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Review Article

Generation and clinical applications of stem cells in regenerative medicines

Ranjanee Chattopadhyay
Amity Institute of Biotechnology (AIB), Amity University, Noida (Uttar Pradesh), India

Email: missranjanee@gmail.com

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Abstract
Since the discovery of stem cells, they have been claimed to be nature's miracle due to their unique properties, which set them apart from other cell types. It has fascinated the world with its myriad of opportunities in regenerative medicine, such as treating blood-borne cancers, neurodegenerative diseases and disorders, immune system diseases, heart diseases, etc. Although Stem Cell Therapy is yet to be commercialized in India, research and clinical trials using Hematopoietic Stem Cell Transplantation are being conducted to understand the efficacy of stem cells in the remission of various malignant and non-malignant diseases such as Thalassemia, Acute Lymphoblastic Leukemia, Sickle Cell Disease, Aplastic Anemia, Hodgkin's Lymphoma and Juvenile Rheumatoid Arthritis among other diseases. Stem cell research has bloomed over the years and has demonstrated immense potential in Embryonic Stem Cell Therapy, Somatic Stem Cell Therapy, Induced Pluripotent Stem Cell Therapy and, with further understanding of stem cells, it could also be applied in drug modelling and drug development, skin replacement and regeneration, organ repair and regeneration and disease modelling for drug testing, revolutionizing the healthcare industry in the future. The present review discusses the research on stem cells, their applications in regenerative medicine, and economic, regulatory and ethical concerns.

Keywords: Embryonic Stem Cells (ESCs), Hematopoietic Stem Cell Transplantation (HSCT), Induced Pluripotent Stem Cells (iPSCs), Regenerative Medicine, Somatic Stem Cells (SSCs)

INTRODUCTION

The history of stem cells dates back to the late 19th century when the term 'Stem Cell', was first introduced, by Theodor Boveri and Valentin Haecker (Ramalho-Santos and Willenbring, 2007). After that, theoretical developments continued in the early 20th century and a major milestone was achieved with the discovery of HSCs by Canadian scientists Ernest A. McCulloch and James E. Till, in 1961, at the University of Toronto and the Ontario Cancer Institute. They successfully listed the essential properties of stem cells by demonstrating the same in the bone marrow of mice (Weissman and Shizuru, 2008). This became the principle and base of stem cell research. Although the HSCs were discovered in 1961, their isolation was first carried out in 1968, from the bone marrow and thus, HSCs became the first SSCs to be ever discovered and isolated.ESCs were first identified, isolated and cultured from the inner cell mass of blastocysts of embryos of mice in 1981, by British biologists Martin Evans and Matthew Kaufman (Khan *et al.*, 2018). In 1998, James Thomson and his research team successfully isolated hESCs from human embryos, revealing immense potential for regenerative medicine. In the same year, John Gearhart and his colleagues at Johns Hopkins University, identified and isolated the Human Pluripotent Stem Cells, which include both hESCs and hiPSCs, successfully, providing a promising source of stem cells for research, indicating opportunities for new transplantation techniques and a possibility of preventing birth defects, genetic deformities and/or illnesses, in the future (Zhu and Huangfu, 2013).

At Kyoto University, Japan, Shinya Yamanaka and his team 2006 reprogrammed SSCs into iPSCs in to retrieve their pluripotent state and properties of ESCs, marking the origin of iPSCs (Ferreira, 2014). They have also successfully bypassed the controversies encompassing the source of ESCs. The generation of iPSCs from Adult Human Dermal Fibroblasts was first ob-

served in 2007 by Kazutoshi Takahashi and others (Takahashi *et al.*, Nov 2007).The year 2008 marks the first clinical trial of using hESCs to treat spinal cord injury in the United States of America. In 2011, a female maned wolf from Brazil became the first animal to be treated using stem cell therapy after she was hit by a truck. Although she suffered from high-level injuries, she showed exceptional recovery, taking half the time it normally takes for recovery under normal clinical practices, and thus, it marked the success of this process (Boyle and Rebecca, 2011). Japan, in 2012, marks a significant step towards the formation of entire mouse kidneys using mouse blastocysts by replacing the defective cells with Pluripotent Stem Cells (Usui *et al.*, 2012). This exhibits the potential towards organogenesis.

In 2014, Doug Melton and his colleagues produced functional human pancreatic beta cells from hESCs, marking another step towards eradicating Diabetes (Pagliuca *et al.*, 2014). The year 2017 marks the success of using iPSCs to treat monkeys suffering from Parkinson's disease (Callaway, 2017). In 2020, Evan Synder along with a team, created a drug - SDV1a, which guides stem cells to the desired location, luring them to damaged tissues and increasing the treatment efficacy (Lee *et al.*, 2020) (Fig. 1).

Stem cells are partially differentiated or undifferentiated cells that typically do not have a specific function but have the potential to develop into specialized cells such as myocytes, hepatocytes, cardiomyocytes, RBC, WBC, platelets, glial cells, neurons, etc. They are found in multicellular organisms and are found to be the earliest type of cell in the cell lineage (Atala and Lanza, 2012). Stem cells are primarily employed in the organism's repair mechanisms. Also, they can divide indefinitely to create replicas of themselves for future use. The stem cells possess unique properties such as self-renewal, potency, and regenerative abilities. Hence, they are also known as nature's miracle.

With the discovery of Embryonic Stem Cells **(**ESCs) in 1981 by Martin Evans and Matthew Kaufman (Khan *et al.*., 2018), followed by the successful isolation of hESCs by James Thomson in 1998, scientists have been extracting hESCs from unused embryos for conducting stem cell research. In 2006, Shinya Yamanaka and his team at Kyoto University, Japan, reprogrammed Adult Stem Cells into iPSCs. In 2007, Kazutoshi Takahashi and his team generated hiPSCs from Adult Human Dermal Fibroblasts (Takahashi *et al.*, 2007).

It has immense potential and is being applied in SSC Therapies, ESC Therapies, iPSC Therapy, drug modelling and development, disease modelling for drug testing (Turhan *et al.*, 2021), regenerative medicine, etc. CRISPR-Cas9 is a revolutionary gene-silencing technology that has become a leading tool in the therapeutics of genetic diseases and disorders (Chattopadhyay and Srivastav, 2024). They may be coupled with stem cell technology to precisely redesign the stem cells, providing advanced therapeutics tailored to specific diseases.

