Stem Cell Rev and Rep (2017) 13:725–740
DOI 10.1007/s12015-017-9760-2

Gene Delivery Approaches for Mesenchymal Stem Cell Therapy: Strategies to Increase Efficiency and Specificity

Gopi Suresh Oggu[1] · Shyama Sasikumar[1] · Nirosha Reddy[2] · Kranthi Kiran Reddy Ella[2] · Ch. Mohan Rao[1] · Kiran Kumar Bokara[1]

Published online: 16 August 2017

Abstract A significant number of clinical trials have been undertaken to explore the use of mesenchymal stem cells (MSCs) for the treatment of several diseases such as Crohn's disease, diabetes, bone defects, myocardial infarction, stroke etc., Due to their efficiency in homing to the tissue injury sites, their differentiation potential, the capability to secrete a large amount of trophic factors and their immunomodulatory effects, MSCs are becoming increasingly popular and expected to be one of the promising therapeutic approaches. However, challenges associated with the isolation of pure MSC populations, their culture and expansion, specific phenotypic characterization, multi-potential differentiation and challenges of efficient transplantation limit their usage. The current strategies of cell-based therapies emphasize introducing beneficial genes, which will improve the therapeutic ability of MSCs and have better homing efficiency. The continuous improvement in gene transfer technologies has broad implications in stem cell biology. Although viral vectors are efficient vehicles for gene delivery, construction of viral vectors with desired genes, their safety and immunogenicity limit their use in clinical applications. We review current gene delivery approaches, including viral and plasmid vectors, for transfecting MSC with beneficial genes. The review also discusses the use of a few emerging technologies that could be used to improve the transfer/induction of desirable genes for cell therapy.

✉ Kiran Kumar Bokara
bokarakiran@ccmb.res.in; bokarakiran@gmail.com

[1] CSIR-Center for Cellular and Molecular Biology, Medical Biotechnology Complex, Uppal Road, Hyderabad, Telangana 500007, India

[2] MNR Dental College and Hospital, Fasalwadi, Sangareddy, Telangana 500076, India

Keywords Mesenchymal stem cells · Viral vectors · Gene delivery · Stem cell applications · CRISPR/Cas · Optogenetics · Pharmacogenomics

Background

Stem cells are primordial, unspecialized and undifferentiated cells possessing the property of self-renewal through continuous cell division and differentiation into various other types of cells [1]. Stem cells are classified as embryonic and adult stem cells based on their origin. Embryonic stem cells (ESCs) are totipotent and are derived from the inner cell mass of the blastocyst [2]. Adult stem cells (ASCs) with multipotency can be obtained from most of the adult tissues including bone marrow, adipose, cord blood, peripheral blood, amniotic fluid, dental tissues etc., [3, 4]. ESCs possess the ability to give rise to several cell types and can be used in clinical applications. However, the use of ESCs is beset with several ethical issues and the risk of adverse reactions such as tumor formation or immune rejection [5]. Patient-derived ASCs are harvested and are used within the same patient, thus eliminating the ethical concerns and the risk of immune rejection. The limited differentiation ability, however, restricts the universal use of ASCs in the patient [6].

The method of "Induced Pluripotent Stem Cells (iPSCs)" provides a means to achieve better control and refined differentiation of cells of non-stem cell origin. In this process, specific transcription factors, such as *Oct4, Sox2, Klf4*, and *c-Myc* genes are introduced into the cells to induce pluripotency [7]. Although iPSC approach has great potential, its clinical use has been rather limited, primarily because of the requirement of a large number of starting cells and the risk of teratomas [8].

Cell therapies utilizing mesenchymal stem cells (MSCs) are currently being explored in a large number of clinical trials. MSCs exhibit the property of plasticity and fibroblast-like morphology and are identified by their plastic adherence, exhibiting certain cell surface antigens such as $CD105^+$, $CD73^+$, $CD90^+$, $CD34^-$, $CD45^-$, HLA. MSCs possess the ability to differentiate into multiple lineages [9] such as chondrocytes, osteoblasts, adipocytes, tenocytes and skeletal myocytes [10–12]. In specific differentiation media, they have the exquisite capability to differentiate into cells of endodermal and ectodermal origin, such as hepatocytes [13, 14],cardio myocytes [15, 16] and neurons [17, 18].

