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New insights on stem cells modeling and treatment of human diseases

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TABLE OF CONTENTS

1. ABSTRACT

Stem cells exist in many niches throughout the body and have the ability to self replicate and to differentiate to many lineages. As of a result of the advances in stem cell-based therapies, regenerative

medicine is witnessing remarkable development. Encouraging positive outcomes from the use of stem cells in various diseases are extremely promising.. The popularity of stem cell-based therapy is due to its flexibility and potent approach in the treatment of numerous diseases. Treatment with genetically configured HSCs favors the engraftment of transplantation without rejection. MSCs hold an immunoregulatory capacity, elicit immuno-suppressive effects and are immune-privileged cells, due to the low expression of MHCII and costimulatory molecules on their cell surface. Encouraging, positive outcomes from the use of stem cells in immunodeficiency, cancer, hemoglobinopathy, bone, cartilage repair, autoimmune disorders, cardiac and neuronal diseases are extremely promising. Successful stem cells based clinical trials are the game changers in the progress of clinical use of stem cells. This review provides an up to date comprehensive overview of the clinical efficacy of stem cells.

2. INTRODUCTION

The last decade has proved to be a period of profound advancement in stem cell research and its clinical translation. The exciting part is that these cells lend themselves for tissue repair or regeneration, raises hopes for curing diseases earlier tagged as untreatable. To utilize their full potential for regeneration and repair, information on their origin, fate and functional abilities need to be known. The nature and functions of HSCs and MSCs are distinct and they vary in their origin, lineage differentiation commitments, culture conditions and secretory factors and surface markers present on them. They are currently serving as putative therapeutics, and several findings underline their efficiency in the treatment of different diseases. For the efficient therapeutic implication of the stem cells, an in-depth understanding of their maintenance in their *in vivo* niches, methods of their delivery from exogenous sources and expansion of their population is warranted in addition to knowledge on the mechanism employed in the treatment of damaged and diseased tissues. A systematic assessment of organ and disease conditions is mandatory for considering stem cell therapy. Thus far, there are 7,718 stem cell-based clinical trials recorded in the database of the US National Institutes of Health (NIH) (https://clinicaltrials.gov/) as of 25 January 2020.; among which 3,015 trials appear to be completed. Of the ongoing studies 3,7048 studies are based on HSCs and 1,035 based on MSCs.

In this article, we provide a comprehensive review of stem cells (HSCs and MSCs) based clinical trials conducted worldwide, recent clinical findings and highlighting recent technological advancements and therapeutic challenges.

3. HSCS, PROGENITORS & THEIR CHARACTERIZATION

HSCs are present in an enriched and diverse niche in the body, eventuallydifferentiating into mature blood cells. In previous years, the role of HSCs has been extensively studied for their therapeutic potential in treating blood-related disorders. The hematopoietic system is well characterized in terms of its stem and progenitor cell biology. HSCs with their committed downstream progenitors generate at least eight different blood cell lineages throughout life. The maintenance of these multiple lineage fates perhaps control regulatory moleculesthat simultaneously coordinate proliferation, quiescence, and programmed cell death. Disturbance in this coordination which governs stem and progenitor cell behavior can lead to major clinical consequences like leukemia or aberrant hematopoiesis (1).

Characterization of Human HSCs relies on various cell-surface marker phenotypes present on cells at their different stage of differentiation, coupled with some functional assays. CD34, which is a ligand for L-selectin is identified as the first cell surface markers. It is present on 0.5–5% of total blood cells in cord blood, human fetal liver, and adult bone marrow (2-4). While heterogeneous in nature, *in vitro* assays confirmed that almost all $CD34^+$ cells are multipotent or oligopotent. Human HSCs exhibiting $CD34^+CD90^+Lin^-$ phenotype were the first cell population to be isolated, whereas the B, T, natural killer (NK), and myeloerythroid cells bear Lin markers (5). *In vitro* colony-forming assays and *in vivo* SCID (severe combined immunodeficiency)-hu mice model with human fetal bones implants have shown that

these cells could differentiate into lymphoid and myeloid progeny however the stem cell population with $CD34^{+}CD90^{-}Lin^{-}$ phenotype could not differentiate into myeloid and lymphoid cell types (5). It has been reported that 90–99% of $CD34^{+}$ cells co-express CD38 yet,it is the $CD34^{+}$ $CD90^{+}CD38^{-}$cell population that can differentiate into both myeloid and lymphoid cell subsets (5-9). Interestingly, $CD34^{+}CD38^{-}$cells can develop into long term populating cells and thus $CD34^{+}CD38^{-}$ SCID-repopulating cell (SRC) cells can generate lymphoid cell subsets in SCID mice and non-obese diabetes-SCID immunodeficient mice (5, 6, 10 -12). Human HSCs coexpressing CD34 and CD90, isolated from peripheral blood after mobilization by the granulocyte-colony stimulating factor (G-CSF) can generate new cells, enriching the immune reconstitution and long-term engraftment. Notably, contamination of immunoreactive T and host cancer cells can diminish the proficiency of transplantation (13-15).

A recent study reported that the multi-Potent Progenitor (MPP) population with Lin^{-} $CD34^{+}$ $CD38^{-}CD90^{-}CD45RA^{-}$ phenotype in human cord blood possesses multipotent capacity, but an inadequate self-renewal activity (16). Early myeloid and lymphoid committed progenitors generated from MPPs further differentiate into common myeloid progenitor (CMP), granulocyte/macrophage progenitors (GMP), megakaryocyte/erythrocyte progenitors (MEP), and common lymphoid progenitor(CLP) in the human hematopoietic system (17-21). CD45RA (an isoform of CD45) and IL-3Ra (an isoform of CD45, which reversely regulates few classes of cytokine signaling) are used to characterize human CMP, GMP, and MEP cell populations by *in vitro* and *in vivo* studies (21).

3.1. Therapeutic potential of HSCs

Genetically inherited blood disease like, primary immune deficiencies, hemoglobinopathies, various types of cancer, metabolic and storage diseases, congenital cytopenias along with stem cell defects, can be cured by allogeneic HSCs transplantation (22, 23). The transplantation of allogeneic HSCs serves as a constant source of blood cells of all lineages, eliminating defective cells, thus providing a lifelong cure to patients (Figure 1) (24).

3.1.1. Conventional Allogeneic HSCs transplantation

3.1.1.1. Leukemia and Lymphoma

HSCs continue to be the wonder cure of leukemia and lymphoma, resulting from the unregulated proliferation of white blood cells. After the patient's cancerous hematopoietic cells are destroyed via radiation or chemotherapy,, aa compatible HLA typing bone marrow transplantrescues the patient. To overcome the often-difficult nature of finding a compatible donor, the Food and Drug Administration approved Gleevec, also known as imatinib mesylate, an oral drug that can be used as an alternative to a bone marrow transplant when it comes to treating patients with CML. Gleevec only targets the mutant protein produced exclusively by CML cancer cells that disrupt the cell signals controlling the orderly division of progenitor cells. By blocking this protein, the drug restricts the overproduction of cancerous white blood cells (25).

3.1.1.2. HSC recovery in Cancer Chemotherapy

Chemotherapy although aimed at destroying only cancer cells, harms normal cells in circulation. To overcome this issue autologous transfer of stem cells was suggested. In this method, before chemotherapy, the body’s HSCs are mobilized and isolated from peripheral blood. After chemotherapy, these cells are transferred back to the patient, greatly reducing the risk of immune mismatch or graft-versus-host disease. However, there is a chance that some cancer cells get collected during the HSC isolation and can be re-infused back into the patient’s body. This limitation can be overcome by eliminating cancer cells through rigorous purification steps to ensure the preservation of HSCs only (26).