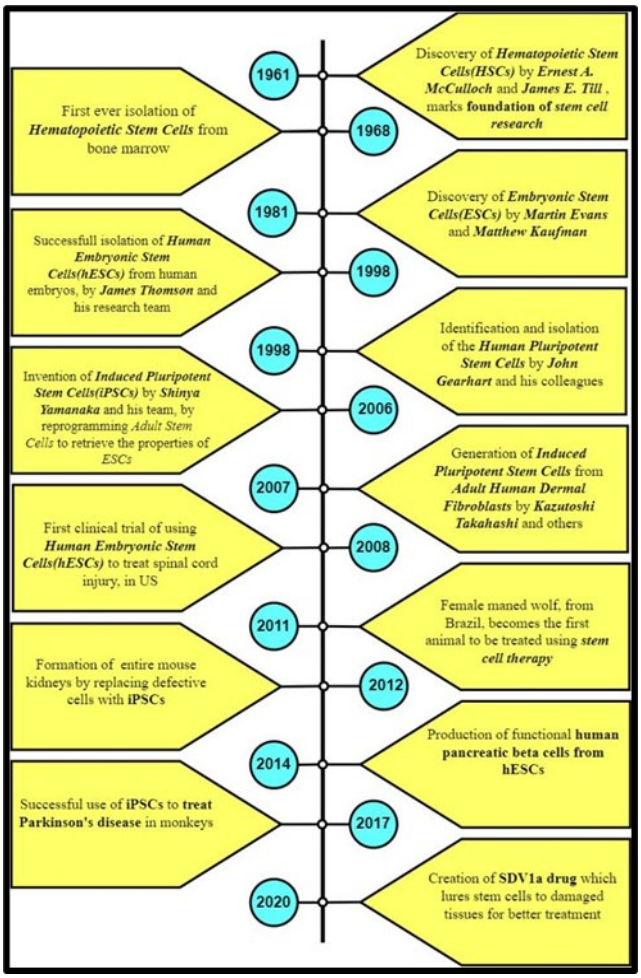

Fig. 1. *Timeline marking the milestones in stem cell research*

TYPES OF STEM CELLS

Stem cells are divided into two main types based on the two main sources: embryo and adult tissues (Fellheimer and Harrison, 2024).

Embryonic Stem Cells (ESCs)

These are acquired from 5 - 14 days mammalian embryos, particularly from the inner cell mass of blastocysts, which are made of around 50 - 150 cells. The inner cell mass of blastocysts are then isolated and cultured using *in vitro* fertilization technique. These are pluripotent stem cells (Varzideh *et al.*, 2023), which have the capacity to evolve into specialized cell types. They are commonly used to repair or regenerate diseased tissues and organs because of their high versatility. ESCs have a high proliferative ability, allowing for rapid replication during ESC culture, which is beneficial for research and development. Since the source of hESCs is human embryos, research involving hESCs is controversial and involves ethical concerns, too (Park *et al.*, 2024).

Somatic Stem Cells (SSCs)

These are also known as adult or tissue-specific stem cells and are found in bone marrow, adipose tissue, brain, blood, gonads, skeletal muscles, liver, and skin. They are multipotent and have a limited capability to evolve into specialized cells. They play a crucial role in the internal repair mechanisms, in replenishing lost cells and maintaining cell count, tissue repair and overall maintenance of the organism. These stem cells are present in developed tissues and are highly tissue-specific. They remain in their undifferentiated state (quiescent stage) until activated (de Morree *et al*., 2023). Examples include Mesenchymal Stem Cells (MSCs), which are involved in the maintenance of bone, cartilage, muscle and fat cells, neural stem cells, Hematopoietic Stem Cells(HSCs), which replenish blood and immune cells, epithelial stem cells, etc. The umbilical cord blood contains both MSCs and HSCs and is known as the purest and the richest form of stem cells.

Induced Pluripotent Stem Cells (iPSCs)

They are another type of adult stem cell that has been programmed to possess the properties of ESCs. It involves artificial reprogramming of adult somatic cells, such as neurons, myocytes, hematocytes, etc., to create stem cells similar to ESCs. This retrieves their pluripotent state, allowing them to evolve into any specialized cell type. It was first observed in research conducted in 2006 by Shinya Yamanaka and his team. It finds various applications in Stem Cell Therapy, such as in Tissue Engineering to repair bone and cartilage damage, Bone Marrow Transplantation used to treat diseases such as leukemia and other blood disorders (Cable *et al.*, 2020), Drug Testing and Disease Modelling to generate more disease-specific therapy, etc. (Turhan *et al.*, 2021). iPSCs have gained popularity because they successfully bypass the controversies encircling the source of ESCs (Aboul-Soud *et al.*, 2021).

PROPERTIES OF STEM CELLS

Stem cells are described by two unique characteristics, viz. self-renewal and potency, which makes them different from other cell types (Zakrzewski *et al.*, 2019). They are also studied for and associated with their regenerative abilities. Stem cells are different from progenitor cells, which are somewhat similar to stem cells but can undergo only limited sessions of cell proliferation and have a lower self-renewal ability than stem cells.

Self-renewal is the ability of stem cells to divide indefinitely to create multiple replicas through cell proliferation, which involves an increase in cell number due to multiple sessions of cell growth and division while maintaining the reserve of undifferentiated stem cells over a long duration (Zakrzewski *et al.*, 2019). As mentioned above, the reserve of undifferentiated stem cells is maintained over a long duration. It is done either by asymmetric cell division or by stochastic differentiation. Asymmetric cell division involves the division of the stem cell into one daughter cell, which gets differentiated into specialized cell types and one mother stem cell, which remains to maintain the reserve. Stochastic differentiation is the mechanism where one stem cell undergoes cell proliferation to form two daughter cells, which are differentiated into specialized cell types. Another stem cell undergoes mitosis to produce two identical stem cells to maintain the reserve of undifferentiated stem cells.

Potency is the ability of undifferentiated stem cells to differentiate into multiple specialized cell lineages, each serving a specific function in the body of the organism. Based on the extent of the ability of undifferentiated stem cells to differentiate into specialized cell types, potency can be divided into Totipotency, Pluripotency, Multipotency, Oligopotency and Unipotency.

Totipotency, also known as Omnipotency, describes the ability of a stem cell to differentiate into embryonic and extra-embryonic cell types. They are obtained from the morula phase of the embryo carrying only 8-16 blastomeres and are capable of constructing complete tissues and organs, further producing a fully functional organism (Bieńko *et al.*, 2023).

Pluripotency describes the ability of a stem cell to differentiate into nearly all types of cells. They are obtained from the inner cell mass of the blastocysts. ESCs, hESCs are pluripotent (Bieńko *et al.*, 2023).