Clinical Advantages of MSCs in Regenerative Medicine

As stated above, despite its potential, stem cell therapy is limited due to the substantial risks of malignant transformation of transplanted cells [19, 20]. In a case study, a boy suffering from a rare genetic disease known as Ataxia Telangiectasia received human neural stem cells. After undergoing neural stem cell therapy, the boy developed abnormal growths in his spine and brain [21]. In patients with hematologic cancers, allogeneic bone marrow transplantation of stem cells have some side effects including myeloid, lymphoid leukemias, lymphomas, and multiple myeloma [22–25]. There are several instances of development of bronchiolitis obliterans and other non-infectious pulmonary complications upon stem cell therapy [26]. It was found that the risk of developing malignant disease was less when MSCs were used in regenerative therapies [27]. Thus, MSCs have emerged as a new therapeutic strategy as cellular vehicles for the targeted delivery and local production of biologic agents [28]. MSCs have the unique property of tropism, where they can migrate to inflammatory sites and tumor microenvironments. They exert immunosuppressive effects upon cell-to-cell contact through regulatory T cells (Tregs), as shown with in-vitro and in-vivo models [29–33], by secreting soluble factors such as β-growth factor [34], hepatocyte growth factor [35], nitric oxide (NO) [36], indoleamine 2,3-dioxygenase (IDO) [37], inter cellular adhesion molecule 1 (ICAM1), chemokine (C-X-C motif) ligand 10 (CXCL10), chemokine (C-C motif) ligand 8 (CCL8) [38–40].

These unique properties, in addition to better efficiencies, make them an attractive material for cell therapy to target various disease models [41–44]. For example, there have been some positive findings regarding the transplantation of MSCs to the infarcted heart to promote repair and regeneration of the damaged cardiac tissue [45]. Systemic administration of MSCs to patients suffering from osteogenesisimperfecta showed significant improvement in the mechanical strength of the bone (bone calcium and collagen) and improved locomotory functions [46]. Another study suggested that transplanting MSCs at the site of injury in stroke patients resulted in significant increase in neurogenesis, angiogenesis and synaptogenesis, thereby normalizing microenvironmental proliferation and replacing damaged brain cells, apart from reducing inflammation and scar thickness [47]. These studies, described above, attribute the beneficial outcome with the use of MSCs to paracrine and trophic factors secreted by the transplanted MSCs.

Despite the beneficial effects of transplanted MSCs observed in some clinical applications, limited tendencies to engraft and differentiate [48] as well as gradual deterioration of functional stem cells with age [49] are some factors that limit their usage in therapies. The mode of action of donor cell populations in the recipient still needs investigations.

New studies to investigate the role of different growth factors in enhancing the therapeutic efficiency of MSCs are emerging. studies have shown that MSCs pre-conditioned with certain growth factors showed improvement in in-vivo cell functions, there by improving therapeutic efficiency, albeit with some limitations [50].

MSCs have been shown to be beneficial for treating ischemic strokes. Experimental evidence suggest that priming the MSCs with the serum of ischemic stroke rats had resulted in a better yield of stem cells that had increased proliferative capacity and neuro-restorative capacity compared with MSCs cultured in fetal bovine serum [49, 51]. It has been demonstrated that treatment of MSCs with trophic factors increased cell viability and proliferation without changing the morphology and expression of surface markers. The beneficial outcome of the pre-treated cells has been attributed to increase in production of BDNF (brain-derived neurotrophic factor), VEGF (vascular endothelial growth factor) and HGF (hepatocyte growth factor) [52].

In another study, preconditioning of cells with hypoxic conditions before transplantation increased the resistance to ischemia and stimulated the production of angiogenic factors, thus improving the ability of cells to survive in damaged tissues. The viability, proliferation, migratory properties, and therapeutic potential of these cells were reported to be increased by adding basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), tumor necrosis factor-alpha (TNFα), insulin-growth factor-1 (IGF-1) and bone morphogenetic protein-2 (BMP-2) to the culture medium [53].

Human derived adipose stem cells (hASCs) cultured in low concentrations of bFGF and EGF showed a significant increase in proliferation [54]. These hASCs exhibited typical spindle-shaped cell morphology and enhanced differentiation into the neural lineage with impaired mesodermal differentiation. These findings suggest that even low concentrations of EGF and bFGF may limit the differentiation ability of stem cells during stem cell expansion in-vitro [54].

Modifying MSCs with beneficial genes which can resist the uncongenial conditions such as hypoxia, ischemia,

oxidative stress and acute or chronic inflammations [38] that are associated with the disease conditions is important to improve their efficacy. However, in-vitro studies have shown that the MSCs are capable of surviving for only a limited period of time in the absence of oxygen [55]. Their survival capacity in the in-vivo environment is an important factor that determines the therapeutic effect exerted [56, 57]. Several clinical studies are being conducted using MSCs, which are in different phases of clinical trials as shown in Table 1.