3.1.1.3. HSCs in Graft-Versus-Tumor Treatment of Cancer

The most exciting aspect of HSC transplantation is their role in treating untreatable tumors. A group of researchers in NIH recently applied a new approach to treat metastatic kidney

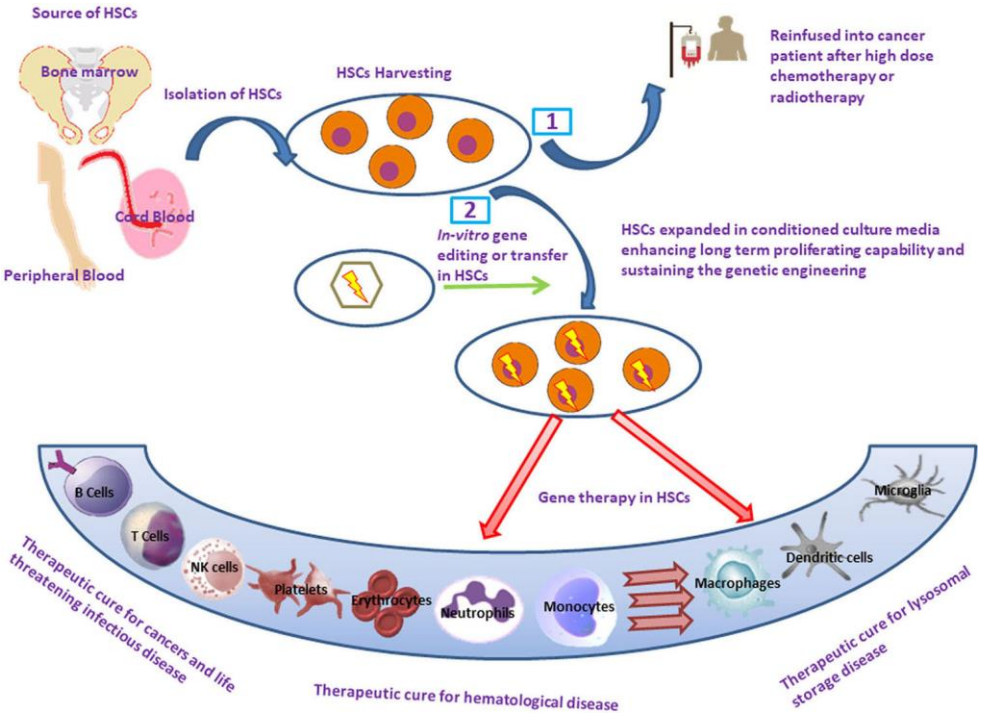

Figure 1. A schematic illustration of therapeutic potentials of HSCs. HSCs isolated from various sources like bone marrow, peripheral blood or umbilical cord. 1. Pure HSCs are then harvested to transplant into a cancer patient through a catheter placed in a blood vessel after a high dose of chemotherapy or radiotherapy. HSCs then traverse to the bone marrow to produce new blood cells.2. HSCs cultured in appropriate conditioned medium with cytokine supplementation promoting their long term proliferative capacity, grooming them for genetic engineering. The genetically engineered cells can be infused in patients, where they settle in the bone marrow and sustain their self-renew capability and differentiate into myeloid, erythroid and lymphoid lineage cells for the lifetime. The gene-modified cells then proliferate in circulations capable of curing the preexisting complex pathogenicity disturbing the myeloid lineage-specific warrior cells present throughout circulations and also in the central nervous system (giving rise to lysosomal storage disease), affecting the lymphoid system, such as primary immunodeficiencies, the erythroid lineages, like sickle cell disease, thalassemia, etc.

cancer. This experimental treatment involved allogeneic stem cell transplantation from an HLA-matched sibling, whose HSCs were mobilized and collected peripherally. After neutralizing the patient's immune system the donor's cells were transfused into the patient. Suppression of the patient's immune system is necessary to reduce graft-versus-host disease (GVHD). Without this step, self T cells will attack the incoming donor cells and destroy them (27). Joshi *et al.* (2000) show that umbilical cord blood-derived HSCs and peripherally harvested HSCs have antitumor activity in the test tube against leukemia cells and breast cancer cells(28).

3.1.1.4. Aplastic Anemia (AA)

AA is a life-threatening form of acquired bone marrow failure affecting the immune system (29). It is characterized by the development and expansion of self-reactive T cells. Due to this abnormal expansion of T cells, HSCs, progenitors, and mature blood cells get destroyed, leading to severe consequences and if untreated causes fatal marrow hypoplasia and pancytopenia. Immunosuppressive therapy (IST) with anti-thymocyte globulin and cyclosporine A, which targets self-reactive T cells and bone marrow transplants, are considered to be the standard of care treatment for AA. Yet, IST approach is often not applicable due to poor response or relapse post IST. As with leukemia, bone marrow transplants are not an ideal option due to lack of histocompatible donor, age, co-morbidities, etc (30-32). Maiguel *et al.* (2011) have discovered a drug named leukadherins, which protects HSCs from self-reactive effector T cell in AA condition, thus reducing the loss of HSCs and its progenitor cells in immune-mediated AA, improving

the disease condition (33). Although allogeneic HSCs transplantation is considered to be an excellent approach to treat genetic blood disorders, the non-availability of suitable matched donor results in immunological complications. In autologous HSCs transplantation, the patient's HSCs are genetically engineered to escape the risk of immunological complications leading to a better cure for patients with genetic blood cell disorders (24).

3.1.2. Genetically engineered HSCs

Genetically Engineered or modified HSCs derived from adult or cord blood are the new therapeutic strategy to treat critical diseases. In this field, Takahashi *et al.* (2006) have made excellent contributions by successfully reprogramming mouse fibroblast into induced pluripotent stem cells by gammaretroviral mediated introduction of the transcription factors Oct4, Sox2, Klf4, and c-myc. This groundbreaking discovery has opened new avenues for therapeutic stem cell engineering. The possibility of harvesting pluripotent stem cells without affecting their stemness properties makes it possible to subclone and select genetically modified cells, as well as to perform extensive efficacy and safety testing in the selected clonal derivatives. Hanna *et al.* (2007) has conducted an interesting study using sickle cell anemia mouse model.They have corrected the β^S-globin gene by homologous recombination in fibroblast-derived from humanized sickle cell transgenic mouse, which was later reprogrammed by retroviral transduction to a pluripotent state,encouraging hematopoietic differentiation in the presence of HoxB4 protein. However, transplantation of the engineered hematopoietic cells were unable to achieve full hematopoietic reconstitution, but diminished the sickle cell syndrome (34-36). There are many retroviral generated vectors that have been used for *ex vivo* gene transfer vectors, including the gammaretroviral vector derived Moloney murine leukemia vectors as well as lentiviral vectors (LVs) (37,38), of which LVs based autologous transplant/gene therapy have generatedclinical advances similar to those from allogeneic transplant for several disorders (39-42). In most clinical trials, LV based therapies have constant stability in the correction of genes of all blood cell lineages, indicating long-term persistence, committed engraftment and ongoing generative capacity of gene-modified HSCs, with no significant reduction in humans over time (24).