Multipotency describes the ability of a stem cell to differentiate into a more limited variety of specialized cell types, often of a closely related cell family. Adult Stem Cells are multipotent (Kolios and Moodley, 2013).

Oligopotency describes the ability of a stem cell to differentiate into an even more limited variety of specialized cell types, having more restrictions than that in the Multipotent stem cells. Examples are Lymphoid Stem Cells, Myeloid Stem Cells, etc (Kolios and Moodley, 2013).

Unipotency describes the ability of a stem cell to differentiate into only one single cell type, performing only one function. They are highly specific and have the properties of other stem cells, including self-renewal and regenerative ability (Kolios and Moodley, 2013). Adult Muscle Stem Cell is an example.

GENERATION OF STEM CELLS

Human Embryonic Stem Cells

The preliminary step involves in vitro fertilization, leading to the formation of the zygote, and the process continues up to the formation of blastocysts. Blastocyst is a rapidly dividing structure having an inner layer of inner cell mass (50-100 cells), which later forms the embryo, the outer layer of trophoblasts, which later forms

chorion and amnion and the blastocoele, which is a fluid-filled portion separating the inner and the outer portion of the blastocysts. During the generation of hESCs, the inner cell mass of blastocysts is isolated and cultured. hESCs possess high proliferative ability, allowing for rapid replication during culture and allowing their use in scientific studies and disease models. These pluripotent stem cells can evolve into nearly all specialized cell types. Embryonic Stem Cell Therapies provide a viewpoint to treat diseases such as Diabetes, Spinal Cord Injury (Aly, 2020), Muscular dystrophy, Heart Diseases such as Heart Failure, Stroke and Myocardial infarction, Blindness, Deafness, etc. They are commonly used to repair or regenerate diseased tissues and organs because of their versatility. Another advantage of hESCs is that they can be differentiated into ectoderm, mesoderm, and endoderm. Since the source of hESCs is human embryos, research involving the same is controversial and involves ethical concerns, too (Park *et al.*, 2024) (Fig. 2).

Human Induced Pluripotent Stem Cells

The first step is the isolation of SSCs. Some are MSCs, Keratinocytes,Neural Stem Cells, HSCs, epithelial stem cells, Fibroblast, etc. The next step involves reprogramming the isolated SSCs, and converting them into hiPSCs. Yamanaka and Takahashi discovered the core transcriptional factors Oct4, Sox2, Klf4, and c-Myc (OSKM), which are crucial for reprogramming (Teshigawara *et al.*, 2017). After the generation of the hiPSCs, OSKM factors help to retrieve the pluripotent state and control their evolution into any specialized cell type (Baghai Naini *et al.*, 2019), such as adipocytes, cardiomyocytes, red blood cells, granulocytes (neutrophils, eosinophils and basophils), monocytes, lymphocytes (B & T cells), platelets, neuronal cells, etc. iPSCs have gained popularity because they, not only can retrieve the pluripotent state and properties of ESCs, they also can successfully bypass the controversies encircling the source of hESCs (Aboul-Soud *et al.*, 2021). It finds various applications in Stem Cell Therapy, such as in Tissue Engineering to repair bone and cartilage damage in Bone Marrow Transplantation to treat diseases such as Leukemia, Lymphoma and other blood disorders (Cable *et al.*, 2020). iPSCs can be applied in regenerative medicine to create scaffolds and organoids, mimicking the characteristics of the target organism, which will aid in drug modelling and development and reduce the use of animals involved in this process. They can be applied to designing disease models for drug testing and developing more disease-specific therapies (Aguirre *et al.*, 2023) (Fig. 3).

RESEARCH AND CLINICAL APPLICATIONS OF STEM CELLS

According to the National Guidelines for Stem Cell Research (NGSCR)-2017, India acknowledges HSCT as the only type of stem cell therapy (National Guidelines for Stem Cell Research, 2017), (Pg 36) and annexure-III lists the diseases and conditions in which HSCT can be applied. Although hospitals or medical practitioners, at present, cannot claim to commercially provide stem cell therapy as a cure for any specific disease or condition, it must be noted that the clinical trials and studies of stem cell therapy for various diseases show remission in the intensity of the same, and in many cases, improving the health of the patient significantly, hence demonstrating the potential of further advancements in stem cell research.

Thalassemia

It is an autosomal recessive disease, which occurs due to mutation of genes, resulting in a reduced rate of synthesis of one of the globin chains of haemoglobin and are two forms. α-thalassemia is a form of thalassemia which results in impaired production of α-globin chain of haemoglobin and involves genes HBA1 and HBA2 located on the 16th chromosome. β-thalassemia is anoth-

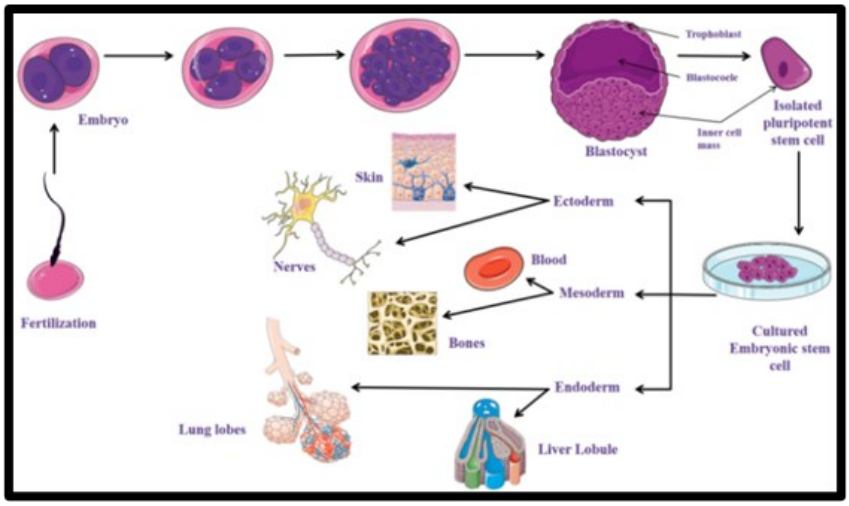

Fig. 2. *Generation of Pluripotent human embryonic stem cells (hESCs)*

er form of thalassemia which results in impaired production of β-globin chain of haemoglobin and involves a gene on the 11th chromosome. The severity of thalassemia is dependent on the number of genes out of 4 genes for α-globin and 2 genes for β-globin are affected. Thalassemic patients require periodic red blood transfusions to promote normal growth and development and to avoid extreme skeleton deformity.