Main Text

Physical and Chemical Methods of Gene Delivery in MSCs

Different methods such as non-viral (physical and chemical) and viral vector-based methods are used to introduce beneficial genes.

Several physical methods (non-viral) such as electroporation [62], nucleofection [63], sonotransfection [64] and nanoparticles [65] are used for delivery of beneficial genes into MSCs. In-vitro studies suggested that electroporation-mediated transfer of SOX-5, SOX-6 and SOX-9 [66] and BMP-2 [67] genes enhanced the migration and differentiation potential of MSCs into chondrogenic and osteogenic lineages respectively. Another study showed that the homing efficiency of MSCs in glioma patients was improved by delivering C-X-C gene for chemokine receptor type 4 (CXCR4) using electroporation [68, 69]. Transfection of recombinant human bone morphogenetic protein (rhBMP-6) into adipose derived stem cells using nucleofection method (electroporation-based transfer of DNA or RNA into cells) resulted in efficient bone formation and spinal fusion in a lumbar paravertebral muscle in immune deficient mice [70].

Another physical method, sonofection, exploits ultrasonic waves to create cavitation for delivering DNA into the cells [71]. Transfection of plasmid DNA containing the growth/differentiation factor 11 (Gdf11)/Bmp11 in-vivo into dental pulp stem cells using sonofection significantly induced the expression of dentin sialoprotein (Dsp) and restored the amputated dental pulp in canine teeth [72]. Delivery of small interfering RNA (siRNA) into MSCs using sonofection significantly knocked down phosphatase and tensin homolog on chromosome 10 (PTEN), suggesting that this technique can be used for induced overexpression or knockdown of genes [64].

Nanoparticles such as magnetic nano-beads [73, 74], silica [75], carbon nanotubes [76], gold nanoparticles [77] and quantum dots [78] have been in use for gene delivery into MSCs. The gene for glial cell line-derived neurotrophic factor (GDNF) was successfully delivered through magnetized synthetic hydroxyapatite and natural bone mineral nanoparticles by magnetic field [79]. In another study, plasmid containing BMP-2 gene was effectively transferred into MSCs using mesoporous silica [80]. Carbon nanotube-based composites have been shown to favor the attachment and osteogenic differentiation of MSCs and facilitate bone repair in rats [81–83]. It has been reported that carbon nanotube scaffolds enhance the neural differentiation of hMSCs, improve neural cell branching and the synaptic activity of cells in a monolayer culture as well as in tissue explants [84, 85]. In one case study, functionalized gold nanoparticles with polyethyleneimine (PEI) were successfully used to

Table 1 Showing the use of MSCs to treat different diseases and the stage of the clinical trials (Source: ClinicalTrials.gov)

Condition	Intervention	Phase	Status	Reference
Ovarian cancer	MSC INFβ	Phase 1	In-progress, Sep 2016	
CMV infection	MSC	Phase 2	In-progress, Jan 2015	[58, 59]
Graft-versus-host-disease	MSC (hPPL)	Phase 1 Phase 2	Completed, Nov 2014	
Bone atrophy	BCP with autologous mesenchymal stem cells (MSC)	Phase 1	In-progress, May 2016	
Chronic myocardial ischemia	Autologous mesenchymal stem cells	Phase 1	In-progress, July 2016	
Renal transplant rejection	Kidney transplantation with MSCs infusion		Completed, Feb 2011	[60]
Erectile dysfunction Type 1 diabetes mellitus Type 2 diabetes mellitus	Biological: HUC-MSCs Biological: injectable collagen scaffold + HUC-MSCs	Phase 1	In-progress, April 2016	
Skin burn degree second	Allogeneic (MSC's) Application to the Burn Wounds	Phase 1	In-progress, Aug 2016	
Prostate cancer	Allogeneic human mesenchymal stem cells	Phase 1	In-progress, April 2016	[61]
Focal segmental glomerulosclerosis	Intravenous injection	Phase 1	In-progress, Nov 2015	
Brain injury	Procedure: stereotactic hematoma evacuation Biological: MSCs transplantation Biological: injectable collagen scaffold with MSCs transplantation	Phase 1	In-progress, April 2016	
Nerve and spinal cord injuries	Umbilical Cord mesenchymal stem cells	Phase 3	In-progress, June 2015	

transfect hMSCs with the CCAAT/enhancer binding protein (C/EBP) beta gene, fused to enhanced green fluorescence protein (EGFP), to induce adipogenic differentiation [86].