3.1.2.1. Lentiviral gene transfer

Gene therapy involving autologous transplant/gene therapy by LVs has shown successful results equivalent to allogeneic transplants for several disorders (24). The first LV clinical testing was done in T cells of human immunodeficiency virus (HIV) infected patients to check for viral resistance (43, 44) before testing in HSCs (45). In the absence of suitable HSCs donor, LV based gene therapy was proposed to allow for broader treatment possibilities due to its higher gene transfer efficiency. The demyelinating leukody-strophies metachromatic leukodystrophy (MLD) (40), adrenoleukodystrophy (ALD) (45) and the immune and platelet deficiency Wiskott Aldrich syndrome (WAS) (39, 46) were the first diseases to be treated. High-titer lentiviruses have successfully cured sickle cell disease and β-thalassemia (47, 48) in the mouse model. The human multidrug resistance 1 gene (MDR1), the P-glycoprotein product of which is required for the removal of certain drugs from cells (49, 50) is used as the selection marker for further expansion of gene-corrected HSCs and their progeny by drug treatment. Anthracyclines and taxanes are the most commonly used in the selection method, as they are toxic to untransduced cells without significant endogenous MDR1 activity. This led to a discreet selection as well as an expansion of the MDR1- transduced human HSCs (49-51). Nowadays more than 200 patients worldwide have been treated by LV based HSC gene therapy for several diseases, including ALD (52), MLD (53), WAS (54), chronic granulomatous disorder (CGD), some primary immunodeficiencies (42, 55), mucopolysacc-haridoses, thalassemia sickle cell disease (56-59), Fanconi anemia (60) and HIV infection (61), with a follow up including up to 10 years for the earliest treated patients. Due to higher therapeutic potential and safety measurements LT based HSCs gene therapy is now a widely accepted approach (55).

3.1.2.2. Gene therapy using genetically engineered HSCs for various diseases

Gene therapy trials of genetically engineered HSCs and progenitor cells have shown promising results as an alternative strategy of allogeneic transplantation in the treatment of primary immunodeficiency. However, early clinical trials have some limitations like the risk of oncogene transactivation and insertional mutagenesis related

to gamma retroviral vectors. Therefore, safer and more stable, self-inactivating vectors. LVs have been successfully used to treat almost 10 different hematological disorders like haemoglobinopathies, primary immunodeficiencies, and metabolic disorders (38).

3.1.2.2.1. SCID

The first gene therapy trials for SCID, done 20 years back, established the future prospective cure of *ex vivo* gene addition to HSPCs (62). SCID is caused by defects in immune system development and function, resulting in life-threatening infections in children. SCID is considered a heterogeneous condition as approximately 20 different genetic causes have been identified (63). X-linked SCID (X-SCID) and SCID caused by adenosine deaminase (ADA) deficiency (ADA-SCID) account for 40% and 10% of all SCID forms, respectively. \All forms of SCID are primarily characterized by the absence of functional, circulating polyclonal T cells. If untreated, children with SCID die within their first year of life, however both X-SCID and ADA-SCID have been successfully treated with gene therapy. Furthermore, a gene therapy product for ADA-SCID is the second gene-based product to obtain market approval from the European Medicines Agency (Strimvelis; GlaxoSmithKline) (38).

X-SCID occurs due to mutations in interleukin-2 receptor subunit-gamma, causing the complete elimination of T cells and natural killer (NK) cells and contributing to the presence of functionally impaired B cells. Before the advent of gene therapy, the only treatment available was HSCs transplantation. Later, major gene therapy trials were conducted in France and UK where almost 20 infants with SCID and no matched sibling donor were treated with autologous $CD34^+$ bone marrow cells infusion after transduction with a gammaretroviral long terminal repeat (LTR)-driven vector. The gene therapy trial relied on complementary DNA (cDNA), comprised of a defective gamma chain Molony retrovirus derived vector with $CD34^+$ cells. Interestingly, after monitoring for10 months gamma chain transgene expressing T and NK cells were observed in patients (62, 64). These two trials established the worthwhile therapeutic potential of this approach (65). It has been reported that 20 years after gene transfer, 18 of the 20 treated patients were alive and had full or nearly full correction of T cell immunodeficiency, including normal T cell subset counts, along with a presence of naive T cells (even in leukemia patients who had undergone gene therapy), a diversified T cell repertoire and normal T cell-mediated immune functions (66-68). Further, the third international X-SCID gene therapy clinical trial (performed in parallel in Europe and the USA) was operated with a second-generation self-inactivating (SIN) retroviral vector without any LTR enhancer sequences (69). Seven out of the nine treated patients recuperated a functional T cell compartment that retained its immune function with no genotoxic effects.

3.1.2.2.2. WAS

Wiskott Aldrich syndrome protein (WASP) is important for cytoskeletal reorganization, terminal differentiation and signal transduction in several hematopoietic cell types. Therefore, mutations in the *WAS* gene cause a complex, X-linked primary immunodeficiencies resulting in complicated clinical manifestations, including micro thrombocytopenia, recurrent infections, and eczema. Patients also develop autoimmune manifestations and tumors (68, 70). A genotype-phenotype correlation has been reported by Imai *et al* 2004 (71). The first clinical trial (occurred in Hannover) for WAS involved ten patients who were treated with a WASP-encoding, LTR driven first-generation gamma retroviral vector (72). The trial was successful in nine of ten patients with complete recovery of their immune system, but they developed myelodysplastic syndrome or leukemia at different time points (73). This raised serious concerns about this first-generation vector. Further LV encoding the human WASP cDNA under the control of the human endogenous promoter was used for a second clinical trial, which was performed in Milan (39), Paris, London (46) and most recently Boston (74). As a result, T and B cell immunity was reconstituted; they were free from recurrent infections with less frequent autoimmune conditions. Poor prognosis in patients was also reported and current studies are seeking improvements s (38).

3.1.2.2.3. CGD

CGD is a primary immunodeficiency of innate immunity caused by defects in NADPH

oxidase subunits. Recently clinical trials were conducted using chimeric myeloid-specific promoter expressing *CYBB*, which encodes the NADPH oxidase catalytic subunit cytochrome *b* 245 heavy chain (also known as GP91PHOX) (75, 76). Weisser *et al.* (2016) have reported that chronic inflammation negatively regulates HSPCs in CGD. It has also been reported that both in humans and mice there was a diminution in HSC population in the bone marrow (77). Further studies are being conducted to resolve these problems and to improve the gene therapy outcomes in CGD.

3.1.2.2.4. LSD

Clinical trials for LSD such as MLD, which is caused by a defect in the production of a functional lysosomal enzyme, arylsulfatase A (ARSA) have been reported (38). Enzyme replacement therapy and HSC transplantation have been established as potential treatments for LSD. Gene therapy for MLD involves autologous HSPCs transplantation whichdifferentiates into macrophages and microglia in the central nervous system and then provides the ARSA for cross-correction of the affected nervous tissue (78, 79). Experiments in murine models showed intracerebroventricular transplantation of human HSPCs, along with ARSA-expressing LV, resulting into more stable and more effective delivery of higher levels of ARSA enzyme to the brain relative to standard intravenous transplantation (40).