Allogeneic HSCT (allo-HSCT) for thalassemia shows significant remission of disease and improved patient's health with a 65% cure rate in adult thalassemia patients. Risk class-based HSCT shows 90%, 84% and 78% disease-free survival rates for class 1, 2 and 3 thalassemia patients, respectively (Lucarelli *et al.*, 2012). Although finding HLA-matched related donors is a major hurdle, matched unrelated donors, mismatched related donors, and cord blood may become alternated stem cell sources. A study was conducted on 257 β-thalassemia major patients who underwent HSCT with a modified NF-08-TM conditioning regimen at Guangzhou Women and Children's Medical Centre, China, between January 2013 and January 2019. It demonstrated 92.2% (237 individuals) overall survival and 91.8% (236 individuals) thalassemia-free survival (Huang *et al.*, 2021).

Acute Lymphoblastic Leukemia (ALL)

It is a type of cancer in which the normal differentiation and proliferation of lymphoblasts, which typically develop to produce mature B and T cells, gets affected, leading to the formation of immature lymphocytes in the bone marrow. There are two types viz. B-cell ALL and T-cell ALL. The onset of ALL is typically from 2 to 5 years of age. Here, both the normal development of lymphocytes and the control over the number of lymphocytes become defective, leading to the formation of leukemic lymphoblast, which interferes with the production of blood cells. Environmental risk factors such as exposure to radiation, genetic factors, and abnormal immune responses to infections may be the trigger, but the cause remains unknown.

A study was conducted on 354 ALL patients ranging from 1 to 61 years of age who underwent allo-HSCT between 1995 and 2015 (Bondarenko Sergey *et al.*, 2016) to deduce the effectiveness of allo-HSCT in ALL. Before allo-HSCT, 34% of patients suffered from active disease, 24% were in 1st remission, 26% in 2nd remission and 17% in ≥ 3rd remission. Post allo-HSCT, overall survival (OS) was 47% in patients under remission status and 18% under active disease state. 5 year OS was 48% in children and 47% in adults; non-relapse mortality (NRM) was 32% for children and 37% for adult ALL patients (Bondarenko Sergey *et al.*, 2016).

Sickle Cell Disease (SCD)

It is a group of inherited haemoglobin-related blood disorders and Sickle Cell Anaemia (SCA) is the most common type. SCA is an autosomal recessive disease that is controlled by a single pair of allele. It gets transmitted in heterozygous condition (Hb^A Hb^S) i.e when both parents act as carriers for the gene, and the offspring shows a diseased phenotype in homozygous condition (Hb^S Hb^S). Here, a single base substitution at the sixth codon, from GAG to GUG, leads to amino acid substitution from Glutamic acid (Glu) to Valine(Val) at the sixth position of β-globin chain of haemoglobin. Under normal oxygen concentrations, this benign mutation does not cause any evident repercussions on the secondary (2°), tertiary (3°) and quaternary (4°) structure of haemoglobin. But, under low oxygen concentrations, Hb^S polymerizes, changing the shape of RBCs from a biconcave disc to a rigid and elongated sickle-like structure. Also, the heterozygous condition (Hb^A Hb^S) proves to be beneficial for people living in malarial-prone areas. It increases their chances of survival be-

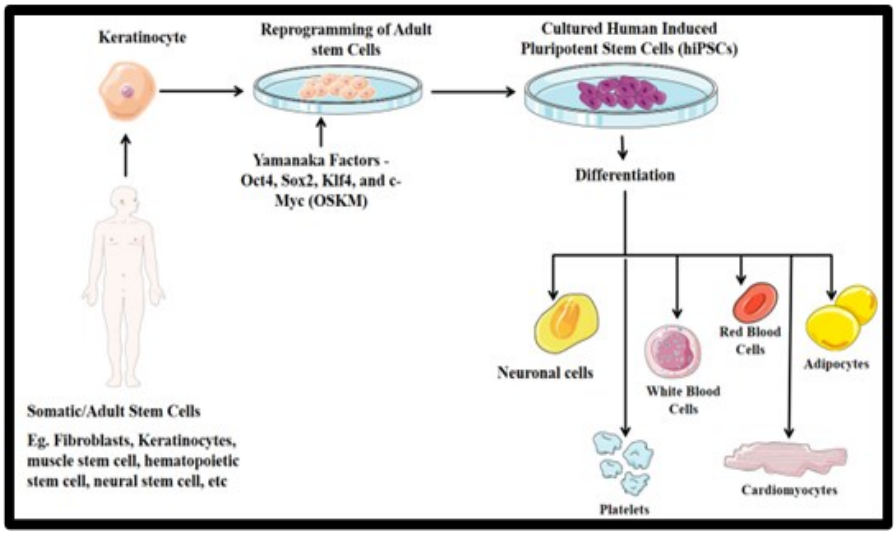

Fig. 3. *Generation of Human induced pluripotent stem cells (hiPSCs)*

cause RBCs having defective haemoglobin ruptures and polymerization of Hb^S affects the parasite's reproductive and digestive ability, respectively. Patients suffering from SCA require periodic blood transfusions to decrease the number of RBCs with defective haemoglobin and increase the normal RBCs, reducing the complications.

Diagnosis of SCA is possible during pregnancy and the first signs and symptoms begin at the age of 5-6 months. For the disease, it affects all the major organs and reduces the individual's life expectancy. allo-HSCT for SCA must be offered early to prevent further worsening of health. Once again, HLA-matching is crucial in finding a suitable donor and in achieving higher success rates of HSCT. In a research conducted between November 2011 and June 2014, 13 adult patients with high-risk sickle cell disease underwent chemotherapy-free allo-HSCT using matched related donors(MRD) as the stem cell source (Saraf *et al.*, 2016).

After allo-HSCT, only 1 out of 13 patients experienced secondary graft failure; thus, the initial success rate was 92%. After one year post-transplantation, 12 of 13 patients (92%) maintained stable donor chimerism, exhibiting normalized haemoglobin concentrations, improved cardiopulmonary and QoL (Quality of Life) parameters and significant improvement in overall health and vitality. The patients under study did not require RBC transfusions, did not develop any sickle cell anaemia-related complications and no transplantation-related mortality was observed throughout the study (Saraf *et al.*, 2016).

