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 Critical Limb Ischemia (CLI)

A. Information for public and patients

What is Critical Limb Ischemia (CLI)?

Critical limb ischemia (CLI) is considered the most severe form of peripheral artery disease (PAD) which is caused by obstruction of peripheral arteries so that the blood supply to the limbs is insufficient to maintain their normal internal functioning. CLI is said to have developed when the blood flow to the limb is insufficient for metabolism of the cells in the limb, even when the person is resting. Patients may experience pain in the feet or in the toes when walking and in some severe cases even when he/she is not walking (rest pain). If the disease is severe, they may develop painful sores / ulcers on the toes or feet or legs. If the circulation does not improve, these ulcers can start as dry, gray, or black sores, and eventually become dead tissue (called gangrene). If no therapeutic intervention is made during this stage of the disease the inevitable outcome is limb amputation. The blockage of arteries is primarily due to atherosclerosis; although in Mediterranean and Asian countries including India this can be due to Thromboangitis obliterance (TAO) or Buerger's disease which has shown to be strongly associated with tobacco / cannabis use.

What is the treatment of CLI?

The treatment options for this condition in general are lifestyle changes such as control of diabetes, hypertension, and hypercholesterolemia, along with dietary restrictions aimed at reducing lipid levels, complete discontinuation of cigarette smoking or other use of tobacco / cannabis in any form, maintaining ideal body weight, and drugs to treat conditions that have primarily caused this condition. There are surgical options to reestablish blood circulation in the limbs. But surgery is usually not possible in all cases.

Have stem cells been used in CLI?

Along with supportive symptomatic therapies, studies have reported their experience with the use of different types of stem cells for patients with CLI. From the published studies, websites and other resources, it has come to our knowledge that many Indian patients with CLI / PAD have been offered different types of stem cell therapies within and outside clinical trial / research. ICMR with inputs from medical / surgical specialists in this field has reviewed the existing scientific and medical literature regarding any evidence based safety and efficacy of stem cells in CLI. Critical review of the studies reported so far and feedback from experts in the field do not support the use of cell therapy in CLI as on date.

Recommendations (2021):

Based on a critical review of the available scientific evidence by the ICMR experts, stem cell based or derived products for CLI should NOT be offered as a standard or routine therapy to patients with CLI unless it is approved by the regulatory agency in India. One such stem cell and cell based product from India, stempeucel® (Adult Human Bone-Marrow Derived, Cultured, Pooled, Allogeneic Mesenchymal Stromal Cells) has been granted manufacturing and marketing license by Central Drugs Standard Control Organisation (CDSCO) for "no-option" patients of CLI due to Buerger's disease and Critical Limb Ischemia due to Atherosclerotic PAD in Rutherford III-5 or III-6, not eligible for or have failed traditional revascularisation treatment, with rest pain and/or ulcers in the affected limb. This is the only approved stem cell and cell-based product for above mentioned conditions in India. These guidelines will be periodically reviewed for any new evidence showing benefit or harm with the use of stem cells for CLI.

CAUTIONARY NOTE

From various websites and other sources, it has come to our knowledge that some doctors and clinics in India continue to offer stem cells as a standard treatment option to CLI patients outside the purview of regulated and approved clinical trials. Patients with CLI from India and those coming from outside India should be aware that any type of stem cells therapy for CLI should be offered only as part of ongoing clinical trials that have all the approvals from the regulatory authorities in India. These trials should follow the national guidelines on stem cells research. As part of regulated clinical trials, patients should be closely monitored not only for objective measures of clinical benefit but also for any possible harms with use of stem cells. As per the ICMR National Bioethics guidelines 2017, clinical trial participants should have read and signed the informed consent form which explains them standard and alternative therapies, possible benefits and harms due to experimental treatments like stem cell therapy. Participants should not be made to pay for any expenses incurred including 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 with 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

Peripheral artery occlusive disease (PAOD) also commonly known as peripheral artery disease (PAD) is a manifestation of atherosclerosis caused by obstruction of peripheral arteries. Critical limb ischemia (CLI) is the severe subset and end stage of PAD which is characterised by severe rest pain, non-healing ischemic skin lesions and finally gangrene of the extremity due to inadequate blood supply to the limb. If no therapeutic intervention is made during this stage of the disease the inevitable outcome is limb amputation. The blockage of arteries is primarily due

to atherosclerosis; although in Mediterranean and Asian countries including India this can be due to Thromboangitis obliterance (TAO) or Buerger's disease which has shown to be strongly associated with tobacco or cannabis use. Other causes include various autoimmune disorders like Systemic Lupus Erythematosus (SLE), and acute conditions like embolism. Various risk factors causally related to PAD include diabetes, hyperlipidemia, hypertension and smoking. Coexistent coronary artery disease (CAD) and cerebrovascular disease (CVD) are highly prevalent in patients with PAD particularly in elderly population.