3.1.2.2.5. β-hemoglobinopathies

The β-hemoglobinopathies- β-thalassemia and sickle cell disease (SCD) are the most common monogenic blood disorders and constitute a global health problem.β-Thalassemia occurs due to mutations that reduce or abolish the synthesis of β-globin chains (80). Patients with severe β-thalassemia suffer from anemia, iron overload, hepatosplenomegaly, and various organ complications and are treated with recurrent blood transfusion and iron chelation. Presently, the only curative treatment for β-thalassemia is allogeneic HLA- HSCs transplantation. HSCs transplantation involves high-dose chemotherapy and immunosuppression. Due to the lack of sibling donors for HSCs transplantation, gene therapy is used as an alternative therapeutic approach. The invention of SIN LVs that have an optimized β-globin gene under the control of the β-globin promoter, a 3 prime enhancer and the DNase-I-hypersensitive sites 2, 3 and 4 from the β-globin locus control region has therapeutic potential to cure this disease. The first clinical trial for β-thalassemia (the LG001 study, authorized in France in 2006) involved nan LV (HPV569) containing a β-globin cassette flanked by two LTRs, each containing two copies of the core 250 bp HS4 chicken insulator (81). The first patient sustained the level of hemoglobin and remained stable for more than 8 years, exhibiting 30% of the total hemoglobin. In 2015, another phase I/II clinical trial was conducted in Italy, involved G-CSF mobilized HSPCs transplantation of HSPCs, along with plerixafor and transduced with the compact β-globin-expressing GLOBE vector (82, 59). A myeloablative, low toxic, conditioning procedure (based on treosulfan and thiotepa) was used to indulge engraftment. Ferrari *et al.* (2017) reported that seven patients with different genotypes had been treated with plerixafor with G-CSF-mobilized, transduced $CD34^{+}$cells at a high dose ($>10 \times 10^{6}$ cells per kg) and a vector copy number per cell ranging from 0.7 to 1.5. The clinical outcome mostly benefitted younger patients (83).

4. MSCS AND ITS THERAPEUTIC APPLICATIONS

MSCs are self-renewing, multipotent cells found in bone marrow, skeletal muscle, dental pulp, bone, umbilical cord and adipose tissue (84). In accordance with the minimum criteria for defining the MSCs as proposed by the International Society for Cellular Therapy, MSCs should exhibit plastic adherence and should have a specific set of cell surface markers, i.e. cluster of differentiation (CD)73, CD90, CD105 and lack expression of CD14, CD34, CD45 and human leukocyte antigen DR (HLA DR). These should also have the ability to differentiate *in vitro* into adipocyte, chondrocyte, and osteoblast (85).

Plating studies indicate MSCs are present as a rare population of cells in bone marrow, representing perhaps 0.001% to 0.01% of the nucleated cells, 10 fold less abundant than HSCs, but MSCs can be readily grown in culture. The advantage associated with MSCs among the many different

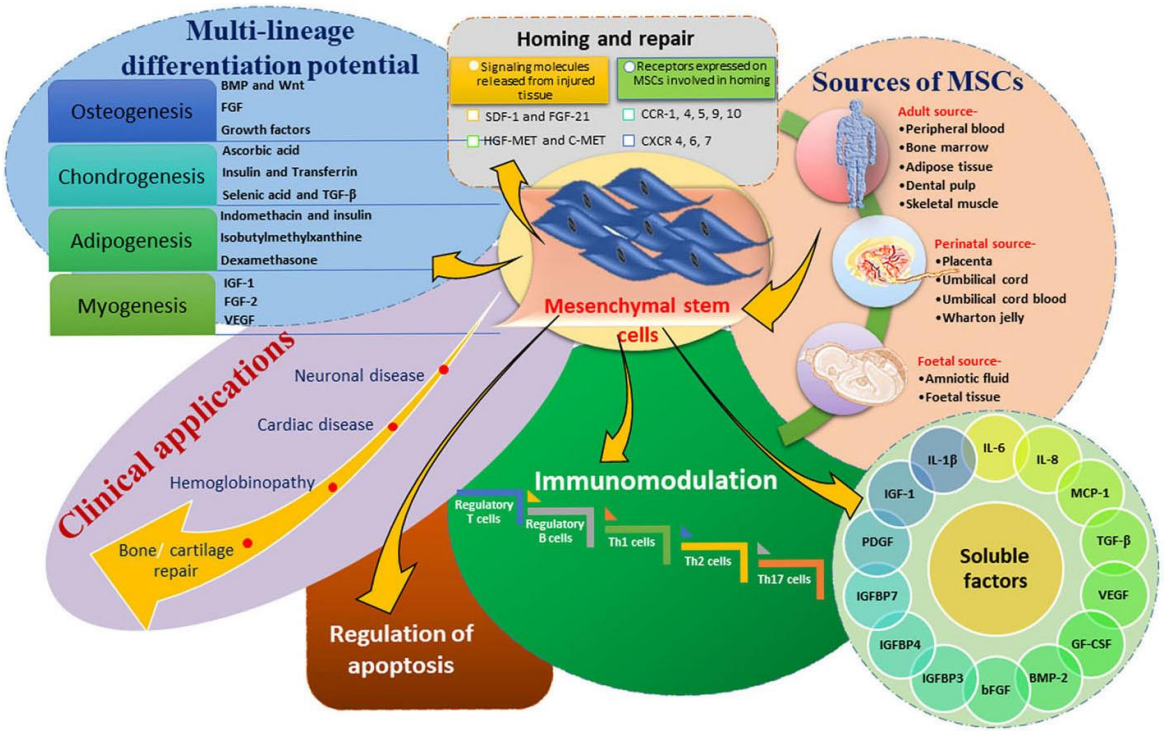

Figure 2. MSCs are derived from different sources like adult, perinatal and fetal tissue. They also have the potential to differentiate into various lineages. MSCs based clinical applications range from neuronal and cardiac disease, hemoglobinopathy to bone/ cartilage repair. They also exert immunomodulatory effects in autoimmunity and involved in the regulation of apoptosis. In response to some signaling molecules from injured tissue MSCs home to the damaged tissue for repair. The secretion of soluble factors (cytokines, chemokines, etc.) by MSCs helps in the synchronization with their microenvironment desirable for transplant maintenance.

stem cells, that lie in their ease of accessibility, handling, and multilineage potential. Outstandingly, cultured MSCs have already been infused in humans for safety and early clinical testing for the sustenance of bone marrow transplantation, treatment of hematological pathologies, cardiovascular diseases, lung, liver, kidney injury, neurological and cardiovascular diseases, chronic inflammation and autoimmune disorders.

In addition to their regenerative properties, MSCs hold an immunoregulatory capacity and elicit immunosuppressive effects in a number of situations. Not only are they immune privileged cells, due to the low expression of class II major histocompatibility complex (MHC II) and costimulatory molecules in their cell surface, but they also interact with several immune pathways through direct cell-to-cell interactions and soluble factor secretion (86). The therapeutic potential of these cells emerges from several properties, including their ability to differentiate into various cell lineages and commitment, secrete soluble factors crucial for cell survival and proliferation, modulate the immune response, help in home and repair and serve as a regulator of apoptosis (Figure 2).