Aplastic Anemia (AA)

It is a rare, non-malignant, severe hematologic disorder in which the bone marrow fails to generate an adequate amount of hematopoietic stem cells necessary for the survival of the individual and also alters the hematopoietic niche, thus hampering the self-renewal and differentiation of HSCs creating a high deficiency of all types of blood cells. Although in half of the cases, the cause remains unknown, known causes are exposure to chemicals, drugs, toxins, ionizing radiations, radioactive substances, heredity and immune system disorder, and it is observed in individuals in their twenties and in later stages of life.

Allo-HSCT for Severe AA provides remarkable outcomes and offers significant remission. Medical records of eighty Malaysian patients suffering from SAA who underwent allo-HSCT were collected from 1st January 1999 to 31st December 2019 (Wilfred *et al.*, 2022). Seventy-nine patients received HLA-matched sibling donor (MSD) and one patient received an HLA-matched unrelated donor (MUD) with an ABO compatibility match score of 65%. Upon analysis, it was observed that 23 patients developed graft versus host disease (GVHD) post-transplantation, and 27.5% of patients died after 100 days post HSCT. Although SAA relapsed in 2.5% of patients, 61.3% of patients achieved complete remission and 10% achieved partial remission after 54 months post allo-HSCT. The OS for 5 years was 63% (Wilfred *et al.*, 2022).

Hodgkin's Lymphoma (HL)

It is a type of malignant blood and lymph tumor that originates from lymphocytes and is characterized by the presence of multi-nucleated Reed-Sternberg (RS) cells in the lymph nodes. There are two major types: Classic HL and nodular lymphocyte-predominant HL. In most cases, the cause of HL is Epstein-Barr virus (EBV). Other risk factors include family history of HIV/AIDS.

In a study conducted by the University of Medical Sciences, Tehran, Iran, 116 patients suffering from HL received auto-HSCT between 2007 and 2014 (Kadkhoda *et al.*, 2023). Although 19.6% of patients died, 87.9% of patients experienced non-recurrence of HL post auto-HSCT. The Cure rate (CR) for HL patients aged more than 32 years was 0.56, and for those aged less than 32 it was 0.80, demonstrating the role of age in lower survival post-transplantation. Also, CR for obese HL patients (25.9%) was 0.83, whereas the CR for non-obese patients (74.1%) was 0.57, indicating the role of obesity in increasing the curing potential of HL post auto-HSCT (Kadkhoda *et al.*, 2023).

Juvenile Rheumatoid Arthritis (JRA)

Also known as Juvenile Idiopathic Arthritis (JIA), JRA is an autoimmune and inflammatory joint disease which is observed in patients aged less than 16 years. It is a non-malignant and non-infective disease where the patient's immune system loses its ability to differentiate between self-antigens and foreign antigens, attacking and destroying its cells and tissues. Environmental changes or genetic mutations may trigger them; however, the exact cause remains to be entirely comprehended.

Systemic JRA (sJRA) is a subtype of JIA that affects the body's skin, organs, and joints. A clinical study between 2007 and 2016 treated 16 patients, out of which 11 were suffering from sJRA and 5 with Rheumatoid factor-negative polyarticular JIA using allo-HSCT (Silva *et al.*, 2018). Fourteen out of sixteen patients survived, and while all patients demonstrated significant improvement in arthritis and overall improvement in QoF parameters, eleven patients exhibited complete drug-free remission (Silva *et al.*, 2018).

Another study reports how a three-year-old girl suffering from sJIA was successfully treated with haploidentical allo-HSCT (Morelle *et al.*, 2021). Pre-transplantation, the patient was suffering from growth retardation, systemic hypertension, etc and underwent transplantation at the age of 3.7 years. Post transplan-

tation, the patient suffered from severe infections, and autoimmune thyroiditis, among other complications, which finally stopped after 6 months. Three years post-transplant, she survived with significant improvement in health and vitality and sJIA was in complete remission even after the discontinuation of immunosuppressive drugs (Morelle *et al.*, 2021).

Ewing's Sarcoma (EWS)

This type of cancer forms in soft tissues and bone, mostly observed in children. It could be caused by reciprocal translocation, which is a type of chromosomal mutation; however, the exact cause of this disease remains unknown. This type of cancer commonly develops in the legs, the wall of the chest and the pelvis.

A study was conducted on 47 patients suffering from EWS who underwent auto-HSCT between February 1997 and September 2018 (Pawlowska *et al.*, 2021). The ten-year OS for patients without relapse, late relapse (≥2 years from diagnosis) and early relapse (<2 years from diagnosis) was 75%, 50% and 18%, respectively. Disease-free Survival (DFS) for 10 years and 15 years remained the same (37%). The OS for 10 years and 15 years were 46% and 42%, respectively. It is the largest single-institution study involving sustained long-term follow-up exceeding ten years (Pawlowska *et al.*, 2021).

Systemic Sclerosis (SS)

It is a non-malignant autoimmune rheumatic disease characterized by excessive formation and accumulation of collagen in the skin and internal organs. There are two types viz. Limited SS and Diffuse SS. While the limited form doesn't affect areas above the elbows and knees and doesn't involve the face, the diffuse form may spread over the entire torso. The exact cause is yet to be completely understood.

From March 2001 to October 2009, 156 patients suffering from early diffuse cutaneous SS underwent auto-HSCT (van Laar *et al.*, 2014). During a median follow-up of 5.8 years, 19 deaths and 3 irreversible organ failures were recorded and it was also observed that although there was an increase in the treatment-related mortality in the first year post auto-HSCT, it was successful in providing significant long-term disease-free-survival, indicating its efficacy in SS phase 1 and small phase 2 Autologous Stem Cell Transplantation International Scleroderma (ASTIS) trials (van Laar *et al.*, 2014).

Chronic Lymphocytic Leukemia (CLL)

It is a type of cancer with an abnormal increase in the production of lymphocytes in the bone marrow. Multiple epigenetic changes may cause it, and the risk factors include having a family history of the disease, exposure to Agent Orange, sun, Hepatitis C virus, insecticides, common infections, etc. CLL is asymptomatic and its typical onset is in patients aged over 50. The complications including low levels of antibodies in the bloodstream, frequent infections, and conversion of CLL into a more aggressive disease (Richter's transformation) leads to early death (Hallek *et al.*, 2021).

A clinical study was conducted where 58 patients suffering from CLL underwent Reduced Intensity Conditioning (RIS) allo-HSCT between September 2006 and April 2017 at Memorial Sloan Kettering Cancer Center, New York (Oscar B *et al.*, 2021). After one year post RIS allo-HSCT, Graft versus host disease/relapse-free survival (GFRS) was 38%. After 5 years post-transplantation, OS and 5-year year progression-free survival (PFS) was recorded to be 58% and 40%, respectively. These records provide significant evidence supporting the early remission of CLL in patients using RIS allo-HSCT (Oscar B *et al.*, 2021) (Table 1).