In addition to the physical methods of gene delivery, several chemical (non-viral) methods of transfection mediated by calcium phosphate [87], cationic lipids [88], cationic polymers [89], cationic peptides [90] and cationic polysaccharides [91] have been used for gene delivery.

Calcium phosphate-mediated delivery involves the co-precipitation of positively charged Ca^{2+} ions and negatively charged DNA [92]. TGF-β1 was successfully expressed in MSCs by encapsulating DNA into calcium phosphate, and induced chondrogenesis [87].

Cationic lipid-based transfection or lipofection utilizes nano-sized lipoplex particles formed by the spontaneous assembly of DNA and cationic liposomes [93–96]. Lipofectamine 2000, a commercially available lipoplex complex, was used to introduce forkhead box A2 (Foxa2) gene into MSCs to enhance damaged liver tissue regeneration [88]. Cationic polymers such as polyethylenimine (PEI) and polyamidoamine dendrimers (PAMAM) enable the release of DNA into the cytoplasm and nucleus [97]. PEI-based transfection was used for introducing Bcl-2 gene in MSCs [98]. Likewise, cationic-based peptides (arginine-rich peptides) and polysaccharides (dextran) have also been used for DNA delivery into the cells [99, 100].

A major advantage of these non-viral mediated (physical and chemical methods) gene delivery techniques is the ease with which they can be carried out. However, both physical and chemical methods are in limited use because of their low efficiency [101], unsuitability for transfection of large population of cells [102] and their possibility of disrupting cellular and nuclear membranes [103]. The use of chemical agents could lead to toxicity at higher concentrations [104] and adverse reactions with negatively charged molecules both in-vitro and in-vivo [105]. Further, safety concerns have been raised because of their undegradable nature of certain polymers [106].

Genetic Modification of MSCs

Because of the limitations and disadvantages of non-viral methods of gene delivery mentioned above, several studies have used viral vectors to improve delivery of genes. The use of viral vectors for gene transfer exploits the natural ability of the viruses to infect the cells including MSCs. Transgenes may be incorporated either in addition to the genome or by replacing one or more genes. Genetic modification of MSCs with different beneficial genes could improve survival, repair and mediate recovery in the in-vivo environment, which may enhance their therapeutic potential during transplantation. The viruses that are currently being used as vectors are lentiviruses, retroviruses, adeno-associated viruses, adenoviruses and baculoviruses [107].

Retroviruses

Retroviruses are double-stranded RNA viruses having reverse transcriptase and a lipid envelope with receptor-binding proteins. After binding to the receptor, the external layer of viral envelope integrates with the cellular membrane, internalizing the virus and releasing the contents into the cytoplasm. The viral RNA reverse transcribed into DNA using reverse transcriptase, integrates into the host genome [108].

Retroviral vectors can only infect proliferating cells with very high efficiency. Post-mitotic cells such as myocytes or neurons are not susceptible to retroviral infection, thus limiting the spectrum of cells that can be targeted. Integration of the viral genome into the host enables long-term expression of the transgenes.

In clinical trials on X-linked severe combined immunodeficiency (X-SCID), $CD34^+$ HSCs transduced with MLV retroviral vectors encoding the ADA gene were used. In the initial trial, patients transplanted with the HSCs exhibited sustained engraftment and their differentiation into different lineages, enhanced lymphocyte count, reduced toxic metabolites and increased immune function [8]. In an another trial, 9 out of the 10 patients involved in the trial, showed increased T cell counts and functions; 8 of them nolonger required enzyme replacement therapy and 5 showed an antigen-specific immune response to vaccines [109].

However, silencing of viral transgenes due to methylation of the viral promoter during cell differentiation is one of the limiting factors [110]. Another important feature that must be considered while using retroviral vectors is the safety factor [111]. Due to random integration of the viral genome into the host genome, there is a risk of disruption of proto-oncogenes and tumorigenesis. An example of such a scenario is the clinical trial conducted for X-SCID [112] in which $CD34^+$ bone marrow cells were isolated and transduced in-vitro with Moloney murine leukemia virus carrying a common γ chain cytokine receptor [113] and the altered cells were transplanted back into the patients. Though the immunodeficiency was corrected in eight of the nine patients and the study was considered successful, four of the patients developed leukemia over the next nine years due to insertional oncogenesis [112].

