchenne Muscular Dystrophy: Safety and Feasibility Study in India. J Stem Cells. 2015;10(2):141-56 Single blinded study on 11 patients. One of the inclusion criteria - mutation in dystrophin gene in cytogenetic analysis. As this is not possible the definitive diagnosis is uncertain. Small underpowered study.
iii.	Source: Allogeneic, umbilical cord donors RoA: peripheral IV infusion 6 Kang PB, Lidov HG, White AJ, Mitchell M, Balasubramanian A, Estrella E, Bennett RR, Darras BT, Shapiro FD, Bambach BJ, Kurtzberg J, Gussoni E, Kunkel LM. Inefficient dystrophin expression after cord blood transplantation in Duchenne muscular dystrophy. Muscle Nerve. 2010 Jun;41(6):746-50. Single case report. Primary stem cell transplant to treat chronic granulomatous disease. On follow up he was diagnosed to have DMD as well. Analysis of myofibers demonstrated no definite donor cell engraftment. Even though this is done on one patient it shows that the concept of stem cell transplant is not efficacious on its own.
iv.	Source:Autologous bone marrow-derived mononuclear cells RoA: intrathecally and intramuscularly Sharma A, Sane H, Badhe P, Gokulchandran N, Kulkarni P, Lohiya M, Biju H, Jacob VC. A clinical study shows safety and efficacy of autologous bone marrow mononuclear cell therapy to improve quality of life in muscular dystrophy patients. Cell Transplant. 2013;22 Suppl1:S127-38 This non-randomized, open-label, single center trial from India on 150 patients with muscular dystrophy. Limb girdle muscular dystrophies and DMD and BMD are clubbed together. No molecular diagnosis of the dystrophies is provided. 86.67% of cases showed symptomatic and functional improvements. However specific muscles tested are not defined. Patients were not grouped based on the severity of affection at the onset of treatment. Long term follow-up of this cohort is not available. The author acknowledges that this is a single-center study with no control group and a limited follow up
v.	Source: Autologous transplantation of muscle derived CD133+ stem cells RoA: Intramuscular Torrente Y, Belicchi M, Marchesi C, D'Antona G, Cogiamanian F, Pisati F, Gavina M, Giordano R, Tonlorenzi R, Fagiolari G, Lamperti C, Porretti L, Lopa R, Sampaolesi M, Vicentini L, Grimoldi N, Tiberio F, Songa V, Baratta P, Prelle A, Forzenigo L, Guglieri M, Pansarasa O, Rinaldi C, Mouly V, Butler-Browne GS, Comi GP, Biondetti P, Moggio M, Gaini SM, Stocchetti N, Priori A, D'Angelo MG, Turconi A, Bottinelli R, Cossu G, Rebulla P, Bresolin N. Autologous transplantation of muscle-derived CD133 + stem cells in Duchenne muscle patients. Cell Transplant. 2007; 16 (6): 563-77 Small sample size – 5 patients; double-blind phase I clinical trial. Increased capillaries per muscle fibre - angiogenic potential of the injected CD133+ stem cells. But not clear how the cells will promote the switch of slow-to-fast muscle fiber type.This is the first step for future clinical trials for DMD based on the autologous transplantation of engineered stem cells and need at least four potential improvements. • isolate cells from easily accessible site as blood • Expand in vitro without loss of stem cell property

	• transduce them with viral vectors that promote the expression of dystrophin by exon skipping • to deliver them to diseased muscle through arterial circulatory routes. The authors say that better understanding of the stem cell behaviour in the human muscle structures is required to gain the route for stem cell therapy.
vi.	Source:Autologous bone marrow mononuclear cell transplantation RoA: Intrathecal ClinicalTrials.gov Identifier: NCT02241434. The Role of Autologous Bone Marrow Mononuclear Cell Therapy in Duchenne Muscular Dystrophy Recruitment Status: Withdrawn First Posted: September 16, 2014 Last Update Posted: October 25, 2018 Study was to start in January 2009. Completion date 2016 There are no updates of any results and recruitment withdrawn

None of the studies are of an appropriate level of evidence based on the methodological quality of their design, validity, and applicability to patient care. None of them are randomized double blind studies or multicentric studies as are usually performed for rare genetic disorders. Many are single case reports or use a small number of patients. The criteria to assess benefit for use of stem cell therapy in the studies are not well defined or uniform and thus the presence or absence of improvement in muscle function is ambiguous.

In summary, the studies, case series, including one aborted clinical trial reported so far do not show clear scientific evidence to support the use of stem cell therapy in DMD. Hence, based on current knowledge, stem cell therapy should not be offered as one of the standard or routine therapy to patients with DMD. Currently the role of stem cell use in DMD should be in a research setting with a rigorously designed and executed protocol as per the national guidelines, with appropriate approval from recognized regulatory authorities in India. These studies should be closely monitored for the possibility of any harm to the patients by the use of stem cells. Participants of any such trials should have read and signed the informed consent form which has to clearly explain the alternative therapies, the design and phase of the clinical trial, possible benefit and harm due to stem cell therapy and what compensation will be provided to the patient or the family in the event of any harm or death due to the use of experimental stem cell therapy. The real utility of stem cells is where they are combined with genome editing to correct the patient mutation in the induced pluripotent stem cell.

Summary of Evidence and Recommendations for Medical / Scientific Professionals (2021)

Based on the review of available scientific evidence, stem cell therapy should NOT be offered as a standard or routine therapy to patients with Duchenne Muscular Dystrophy.

The experts observed that Duchenne Muscular Dystrophy is a severe disorder with premature death and can have a major impact on the quality of life of the affected child and the family. There are standards of care that are published for the care and patients of patients with DMD. Recently definitive therapy targeting the type of mutation has also recently been approved for use. In September 2016, the US Food and Drug Administration (FDA) granted accelerated approval of Eteplirsen, an exon skipping drug that has shown to increase dystrophin in patients with a mutation of the dystrophin gene amenable to exon 51 skipping. Ataluren is licensed in the European Union and United Kingdom to treat patients aged 2 years and older with DMD caused by nonsense mutations.Golodirsen and Viltolarsen, drugs for use in patients with mutations amenable to exon 53 skipping were approved by FDA in December 2019 and August 2020 respectively.

Currently there is no recommendation of the use of stem cells treatment in patients with DMD. The real utility of stem cells is where they are combined with genome editing to correct the patient mutation in the induced pluripotent stem cell. It is therefore imperative that use of any type of stem cell in DMD should be restricted to clinical trials with due approvals from regulatory authorities in India and as per the national guidelines on stem cell research. As per the ICMR National Bioethics guidelines 2017, trial participants should have read and signed the informed consent form which explains them alternative therapies, possible benefits as well as harm due to experimental treatments like stem cell therapy. Participants should not be made to pay for any expenses incurred beyond routine clinical care and which are research related including tests, investigations and any interventions (such as stem cells). This is applicable to all participants, including those in comparator/control groups. Participants in a clinical trial should be provided compensation in the event of any harm or permanent injury or death due to the use of experimental stem cell therapy.

These guidelines will be periodically reviewed for any new evidence showing benefit or harm with the use of stem cells for Duchenne Muscular Dystrophy.