4.1. MSCs and their multi-lineage differentiation potential

MSCs differentiate both *in vitro* and *in vivo*, into various mesenchymal tissues, including those of bones, cartilage, fat, muscles, tendons, and bone marrow showing multipotency and can transdifferentiate into non-mesoderm-like cells, including neuronlike cells, hepatocytes, and pancreatic islet-like cells. Osteogenic differentiation is stimulated by BMPs, WNTs, FGFs and growth factors and other heparan sulfate sensitive morphogens at a specified concentration of

dexamethasone and ascorbic acid. (87-92) Indomethacin, insulin, and isobutylmethylxanthine along with dexamethasone stimulate adipogenesis (93) whereas ascorbic acid, insulin, transferrin, selenic acid, and TGFbeta are known to induce chondrogenesis (94-96). During autologous transplantation, synovial fluid-derived mesenchymal stem cells (SF-MSCs) show huge potential for cartilage regeneration. Jia *et al.* (2019) showed that TGF beta3 and kartogenin work synergistically and help repair cartilage defects through the regeneration of hyaline cartilage by promoting the process of chondrogenesis of rabbit SF-MSCs (97). Under defined conditions MSCs exhibit the potential to differentiate into myocyte, neural, and epithelial cells indicating endodermic and neuroectodermic differentiation. (98-101) MSCs also differentiate into pancreatic islet cells (102) and contribute to wound repair by differentiating into keratinocytes and multiple skin cell types (103).

4.2. State of MSCs in clinical research

Promising shreds of evidence supporting differentiation and trans-differentiation potential of MSCs promote new clinical perspectives based on their function and will help repair tissue and organ damage by replacing the damaged resident cells. Here, we provide an update on successful clinical applications of MSCs in various diseases.

4.2.1. MSCs in Neuronal diseases

In neurodegenerative processes and traumatic disorders, demyelination plays central role. The exogenous supply of myelinating cells via transplantation can be achieved by using MSCs. Using a cytokine cocktail Keilhoff *et al.* (2006) were able to form rat MSC derived myelinating cells. These transdifferentiated MSCs had increased expression of LNGF-receptor, Krox20, and CD104 with the expression of BMP receptor-1A downregulated as compared to untreated MSCs.. They co-cultured PC-12 cells with MSCs and grafted the transdifferentiated MSCs and Schwann cells into an autologous muscle in the sciatic nerve of the rat. After 14 days transdifferentiated MSCs and Schwann cells could myelinate PC12 cells *in vitro*. Autologous nerve grafts were appropriately myelinated both in the Schwann cell groups and in the transdifferentiated MSC groups *in vivo*, whereas regeneration was impaired in the cell-free and the untreated MSC groups (104).

Studies in spinal cord injury (SCI) patients have shown that for a treatment to be effective it must primarily improve their quality of life. Numerous studies have shown that stem cells represent an alternative treatment for various disorders and have shown promise in several disease/trauma states. For instance, the use of autologous $CD34^{+}$stem cells has been shown to ameliorate symptoms of several disorders such as leukemia, cardiomyopathy, diabetes and several autoimmune diseases, including multiple sclerosis. For the first time, Geffner *et al.* (2008) reported eight case studies of SCI (four acute, four chronic) with approximately 2 years of follow-up that were administered bone marrow stem cells (BMSCs) via multiple routes: directly into the spinal cord, directly into the spinal canal, and intravenous. Magnetic resonance imaging illustrated morphological changes in the spinal cord of some of the patients following BMSCs administration. Comprehensive evaluations demonstrated improvements in the American Spinal Injury Association scale, Barthel index (quality of life), Frankel, and Ashworth scoring. They have administered BMSCs into 52 patients with SCI and have had no tumor formations, no cases of infection or increased pain and few instances of minor adverse events. Their study showed that BMSCs administration via multiple routes is feasible, safe, and may improve the quality of life for patients living with SCI (105).

Alhazzani *et al.* (2018) investigated the role of MSCs in neuroprotection and regeneration and identified how MSCs complement the rescue of neuronal cell death mediated by (Ca^{2+}) and reactive oxygen species (ROS). SH-SY5Y-differentiated neuronal cells were exposed to *in vitro* cerebral ischemia-like stress and were rescued from cell death by carrying out MSCs and neuronal cell co-culture experiments. Neuronal cell death is characterized by the induction of proinflammatory tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-12 with corresponding downregulation of anti-inflammatory cytokine transforming growth factor (TGF)-β, IL-6 and IL-10. Increased intracellular

calcium (Ca^{2+}) and ROS confirmed the oxidative stress-mediated apoptosis, while upregulation of nuclear factor NF-κB and cyclo-oxygenase (COX)-2 expressions, along with apoptotic cells, established ischemic stress-mediated cell death. Stressed neuronal cells were rescued from death when co-cultured with MSCs via increased expression of anti-inflammatory cytokines (TGF-β, IL-6and IL-10), significantly downregulated NF-κB and proinflammatory COX-2 expression. Low superoxide dismutase 1 expression at the mRNA level was rescued by MSCs coculture, while no significant changes were observed with catalase and glutathione peroxidase. Interestingly, increased serotonin released into the culture supernatant was proportionate to the elevated Ca^{2+} and corresponding ROS, which were brought down to normal levels by coculture with MSCs.The observed results indicate towards MSCs-mediated modulation of stressed neuronal cell survival *in vitro* (106).

4.2.2. Cardiac diseases

Vrtovec *et al.* (2011) investigated the clinical effects of intracoronary transplantation of $CD34^+$ cells in patients with dilated cardiomyopathy (DCM). The study included 55 patients with DCM, 28 were randomized to $CD34^+$ transplantation (SC group), and 27 patients did not receive stem cell therapy (controls). In the SC group, peripheral blood $CD34^+$ cells were mobilized by granulocyte-colony stimulating factor and collected via apheresis. Patients underwent myocardial scintigraphy and $CD34^+$ cells were injected in the coronary artery, supplying the segments with reduced viability. They found that the intracoronary stem cell transplantation could lead to improved ventricular remodeling, better exercise tolerance and potentially improved survival in patients with DCM (107).

Hare *et al.* (2017) studied chronic non-ischemic dilated cardiomyopathy (NIDCM) in thirty-seven patients at the University of Miami Hospital between December 2011 and July 2015. Theywere given allogeneic and autogenic hMSCs in 1:1 ratio. Patients were given 100 million hMSCs via transendocardial stem cell injection in the left ventricular sites. The results showed that both allogeneic as well as autogenic were safe, with increase in serious adverse events (SAE) and did not enhance the immunologic reactions during the POSEIDON (The PercutaneOus StEm Cell Injection Delivery effects On Neomyogenesis) pilot study (108).

Ischemic cardiomyopathy (ICM) and DCM have different histopathology as well as diagnosis. Tompkins *et al.* (2018) compared the efficacy of transendocardial delivery of MSCs in ICM and DCM. They conducted 3 single-center, randomized, and blinded clinical trials: (1) TAC-HFT (Transendocardial Autologous MSCs and Mononuclear Bone Marrow Cells in Ischemic Heart Failure Trial); (2) POSEIDON (A Phase I/II, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous MSCs Versus Allogeneic MSCs in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction); and (3) POSEIDON-DCM (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis in Dilated Cardiomyopathy). It was found that MSC therapy was beneficial in ICM and DCM patients, though the effects were variable on the phenotypic outcome. MSC therapy enhanced functional capacity and quality of life in both DCM and ICM patients (109).In another study, DCM patients with the pathophysiology of endothelial dysfunction showed improvement with allogeneic but not autologous MSCs therapy. Following the transplant of autologous (n = 11) or allogeneic (n = 10) MSCs in a subset of POSEIDON-DCM patients, their plasma, TNF alpha and endothelial progenitor cell-colony forming units were assessed. Aside fromendothelial cells, TNF alpha mRNA expression, Stromal derived factor 1 (SDF-1), alpha secretion by MSCs and levels of ROS in response to SDF-1 alpha were assayed. It was observed that the MSC secretion of SDF-1 alpha inversely correlated with EPC-CFU production in DCM patients and therefore may be a modulator of MSC therapeutic effect in this clinical setting (110).