ADVANTAGES & DISADVANTAGES OF STEM CELLS

The discovery of stem cells has elucidated massive potential. With further advancements in stem cell research, it may become possible to develop effective therapeutic strategies for various diseases, which might revolutionize the medical techniques applied today.

ADVANTAGES

Stem cells can reform the health and pharmaceutical industry with effective patient-specific medicines having low to no effects. Stem cell technology may enhance the body's ability to repair itself. Skin stem cells express multipotency and have successfully repaired the skin of burn victims, paving the way for skin replacement and regeneration (Jo *et al.*, 2021). The ESC Therapies provide a viewpoint to treat diseases such as Diabetes, Spinal Cord Injury (Aly, 2020), Muscular dystrophy, Heart Diseases, etc. SSCs such as HSCs are already used in bone marrow transplantation to treat blood-borne cancers and immune system diseases that affect bone marrow (Cable *et al.*, 2020).

Although ESCs express pluripotency, the source of hESCs is human embryos; hence, research involving hESCs is a matter of controversy and involves bio-ethical concerns, too (Park *et al.*, 2024). It also poses a threat to female donors. hiPSCs has appealed to the researchers because it possesses the properties of hESCs but does not involve embryo destruction as observed during the generation of ESCs via in-vitro fertilization. iPSCs can be applied to create scaffolds and organoids, as biomedical models, mimicking the characteristics of the target organism, which will aid in drug modelling and development (Okano *et al.*, 2022). Further, they can be applied to constructing organs for transplantation surgeries. They can also be applied to designing disease models for drug toxicity testing and

Table 1. Research and clinical trials conducted for different malignant and non-malignant diseases using hematopoietic stem cell transplantation

DISEASE	BRIEF	RESEARCH	REFERENCE
Thalassemia	A non-malignant, autosomal recessive disease of two major types, α-thalassemia and β-thalassemia leading to impaired production of α-globin or β-globin chain of haemoglobin, respectively	Clinical study involving 257 β-thalassemia major patients who underwent HSCT with a modified NF-08-TM conditioning regimen demonstrated 92.2% overall survival and 91.8% thalassemia-free survival.	Huang *et al.*, 2021
Acute Lymphoblastic Leukemia (ALL)	A type of cancer of two major types, B-cell ALL and T-cell ALL, where the normal differentiation and proliferation of lymphoblast get affected, leading to the formation of leukemic lymphoblast, which interferes with the production of blood cells	The study was conducted on 354 ALL patients (34% suffering from active disease, 24% in 1st remission, 26% in 2nd remission and 17% in ≥ 3rd remission, prior to allo-HSCT), aged 1 to 61 years who underwent allo-HSCT between 1995 to 2015, expressed OS of 47% in patients under remission status and 18% in patients under active disease state post allo-HSCT, 5 years OS of 48% in children and 47% in adults and NRM of 32% for children and 37% for adult ALL patients	Bondarenko Sergey *et al.*, 2016
Sickle Cell Anaemia (SCA)	A non-malignant, autosomal recessive disease that gets transmitted in heterozygous condition (HbA HbS) and offspring shows diseased phenotype in homozygous condition (HbS HbS). Here, a single base substitution at the sixth codon leads to amino acid substitution from Glu to Val at the sixth position of β-globin chain of haemoglobin, changing shape from a biconcave disc to a rigid and elongated sickle-like structure under low oxygen concentration. It affects all the major organs and reduces the life expectancy of the individual.	Research involving 13 adult patients suffering from high-risk SCD who underwent chemotherapy-free Allo-HSCT using MRD as the stem cell source initially exhibited a 92% success rate. After 1 year, 92% of patients maintained stable donor chimerism, improved cardiopulmonary and QoL parameters, normalized haemoglobin concentrations, did not require RBC transfusions and did not develop any SCD-related complications. Also, no transplantation-related mortality was observed throughout the study	Saraf *et al.*, 2016
Aplastic Anemia (AA)	A rare, non-malignant, severe hematologic disorder in which the bone marrow fails to generate adequate HSCs necessary for survival alters the hematopoietic niche, thus hampering its self-renewal and differentiation creating a high deficiency of all types of blood cells	A study involving 80 Malaysian patients suffering from Severe AA who underwent allo-HSCT, 79 of which received HLA-MSD and 1 receiving HLA-MUD, demonstrated 61.3% complete remission and 10% partial remission after 54 months post allo-HSCT and exhibited overall survival for 5 years as 63%	Wilfred *et al.*, 2022
Hodgkin Lymphoma (HL)	A type of malignant blood and lymph tumor originating from lymphocytes, characterized by the presence of multi-nucleated Reed-Sternberg cells in the lymph nodes and of two types viz. Classic HL and nodular lymphocyte-predominant HL	A study conducted on 116 HL patients who underwent auto-HSCT in Iran between 2007 and 2014 shows 87.9% non-recurrence and 19.6% deaths post auto-HSCT; CR represents the role of age in lower survival (CR = 0.56 for age > 32 and CR = 0.80 for age < 32) and how obesity plays a role in increasing the curing potential of HL post auto-HSCT (CR = 0.83 for obese patients and CR = 0.57 for non-obese patients)	Kadkhoda *et al.*, 2023