Diagnostic studies include measurement of hemodynamic changes by Ankle Brachial Pressure Index (ABPI), transcutaneous partial oxygen pressure (TcPO2), toe brachial pressure index, exercise testing to elicit symptoms, segmental pressure monitoring, and Doppler examination of the vascular system. Magnetic resonance angiogram (MRA) and computed tomography (CT) angiography can also aid in the diagnosis. The management of CLI is based on risk factor management and surgical or endovascular revascularization aiming to improve blood flow to the affected extremity. Most patients with CLI are managed with analgesics, antiplatelets and cilostazol in addition to lipid lowering drugs. If revascularization has failed or is not possible, major amputation is often necessary. Approximately 10% of CLI patients will undergo primary amputation with healing rates varying from 30% and 90%, and re-amputation rate stands between 4% and 30%. Overall, approximately 40% and 50% of CLI patients will lose their leg within 6 – 12 months and approximately 15% will also require contralateral amputation within 2 years. In CLI patients, cardiovascular cause of mortality increases substantially with approximately 20% of patients dying during first 6 to 12 months after CLI onset, and 2 -, 5 – and 10 years mortality rates of approximately 35%, 70% and 100% respectively.

Many Indian patients with CLI have been offered different types of stem cells therapies as part of approved clinical trials and also as standard treatment option which is outside the purview of approved clinical trial. ICMR with inputs from experts in this field has reviewed the existing medical and scientific literature regarding level of evidence of evidence and safety of stem cells in CLI.

A critical review of the published human studies supporting the use of stem cells in CLI has been undertaken. Summary of some representative studies are outlined below:

Chronic Limb Ischemia	
S.No.	**Review of Literature** **Critique / Applicability of the study results**
i.	Lafrati MD et al. Early results and lessons learned from a multicenter, randomized, double-blind trial of bone marrow aspirate concentrate in critical limb ischemia. J Vasc Surg. 2011 Dec;54(6):1650-8. doi: 10.1016/j.jvs.2011.06.118. In this multicenter, randomized, double-blind, placebo-controlled trial of autologous bone marrow cell therapy for CLI, the therapy was well tolerated without significant adverse events. The BMAC group demonstrated trends toward improvement in amputation, pain, quality of

	life, Rutherford classification, and ABI when compared with controls.
ii.	Ai M et al. Safety and efficacy of cell-based therapy on critical limb ischemia: A meta-analysis. Cytotherapy. 2016 Jun;18(6):712-24. doi: 10.1016/j.jcyt.2016.02.009. Cell-based therapy has a significant therapeutic effect on CLI, but randomized double-blind placebo-controlled trials are needed to improve the credibility of this conclusion.
iii.	Gupta P K et al.Administration of Adult Human Bone Marrow-Derived, Cultured, Pooled, Allogeneic Mesenchymal Stromal Cells in Critical Limb Ischemia Due to Buerger's Disease: Phase II Study Report Suggests Clinical Efficacy. Stem Cells Transl Med. 2017 Mar;6(3):689-699. doi: 10.5966/sctm.2016-0237. This phase II, open-label nonrandomized study demonstrated the possible effects of allogeneic bone marrow derived Mesenchymal stromal cells in CLI due to Buerger's disease. The patients in the 2 million cells/kg group showed clinical benefit in both the primary endpoints (rest pain relief and ulcer healing) and most secondary endpoints (improvement in total walking distance, ankle brachial pressure index, and quality of life). Hence, it is suggested that in this pathfinder study, BM-MSCs at a dose of 2 million cells/kg body weight may be the best dose in patients with critical limb ischemia due to Buerger's disease.
iv.	Idei N et al. Autologous bone-marrow mononuclear cell implantation reduces long-term major amputation risk in patients with critical limb ischemia: a comparison of atherosclerotic peripheral arterial disease and Buerger disease. Circ Cardiovasc Interv. 2011 Feb 1;4(1):15-25. doi: 10.1161/CIRCINTERVENTIONS.110.955724. BM-MNC implantation reduces major amputation rate and is safe and effective in patients with CLI, especially in patients with Buerger's disease.
v.	Liew A et al. Cell Therapy for Critical Limb Ischemia: A Meta-Analysis of Randomized Controlled Trials. Angiology. 2016 May;67(5):444-55. doi: 10.1177/0003319715595172. In summary, our meta-analysis suggests that cell therapy forpatients with CLI is feasible and safe, with preliminary assessments of efficacy of these cell types, encouraging. However, additional, carefully designed future double-blind, sham, and placebo controlled RCTs in a large, homogenous patient cohort, with a clearly defined cell type, cell number, and potency are needed to confirm its true potential therapeutic effect.
vi.	Wahid FSA et al. Efficacy and Safety of Autologous Cell-based Therapy in Patients with No-option Critical Limb Ischaemia: A Meta-Analysis**.** Curr Stem Cell Res Ther. 2018;13(4):265-283. doi: 10.2174/1574888X13666180313141416. Implantation of autologous cell-based therapy may be an effective therapeutic strategy for no-option CLI patients. BM-MNC and mobilized – peripheral blood stem cells more effective than non-cell-based therapy in improving AR and other limb perfusion parameters. BM-MSC may be beneficial in improving perfusion parameters but not AR, however, this observation needs to be confirmed in a larger population of patients. Generally, treatment using various sources and phenotypes of cell products appeared safe and well tolerated.
vii.	Sharma S et al. Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate Safety and Therapeutic Efficacy of Angiogenesis Induced by Intraarterial Autologous Bone Marrow-Derived Stem Cells in Patients with Severe Peripheral Arterial Disease. J VascIntervRadiol. 2021 Feb;32(2):157-163. doi: 10.1016/j.jvir.2020.09.003. Epub 2020 Nov 25. PMID: 33248918. Intraarterial administration of autologous BMSCs results in significantly greater improvement in hemodynamic parameters such as ABI and TcPO2 in patients with severe PAD and greater freedom from majoramputation among patients with CLI, with no adverse effects or 30-day mortality. Intraarterial autologous stem cell therapy delivered proximal to the most proximal occlusion is a safe and effective alternative in the management of patients with severe PAD.