4.2.3. Hemoglobinopathy

Allogeneic HSCs transplantation for patients with hemoglobinopathy can be curative but is limited by donor availability. Although positive results are frequently observed in those with an HLA-matched sibling donor, the use of unrelated donors has been complicated by poor engraftment, excessive regimen-related toxicity, and GVHD. As a

potential strategy to address these obstacles, a pilot study was designed that incorporated both reduced-intensity conditioning and MSCs. Six patients were enrolled, including 4 with high-risk SCD and 2 with transfusion-dependent thalassemia major. Conditioning consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (60 mg for patients weighing > 30 kg and.9 mg/kg for patients weighing <30 kg). Two patients received HLA 7/8 allele matched bone marrow and 4 received 4-5/6 HLA matched umbilical cord blood as the source of HSCs. MSCs were of bone marrow origin and derived from a parent in 1 patient and an unrelated third-party donor in the remaining 5 patients. GVHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil. One patient had neutropenic graft failure, 2 had an autologous hematopoietic recovery, and 3 had hematopoietic recovery with complete chimerism. The 2 SCD patients with autologous hematopoietic recovery are alive. The remaining 4 died either from an opportunistic infection, GVHD, or intracranial hemorrhage. Although no infusion-related toxicity was seen, the co-transplantation of MSCs was not sufficient for reliable engraftment in patients with advanced hemoglobinopathy. Although poor engraftment has been observed in nearly all such trials to date in this patient population, there was no evidence to suggest that MSCs had any positive impact on engraftment. Because of the lack of improved engraftment and unacceptably high transplant-related mortality, the study was prematurely terminated. Further investigations into understanding the mechanisms of graft resistance and the development of strategies to overcome this barrier are needed to move this field forward (111).

Beta Thalassemia major (beta-TM) is characterized by anemia that is caused by a genetic defect in hemoglobin synthesis and results in ineffective erythropoiesis (IE). During IE, there is a change in the microenvironment of thalassemia bone marrow which causes changes in BMMSCs. Ceren *et al.* (2012) looked into the global structural and compositional changes in BM-MSCs in β-TM that may provide a basisfor understanding interactions of HSCs-MSCs in such a pathological bone marrow microenvironment. They attributed the significant increase in lipid, protein, glycogen, and nucleic acid contents in thalassemic BM-MSCs to enhanced cell proliferation and BM activity. The significant decreases in the content of the mentioned macromolecules in post-transplant group BM-MSCs versus pre-transplant BM-MSCs were interpreted as a restoring effect of BMT therapy on IE and defective BM microenvironment. These alterations were also supported by erythropoietin and growth differentiation factor in bone marrow plasma samples as a reflection of IE (112).

4.2.4. Bone/ cartilage repair

Allogeneic MSC therapy has been proposed as a convincing therapeutic option for chronic knee osteoarthritis. In a study, 30 patients with chronic knee pain were recruited and divided into 2 groups of 15 patients. These patients were unresponsive to conservative treatments and exhibited radiological evidence of osteoarthritis. The control group of patients was given intra-articular hyaluronic acid whereas the test group was given an intra-articular injection of allogeneic bone marrow MSCs. They were evaluated for the nextone year and their pain, disability, and quality of life were assessed. The MSC-treated patients showed improvement in algofunctional indices and improvement in cartilage quality whereas the controls group treated with hyaluronic acid did not. Articular cartilage quality was assessed by quantitative magnetic resonance imaging T2 mapping (113).

Thiazolidinedione (TZD) therapy has been associated with an increased risk of bone fractures. Studies in rodents have led to a model in which decreased bone quality in response to TZDs is due to a competition of lineage commitment between osteoblasts (OBs) and adipocytes (ADs) for a common precursor cell, resulting in decreased OB numbers. Beck *et al.* (2013) studied the effects of TZD exposure on OB-AD lineage determination from primary human BMSCs both *in vitro* and *in vivo* from nondiabetic subjects and patients with type 2 diabetics. Their experimental design included 2 phases. Phase 1 was an *in vitro* study of TZD effects on the differentiation of hBMSCs into OBs and ADs in nondiabetic subjects. Phase 2 was a randomized, placebo-controlled trial to determine the effects of 6-month pioglitazone treatment *in vivo* on hBMSC differentiation using AD/OB colony-forming unit assays in patients with type 2 diabetes. *In vitro*, TZDs

(pioglitazone and rosiglitazone) enhanced the adipogenesis of hBMSCs, whereas neither altered OB differentiation or function as measured by alkaline phosphatase activity, gene expression, and mineralization. The ability of TZDs to enhance adipogenesis occurred at a specific time/stage of the differentiation process, and pretreating with TZDs did not further enhance adipogenesis. *In vivo*, 6-month TZD treatment decreased OB precursors, increased AD precursors, and increased total colony number in patients with type 2 diabetes. Results showed that TZD exposure *in vitro* potently stimulates adipogenesis but does not directly alter OB differentiation/mineralization or lineage commitment from hBMSCs. However, TZD treatment in type 2 diabetic patients resulted in decreased osteoblastogenesis from hBMSCs compared with placebo, indicating an indirect negative effect on OBs and suggesting an alternative model by which TZDs might negatively regulate bone quality (114).

The effect of the critical periods of melatonin treatment required to induce human adipose tissue-derived MSCs into osteoblasts and which osteogenic genes are involved in the process was studied by Sethi *et al.* (2010). The study design consisted of adding melatonin for different times (2, 5, 10, 14 or 21 days) toward the end of a 21-day treatment containing osteogenic (OS+) medium or at the beginning of the 21-day treatment and then withdrawn. The results showed that a 21-day continuous melatonin treatment was required to induce both alkaline phosphatase (ALP) activity and calcium deposition and these effects were mediated through MT_2Rs. Functional analysis revealed that peak ALP levels induced by melatonin were accompanied by attenuation of melatonin-mediated inhibition of forskolin-induced cAMP accumulation. Immunoprecipitation and western blot analyses, respectively, showed that MT_2R/β-arrestin scaffolds complexed to Gi, MEK1/2, and ERK1/2 formed in the differentiated hAMSCs (i.e., when ALP levels were highest) where ERK1/2 resided primarily in the cytosol. It was hypothesized that these complexes form to modulate the subcellular localization of ERK1/2 to affect osteogenic gene expression. Chronic melatonin exposure induced the expression of osteogenic genes RUNX-2, osteocalcin, and BMP-2, through MT_2Rs. No melatonin-mediated changes in the mRNA expression of ALP, BMP-6 or the oxidative enzymes MtTFA, PGC 1alpha, Polγ, NRF-1, PDH, PDK and LDH occurred. The study showed that continuous 21-day melatonin exposure is required to induce osteoblast differentiation from hAMSCs through the formation of MT_2R/Gi/β-arrestin/MEK/ERK1/2 complexes to induce osteogenesis (115).