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Juvenile Rheumatoid Arthritis (JRA) or Juvenile Idiopathic Arthritis (JIA)	An autoimmune and inflammatory joint disease was observed in patients aged less than 16 years. It is a non-malignant and non-infective disease where the immune system of the patient loses its ability to differentiate between self-antigens and foreign antigens, attacking and destroying its cells and tissues and may be triggered due to environmental changes or genetic mutation.	A clinical study between 2007 and 2016 treated 16 patients using allo-HSCT, 11 patients suffered from Systemic JIA and 5 with Rheumatoid factor-negative polyarticular JIA; Fourteen out of sixteen patients survived and while all patients demonstrated significant improvement in arthritis and QoF parameter, 11 patients exhibited complete drug-free remission.	Silva *et al.*, 2018
Ewing's Sarcoma (EWS)	It is a type of pediatric cancer which forms in soft tissues and bone and could be caused by reciprocal translocation, but the exact cause of this disease remains to be discovered. It commonly develops in the legs, the wall of the chest and pelvis.	A study conducted on 47 patients suffering from EWS who underwent auto-HSCT between Feb 1997 to Sept 2018 demonstrated the ten-year OS for patients without relapse, late relapse (≥2 years from diagnosis) and early relapse (<2 years from diagnosis) as 75%, 50% and 18%, respectively; DFS for 10 years and 15 years remained the same (37%) and the OS for 10 years and 15 years were 46% and 42%, respectively.	Pawlowska *et al.*, 2021
Systemic Sclerosis (SS)	A non-malignant autoimmune rheumatic disease characterized by excessive formation and accumulation of collagen in the skin and internal organs. There are two types viz. Limited SS and Diffuse SS, where the limited form does not affect areas above the elbows and knees and does not involve the face, but the diffuse form may spread over the entire torso	From March 2001 to October 2009, 156 patients suffering from early diffuse cutaneous Systemic Sclerosis underwent auto-HSCT. During median follow-up of 5.8 years, 19 deaths and 3 irreversible organ failures were recorded and it was also observed that although there was an increase in the treatment-related mortality in the first year post auto-HSCT, it was successful in providing significant long-term disease free-survival, indicating its efficacy in SS in ASTIS trials	van Laar *et al.*, June 2014
Chronic Lymphocytic Leukemia (CLL)	A type of cancer characterized by abnormal increase in the production of lymphocytes in the bone marrow which may be caused due to multiple epigenetic changes and other risk factors. CLL is typically asymptomatic and the complications include low levels of antibodies in the bloodstream, causing frequent infections, Richter's transformation, leading to early death	A clinical study conducted on 58 patients suffering from Chronic Lymphocytic Leukemia who underwent Reduced Intensity Conditioning (RIS) allo-HSCT between September 2006 and April 2017 at Memorial Sloan Kettering Cancer Center, New York, showed a GFRS of 38% after one-year post RIS allo-HSCT; After 5 years post-transplantation, OS and PFS were recorded to be 58% and 40%, respectively, indicating early remission of CLL patients using RIS allo-HSCT	Oscar B *et al.*, July 2021

bioavailability and developing more disease-specific therapies (Turhan *et al.*, 2021).

By deriving SSCs from patients to creating iPSCs, reduces the chances of graft rejection and provides relief to lifelong use of immunosuppressors by patients who have undergone transplantation surgeries (Volarevic *et al.*, 2018). The use of organoids created using iPSCs will also reduce the use of animal models involved in stem cell research, further helping to diminish the cost and risk associated with clinical trials of new drugs.

It may be possible to find a permanent cure for diseases and conditions which are incurable to date, including Autoimmune diseases, structural and functional deformities, Type I Diabetes, baldness, genetic diseases,

metabolic diseases, blood-borne cancers, brain injury, neurodegenerative disorders (Ford *et al.*, 2020), etc. Researchers have continued to study the proliferation and differentiation of stem cells because, with the ability to regulate these processes, they can involve stem cells in Tissue Engineering and Tissue Regeneration to construct, modify, maintain and repair living tissues.

CRISPR-Cas_9, a revolutionary genome-editing and gene-silencing technology (Chattopadhyay and Srivastav, 2024), may be coupled with stem cell technology to precisely redesign stem cells, providing advanced therapeutics tailored to specific diseases. By studying the proliferation and differentiation of hESCs, it may become possible to develop effective therapeutic strategies to prevent congenital disabilities, infertility, deformities and conditions caused by embryonic stage errors.

It has been observed that Cancer Stem Cells(CSCs), which are present in the tumor, are mostly responsible for tumorigenesis, metastasis and for causing resistance to radiotherapy and chemotherapy (Cianciosi *et al.*, 2021). Thus, scientists are trying to understand the CSCs to develop novel therapies that can prevent metastasis and relapse of cancer.

Stem cells can further be applied in regenerative medicine to repair tissues that have been damaged due to wear and tear or affected by diseases by artificially culturing them into specialized cells. With future advancements, they may also allow organ repair and regeneration. Stem cell techniques may reduce the severity of the disease, thus diminishing the patients' intake of medicines. Scientists have observed that Bone Marrow Mononuclear Cells (BMMNC), which consist of Endothelial Progenitor Cells (EPC) and Mesenchymal Stem Cells (MSC), can repair myocardial damage and improve heart function through myocardial regeneration (Leventhal *et al.*, 2013).

DISADVANTAGES

ESCs exhibit pluripotency, which describes their ability to differentiate into nearly all types of cells. However, the generation of hESCs involves the use of unused human embryos, which raises bioethical and moral concerns. Due to ethical concerns, stem cell therapies involving hESCs are under tight regulation and require a lot of research and testing (Park *et al.*, 2024).

Although SSCs do not involve any ethical concerns, they exhibit multipotency and can differentiate into a more limited variety of specialized cell types, often of a closely related cell family. They are found in sparse amounts, leading to high purification expenses. Adult stem cells can neither be cultured for long or grown in vast amounts. Since it is a newly evolving branch of stem cell therapy, the long-term effects of using SSCs for curing diseases and conditions are yet to be completely understood.

hiPSCs were successful in bypassing the bio-ethical concerns associated with the generation of ESCs, but their reproducibility and long-term maintenance in the form of differentiated cells and tissues remains to be understood. Also, hiPSCs, like the hESCs, can potentially become embryos, if exposed to optimal conditions (Volarevic *et al.*, 2018).

One of the properties that sets stem cells apart from other cell types is their high cell proliferative ability. While it is crucial for stem cell culture and its use in stem cell therapy, excessive or abnormal cell proliferation can occur, which may lead to cancer formation (Aly, 2020). Thus, the generation of an inducible switch would efficiently prevent uncontrolled cell growth.

After transplantation surgery, the transplant stem cells from the donor are often recognized as a foreign body by the patient's immune system, leading to immune rejection (Volarevic *et al.*, 2018). Although introducing hiPSCs has reduced this threat, recent experiments show that most donor stem cells die within one day after transplantation. To avoid this, immunosuppressive drugs are administered, which may also lead to adverse drug reactions and infections. The donor stem cells might sometimes become oncogenic due to excessive cell proliferation (Aly, 2020). Thus, understanding graft tolerance by the host will tremendously improve stem cell therapies.