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

Based on the review of available scientific evidence, there seems to be some role of stem cells in CLI patients, but more studies are required to prove safety and efficacy of stem cells in these conditions. Till date, it is recommended that stem cell therapy should NOT be offered as standard of routine therapy to patients in Critical limb ischemia. One such stem cell and cell based product from India, stempeucel® (Adult Human Bone-Marrow Derived, Cultured, Pooled, Allogeneic Mesenchymal Stromal Cells) has been granted manufacturing and marketing license by Central Drugs Standard Control Organisation (CDSCO) for "no-option" patients of CLI due to Buerger's disease and Critical Limb Ischemia due to Atherosclerotic PAD in Rutherford III-5 or III-6, not eligible for or have failed traditional revascularisation treatment, with rest pain and/or ulcers in the affected limb. This is the only approved stem cell and cell-based product for above mentioned conditions in India. These guidelines will be periodically reviewed for any new evidence showing benefit or harm with the use of stem cells for CLI.

The experts observed that Peripheral arterial disease (PAD) is a common disorder and a major cause of morbidity and mortality. The most severely affected patients, with rest pain, ulcerations, or gangrene, are given a diagnosis of critical limb ischemia (CLI). These patients have a particularly poor prognosis, with high rates of limb amputation and mortality. Despite improvements in medical therapy for atherosclerosis and associated comorbidities as well as improvements in interventional and surgical techniques to improve limb perfusion, CLI continues to carry a major risk of limb amputation. A significant portion of patients with CLI are considered "no option" for revascularization, and no medical therapy has been shown to be capable of reducing the need for amputation. Therefore, novel therapies are needed to treat this disorder. Therefore there is a need to undertake research into the causes and more effective management of CLI especially reducing the amputation rate and overall cardiovascular mortality. Since conventional management fails to significantly delay control symptoms in many cases of no-option CLI cases, such families see a hope in some recovery with the use of stem cells without understanding the risks versus benefit ratio.

Numerous regulatory approved clinical trials (many trials are in phase 2 or phase 3) are being conducted in this condition across the world including India and the patients are being followed up for adequate time to see the safety and efficacy of the stem cells. Till date no stem cells drug is approved by the regulatory agency for this condition. It is therefore imperative that use of any type of stem cells in CLI should be restricted to clinical trials with due approvals from regulatory authority in India and as per the National Guidelines on Stem Cell Research. As part of the regulated clinical trials, patients should be closely monitored not only for objective

measures of clinical benefit but also for any possible harms 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 alternative therapies, possible benefits as well as harm due to experimental treatments like stem cell therapy. Participants should not be made to pay any expenses incurred including 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 with 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 CLI.