4.2.5. Immunomodulatory effects of MSCs in autoimmune diseases

Cell to cell contact in association with the paracrine secretion of a vast array of bioactive macromolecules by MSCs promotes immunomodulation. Though the mechanism by which these cells exert their immunomodulatory function is not completely known, the most approved theory suggests cell-to-cell contact and the release of soluble immunosuppressive factors. Excessive responses of various immune cells like macrophages, dendritic cells, NK cells, T and B cells were suppressed by MSCs by interacting with them in both *in vitro* and *in vivo* conditions (116). MSCs can also induce regulatory T cells (Tregs) and maintain the capability of Tregs to suppress self-reactive T-effector responses. Exogenous injection of MSCs can catalyze the proliferation of antigen-specific Tregs (117), and also adipose-derived- MSCs generate Tregs *in vivo,* which suggests that they have important implications for their immunoregulatory potential and thus, an attractive candidate for cell-based therapy for rheumatoid arthritis (118). Very few studies have been published concerning the modulatory effects of MSCs on B lymphocytes in humans. Cell to cell contact between T cells and MSCs is crucial to inhibit the proliferation of B-cells and antibody secretion. When MSCs isolated from bone marrow were co-cultured with B cells purified from healthy PBMCs in the presence of various B cell stimuli, there was inhibition in B cell proliferation due to an arrested G0/G1 phase and restricted differentiation of B cells as evidenced by reduced levels of IgG, IgM and IgA production (119). Although the immunoregulatory effect of MSCs on Treg and regulatory B cells (Breg) cells have been rigorously studied, yet the mechanisms of Treg and Breg induction by MSCs are not well understood. Whereas some studies propose the role of other immune cells others suggest MSCs-released

cytokines to be driving the expansion of regulatory T and B cell populations, but most of them have come to a congruence on MSCs employing many pathways to generate Breg and Treg cells and the surrounding microenvironment of the MSCs determines operation of the favorable pathway (120). MSCs are also shown to inhibit Th1 and Th17 cell proliferation along with a reduced level of IL-17, IL-2, IL-6, and IFN- gamma and downregulate T cell activation markers like CD38 and HLA-DR (121, 122). Altogether, the inflammatory environment is altered to a level of tolerogenic by modulation of immune cells by MSCs. Wang *et al.* (2016)studied the effect of WJ-MSCs on the Th1, Th2, and Th17 cytokines production and observed the immunosuppressive functioby inhibiting the proliferative response of Th1 and Th17 and enhancing Th2 response along with increased Treg cells. IFN gamma primed WJ-MSCs resulted in a decreased level of IFN gamma and TNF alpha as compared to untreated WJ-MSCs, as well as inhibiting the Th17 along with increasing the Treg cell population. Altogether, the inflammatory environment is altered by MSCs to a level of immunological tolerance through modulating immune cells to attain regulatory phenotype.

Immunoregulatory capacity of MSCs is triggered by the inflammatory environment, which changes during tissue repair. Macrophages are essential in mediating the inflammatory response after injury and can adopt a range of functional phenotypes, exhibiting pro-inflammatory and anti-inflammatory activities. Saldana *et al.*(2019) studied the immunomodulatory functions of MSC primed with TNF alpha and IL-10 secreted from macrophages polarized toward a pro-inflammatory and an anti-inflammatory phenotype respectively and also studied the role of, prototypic pro-inflammatory and anti-inflammatory cytokines, respectively. Immunoregulatory functions of primed MSC were enhanced after encapsulation in hydrogels (123).

4.3. Homing Mechanism of MSCs

MSCs can home to the damaged tissue in response to the presence of some signaling molecules from the injured tissue and related receptors on the MSCs themselves. Allogeneic BM-MSCs were used for *in vivo* reduction of stenosis in a model of rat carotid arteriotomy. Allogeneic BM-MSCs homed in injured carotids after arteriotomy but not in contralateral uninjured carotids which limits stenosis in injured rat carotids and plays a local immunomodulatory action (124). Several cell trafficking-related molecules such as chemokines, adhesion molecules, and matrix metalloproteinases (MMPs) are involved in carrying out the mechanism of homing (125). SDF-1, C-X-C chemokine receptor type 4 (CXCR4), and hepatocyte growth factor (HGF)-MET proto-oncogene, receptor tyrosine kinase (c-MET) are important in hMSC recruitment at sites of tissue regeneration (126). The migration process is highly dependent on the chemokine receptor CXCR4 and its binding partner, the SDF-1 CXCL12. BM-MSCs express CXCR4, CCR1, CCR4, CCR7, CCR10, CCR9, CXCR5, and CXCR6, which all assist in the process of MSCs homing. Indeed, it has been shown that MSCs also express CXCR7, which similarly binds SDF-1 to facilitate homing to various tissues. CXCR4 overexpressing MSCs also show increased homing to the bone marrow in mice, as well as to damaged intestinal mucosa in a mouse model of colitis. CXCR7 overexpression has been demonstrated to promote MSC homing to injured lung tissue (127).

Homing involves adherence of MSCs to vascular endothelial cells and then carry trans endothelial migration in injured tissue (128). Chemokines and adhesion molecules in conjunction with certain MMPs such as MMP-2 and membrane type 1 MMP (MT1-MMP) play a vital role in the invasion of MSCs (129). Remarkably, tumor necrosis factor (TNF) and IL-1 may upregulate homing-related molecules (130). Remarkably, tumor necrosis factor (TNF) and IL-1 may upregulate homing-related molecules, proposing that different inflammation statuses might promote distinct MSC engraftment and therapeutic efficacies. Shahror *et al* (2019) examined whether MSCs overexpressing FGF21 exhibit enhanced homing efficacy in a mouse model of traumatic brain injury or not. Results showed that FGF21 overexpression and Molday IONEverGreen™ (MIEG) labeling of MSC

enhances their homing abilities and enables non-invasive real-time tracking of the transplanted cells (131).

During cell migration, the movement of the nucleus must be coordinated with the cytoskeletal dynamics that influence the efficiency of cell migration. Osteopontin (OPN) significantly promotes the migration of BM-MSCs. Liu *et al.* (2019) studied the mechanism that regulates nuclear mechanics of the cytoskeleton during OPN-promoted BM-MSC migration. Researchers found that the actin cytoskeleton influences nuclear mechanics in BMSCs. It was found that the disruption of actin organization by cytochalasin D resulted in a decrease in the nuclear-projected area and nuclear stiffness. Stabilizing the actin assembly with jasplakinolide resulted in an increase in the nuclear-projected area and nuclear stiffness. (Sad-1/UNC-84 1 is a component of the linker of nucleoskeleton and cytoskeleton complex involved in the connections between the nucleus and the cytoskeleton. SUN1 depletion by RNAi decreased the nuclear stiffness and OPN-promoted BM-MSC migration. Thus, the F-actin cytoskeleton plays an important role in determining the morphology and mechanical properties of the nucleus. They also suggested that the cytoskeletal-nuclear interconnectivity through SUN1 proteins plays an important role in OPN-promoted BM-MSC migration (132).