FUTURE PROSPECTIVES

Stem cells have fascinated the world with their myriad of opportunities. Researchers have continued to study the proliferation and differentiation of stem cells to understand the onset and development of disease in the body, opening new therapeutic techniques. ESC Therapies provide a viewpoint to treat diseases such as Diabetes, Spinal Cord Injury, Muscular dystrophy, Heart Diseases such as Heart Failure, Stroke and Myocardial infarction, Blindness, Deafness, etc (Aly, 2020). iPSC Therapy has appealed to the researchers because it possesses pluripotency but does not involve ethical concerns, as seen in ESCs. It may also cure lifelong use of immunosuppressors by patients who require them due to transplantation surgeries where transplants use donor stem cells. They can also be applied to create organoids, mimicking the characteristics of the target organism and allowing the testing of drugs, which will aid in drug modelling and development (Okano *et al.*, 2022) and will also reduce the use of animals involved in this process. Further, they can be applied to create disease models for drug testing.

Skin stem cells express multipotency and have been successful in anti-aging and repairing the skin of burn victims, paving the way for skin replacement and regeneration (Jo *et al.*, 2021). Stem cells can further be applied in regenerative medicine to repair tissues that have been damaged due to wear and tear or have

been affected by diseases by artificially culturing them into specialized cells. With future advancements, they may also allow organ repair and regeneration. With the ability to regulate the processes of proliferation and differentiation, scientists can involve stem cells in Tissue Engineering and Tissue Regeneration to construct, modify, maintain and repair living tissues.

With advancements in stem cell technology, it may become possible to develop effective therapeutic strategies to prevent congenital disabilities, deformities and conditions that are caused by errors in the embryonic stage. CRISPR-Cas_9, a revolutionary genome-editing and gene-silencing technology, has become a leading tool in the therapeutics of genetic diseases and disorders (Chattopadhyay and Srivastav, 2024). They may be coupled with stem cell technology to precisely redesign the stem cells, providing advanced therapeutics tailored to specific diseases. Extensive stem cell research provides insights into creating personalized treatment plans tailored to the patient's genotype and cater to medical requirements, therefore revolutionizing the medical industry altogether while reducing the rates of side effects.

In the future, it may allow other opportunities such as wound healing, treatment of brain, blood and cardiovascular diseases, cell deficiency therapy, etc. Also, it may be possible to find a permanent cure for diseases and conditions which are incurable to date, including Autoimmune diseases such as Rheumatoid Arthritis (RA), Addison Disease, Crohn's Disease, structural and functional deformities, Lupus, Type I Diabetes, Baldness, genetic diseases, Metabolic diseases, Blood-borne cancers, Retinal degeneration, Traumatic Brain Injury, Tendon ruptures, Neurodegenerative disorders such as Alzheimer's disease, Motor Neuron Disease, Parkinson's disease, etc (Ford *et al.*, 2020).

REGULATORY AND ETHICAL CONCERN

Generation of hESCs involves the use of unused human embryos, which raises bio-ethical and moral concerns. Due to this, stem cell therapies involving hESCs are under tight regulation and require a lot of research and testing (Park *et al.*, 2024). hiPSCs like the hESCs can potentially become embryos if exposed to optimal conditions (Volarevic *et al.*, 2018). Finding stem cell donors might become a difficult process. When introduced into the patient, the cultured stem cells may get contaminated with pathogens, leading to the onset of diseases and thus posing a serious threat to the patient (Vatsa *et al.*, 2022). Stem cell research involves altering gene structures and thus interferes with nature. It might impose greater risks for developing new diseases and thus, its impact must be completely analyzed and quantified.

There is a need for public awareness of stem cells and the potential of stem cell therapy so that they can benefit from them. Stem cell therapy may not be a permanent cure, and may require multiple rounds of therapy for remission or cure for the targeted disease or condition. The ethical, moral, social and bio-safety aspects need to be thoroughly studied and understood before the introduction of stem cell therapy to the public .

The long-term outcome and safety of stem cell therapy are under tight investigation. Thus, standardization of stem cell derivation, culture and differentiation is necessary for exhibiting improvements in the survival, proliferation, and regeneration of transplanted stem cells. It requires a well-defined regulatory framework and guidelines to develop safer and more effective stem cell -based therapies in the future. Before implementing new stem cell therapies, they must be duly tested in multiple in-vitro and in-vivo animal models to avoid lethal combinations.

ECONOMIC CONCERN

Stem cell therapy is a field of biotechnology that is highly important in regenerative medicine. In recent years, it has elucidated tremendous potential in developing tools and therapeutics by engineering stem cells. Stem cells possess unique properties that capture the attention of researchers. With further advancements in stem cell research, they may soon change the future of the healthcare industry altogether. However, there are a few financial concerns attached to stem cell therapy. The cost of stem cell therapy depends on the type, quality, source and quantity of stem cells involved, hospital location, transplantation complexity, patient health, etc. It also depends on the type of stem cell transplant utilized, such as an Autologous stem cell transplant using stem cells collected from the patient or an Allogeneic stem cell transplant using stem cells collected from related or unrelated donors. In India, the cost of an allogeneic stem cell transplant is higher than that of an autologous stem cell transplant because finding a suitable donor has a match with the patient is challenging.

As observed from the cost breakdown for stem cell therapy in India, the main cost of stem cell therapy is the cost of the transplant. Stem cell therapy usually requires multiple sessions for remission of the targeted disease or condition, requiring frequent hospitalization. The patient requires almost a year to recuperate. Immune rejection may occur in the case of allogeneic stem cell transplant. Thus, immunosuppressive drugs are administered to prevent the same, all of which add to the cost. Although many individuals suffer from diseases that can be treated using stem cell therapy, the overall cost of the same often exceeds their budget, leading to financial concerns. Health insurance plans mostly do not cover the cost of these modern treatments, which becomes a great disadvantage for the patients. On 1st October 2020, the Insurance Regulatory and Development Authority of India (IRDAI) released

a list of treatments covered under standard health insurance plans and included modern treatments such as stem cell therapy (Insurance Regulatory and Development Authority of India, 2019). With this opportunity, many individuals will get stem cell therapy, creating awareness of the same, and the world will witness a new era of advancement and prosperity.

Conclusion

Stem cell research has yet to reach its peak. Scientists are trying to understand and control the proliferation and differentiation of stem cells to develop personalized treatment plans tailored to the genetic makeup of patients, all the while reducing the rates of side effects, in an attempt to reduce the rate of mortality and improve the health, vitality and longevity of humans. With the advancement of stem cell research and commercialization of the same, we humans, together as a species, can break yet another boundary set up by Mother Nature. Though immortality still feels utopian and better suited for child follies and fairy tales, the idea of mortality feels like a dawning sun on the horizon. With the onset of a new era of humans, environmental impacts, resource distribution and population control might be the new alarm topics. The truths of life and death will need a redefinition in a world that the religion of money will soon govern.

Conflict of interest

The author declare that she has no conflict of interest.

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