4.4. Regulation of apoptosis

The poor survival of transplanted cells is a limitation for clinical indications that depend on long-term engraftment. Under oxidative stress and inflammatory conditions, the functional activity of MSCs gets decreased leading to apoptosis. Hypoxic conditions enhance the proliferative capacity as well as limits spontaneous differentiation of MSCs. Cellular senescence gets delayed resulting in more frequent population doublings. Priming of MSCs triggers a short term memory *in vitro*, as a consequence, the requirement of *in vivo* activation is not necessary. Priming with oxytocin seems to increase the survival of MSCs in hypoxic conditions by activating AKT and ERK1/2 enzymes, while exposure to apoptotic cytokines stimulates them to secrete stanniocalcin-1, a peptide with anti-apoptotic effects (133). Hypoxic conditioning is a useful strategy for the priming of MSCs within 3D culture systems to improve the therapeutic efficacy of MSCs on transplantation (134). Increased HIF1alpha and manganese superoxide dismutase are expressed by adipose tissue-derived MSCs which were cultured in 3D spheroids and were also found to be resistant to oxidative stress-induced apoptosis (135). Cell survival is also enhanced in injured tissues *in vivo* by priming of MSCs with 17 beta-estradiol which protects MSCs from H_2O_2 exposure *in vitro* (136). Preconditioning of MSCs with IFN-γ (137) and SERPINB9 (138) provides MSCs resistance to NK-cell mediated lysis. Anti-apoptotic factor secretion can be increased significantly by coating the plates with laminin or hyaluronan and on the establishment of cultures on 3D collagen (139). Hemeoxygenase 1, the cytoprotective enzyme helps in cell survival which is induced when MSCs engulf foreign mitochondria leading to mitochondrial biogenesis. The release of mitochondria from dying cells serves as an important environmental cue that controls the cytoprotective function of MSC (140).

4.5. MSCs derived soluble factors based therapeutics

The secretion of various cytokines, chemokines and growth factors by MSCs helps them in the establishment of network with their microenvironment which is an important property being utilized for MSC transplantation. Local cellular dynamics is profoundly affected by active biological factors (141). These released factors may also prevent adjacent cells from undergoing apoptosis and stimulate their proliferation, thereby promoting the regeneration of injured tissue (142). Interaction of MSCs with its microenvironment, comprised of inflammatory cytokines and other local stimuli like ligands of toll-like receptors, and hypoxia play role in tissue regeneration at the site of injury (143, 144). MSC secretome comprising of interleukin-6 (IL-6), IL-8, monocyte chemoattractant protein-1, and transforming growth factor-b (TGF-b)), extracellular matrix remodelers (i.e., TIMP metallopeptidase inhibitor 2 (TIMP-2), fibronectin, periostin, collagen, decorin, and metalloproteinase inhibitors), and growth factors and their regulators (i.e., vascular endothelial growth factor (VEGF), granulocyte-

macrophage colony-stimulating factor, bone morphogenetic protein 2, basic fibroblast growth factor, and insulin-like growth factor binding protein 3 (IGFBP3), IGFBP4, IGFBP7 are involved in regulation of immune system signaling) (141). Bone marrow mononuclear cells produce VEGF, IL-1 beta, PDGF, and IGF-1 cytokines which provide therapeutic benefits post-infarction by restoring contractility of the myocardium, inhibiting cardiomyocyte apoptosis and inducing angiogenesis, thus improving the overall functionality of the infarcted heart (145). The combined use of folic acid and MSC derived soluble factors for the treatment of wound healing showed promising therapeutic effects. The strongest benefits were seen in treatment using folic acid and MDFs together (146). Planella *et al.* (2019), compared the effect of extracellular vesicles (EVs) and soluble protein-enriched fractions (PF) isolated from MSC-conditioned medium (CM) to that of whole MSCs concerning their capacity to modulate B cell activation and their proliferation and B reg induction. Co-culture with MSC and MSC-PF induces naïve phenotype but the shift towards $CD24^{hi}CD38^{hi}$ population was induced by only MSC and not MSC-PF, but both of them fostered IL-10 production by B cells. MSC-EVs failed to promote naïve B cells induced $CD24^{hi}CD38^{hi}$B cells and IL-10 producing Breg phenotypes. The immunomodulatory effect of MSCs is independent of secreted EVs whereas, it is partially mediated by soluble factors (147).

5. CONCLUSIONS

The promise of cures for human ailments by stem cells have been much flaunted but many obstacles must still be overcome. The NIH clinical database (https://clinicaltrials.gov/) suggests that the 340 of 1,390 (24.46%) completed clinical trials using HSCs and 32 of 289 (11%) using MSCs have been terminated due to various reasons. The discrepancy between the consistently encouraging stem cells efficiency in *in vitro* outcomes and failures to demonstrate efficacy in human phase III clinical trials may be due to the use of non-clinical experimental animal models and the procedures followed during the trials. More robust cell research is required as the experiments done with animal models yield different results when replicated in humans during the clinical trial. Additionally, the standardization of *in vitro* culture protocols with stringent criteria for testing of functional parameters is necessary as well.

Based on thousands of clinical trials, the safety of stem cell therapy appears clear; less certain is the efficacy of such cell therapy owing to the fact that the proportion of positive results of the preclinical studies translating into clinical outputs is not large. There is still much to learn and optimize with regards to the *in vivo* interactions of stem cells in human pathological states.

While there is still a broad scope for evolvement in this field, the continued improvement in the clinical treatment modality, decreasing complications, emergence of the newer technologies and development in the field is remarkable, indicating a tremendous potential for stem cell therapy in the future.

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Abbreviations: AA, aplastic anemia; AD, adipocytes; ADA, adenosinedeaminase deficiency; ALD, adrenoleukodystrophy ; ALP, alkaline phosphatase, ARSA, arylsulfatase A; beta-TM, beta- Thalassemia major BMSCs, bone marrow stem cells; B reg, regulatory B cell, cDNA, complementary DNA; CGD, chronic granulomatous disease; CLP, common lymphoid progenitor; CM, conditioned media; CML, chronic myeloid leukemia*;* CMP, common myeloid progenitor ; CXCR, C-X-C chemokine receptor; DCM, dilated cardiomyopathy; EV, extracellular vesicles; G-CSF, granulocyte-colony stimulating factor; GMP, granulocyte/macrophage progenitors ; GVHD, graft-versus-host disease ; HGF, hepatocyte growth factor; HIV, *human immunodeficiency virus;* HSCs, hematopoietic stem cells; IGFBP,insulin-like growth factor binding protein; IL, interleukin; IST, Immunosuppressive therapy; LTR, long terminal repeat ; LSD, lysosomal Storage Diseases; LV, lentiviral vectors; MDR1,multidrug resistance 1 gene ; MEP, megakaryocyte/erythrocyte progenitors; MLD, metachromatic leukodystrophy; MMPs, matrix metalloproteinases; MPP, multi-Potent Progenitor; MSCs, mesenchymal stem cells; NIDCM, non-ischemic dilated cardiomyopathy; NIH, *National Institutes of Health* ; NK, natural killer; NOD, non-obese diabetes; OB, osteoblasts; OPN, osteopontin; PF, protein-enriched fractions; POSEIDON, The PercutaneOus StEm Cell Injection Delivery effects On Neomyogenesis; SCD, sickle cell disease; SCI, spinal cord injury; SCID, severe combined immunodeficiency; SDF-1, stromal derive factor 1, SIN, self inactivating; TIMP-2,TIMP metallopeptidase inhibitor 2;TNF, tumor necrosis factor; Treg, regulatory T cell; TZD, thiazolidinedione; VEGF, vascular endothelial

growth factor; WAS , Wiskott Aldrich syndrome;X-SCID- X linked SCID.

Key Words: Hematopoietic stem Cells, Mesenchymal Stem Cells, Clinical Trials, Transplantation, Therapy, Review

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