Lentiviruses

Lentiviruses have double-stranded RNA as their genetic material and can transduce both quiescent and dividing cells. They integrate their vector genome into the host genome, ensuring long-term expression of the transgenes

[114]. Lentiviral vectors are capable of transducing dividing, non-dividing or slow-dividing cells,without affecting their viability and differentiation potential.

Various strategies have been explored to improve the viability and differentiation potential of MSCs. Transduction of MSCs with lentiviral vectors overexpressing HSP70 increased survival and resistance to apoptosis under conditions of hypoxia and ischemia [56]. Transplantation of MSCs overexpressing PGC-1α using lentiviral vectors attenuated neuronal apoptosis and enhanced the potential of axonal regeneration in a rat model of traumatic spinal cord injury (SCI) [115]. Another study showed that transplantation of MSCs transduced with GREM1 gene reduced apoptosis by oxidative injury, increased the angiogenic properties and enabled restoration of blood flow in a hind limb ischemic model [116]. Transplantation of adipose tissue-derived MSCs expressing HSV thymidine kinase gene using a lentiviral vector, along with ganciclovir treatment, has been shown to decrease the tumor size in mice pre-inoculated with U87 glioblastoma cells [117].

In a clinical trial for X-linked adrenoleukodystrophy (ALD), administering hematopoietic stem cells (HSCs) over-expressing *ABCD1* gene pseudo-typed with a self-inactivating-G glycoproteinto patients showed progressive myelination at 14, 20 and 36 months post-therapy [118]. In another trial, patients with βE/β0-thalassemia were administered with HSCs transduced with self-inactivated lentiviral vector expressing the β-globin gene. Results suggest that the hemoglobin levels of the patients were maintained in the range of 9–10 g/dl for almost 3 years, without any transfusions [119]. In another study transplantation of HSCs transduced with lentivirus encoding WAS gene in patients with Wiskott–Aldrich syndrome resulted in stable and long-term engraftment with enhanced T cell function and protection against severe infections [120]. A phase I/II clinical trial used HSCs transduced with lentiviral vector encoding arylsulfatase-A gene for the treatment of metachromatic leukodystrophy (MLD). Upon transplantation into patients, the HSCs showed a high level of engraftment in bone marrow and peripheral blood and the patients maintained normal and cognitive development for 2 years after therapy [121]. However, the major concern with the use of lentiviral vectors is that, they lack specificity, thus it can lead to the infection of cells that do not need to be transduced. In addition, the majority of developed lentiviruses are HIV-derived, raising safety concerns for in-vivo gene therapy applications [122].

Adeno-Associated Virus (AAV)

Adeno-associated viruses are small non-pathogenic, single-stranded DNA viruses that are dependent on the adenovirus for replication [123]. AAVs enter the cells by endocytosis upon binding to the integrin αVβ5 and FGF4 receptor and integrate at a specific site in the host genome on chromosome 19. The site of integration is called AAVS1. This site specific integration avoids the risk of unpredictable insertional oncogenesis and other deleterious consequences. Another valuable feature of AAV is their low immunogenicity [124].

Although AAVs can infect a broad spectrum of cells, they exhibit certain serotype specificity towards the cell type being used [125]. Over-expression of Insulin-like growth factor I and Transforming Growth Factor β (TGF-β) with AAV vectors in human bone marrow-derived MSCs have been shown to induce differentiation towards chondrogenic and osteogenic lineages, suggesting an effective treatment strategy to treat articular cartilage defects [126, 127]. Human bone marrow-derived MSCs transduced with AAV either expressing SOX9 alone or SOX9 along with TGF-β showed proliferative, and chondrogenic activities [128, 129]. Over-expression of TIMP-1 (which has anti-angiogenic properties) in MSCs enhanced the draining efficiency of lymph nodes in anti-TIMP IgG-1 immunized mice model [130]. In another study, it was shown that bone marrow-derived MSCs over-expressing antisense miRNA-937 significantly reduced the deposition of Aβ, enhanced the levels of BDNF and improved the performance in a social recognition test and plus-maze discrimination avoidance test in an Alzheimer disease model of APP/PS1 transgenic mice [131].

Despite the major advantages such as site-specific integration, low immunogenicity the use of AAVs is limited due to several reasons. Although AAVs can infect a broad spectrum of cells, they exhibit certain serotype specificity towards the cell type being used [125]. One of the major challenges in the clinical use of AAV is that a majority of the human population has antibodies to AAV which reduces the efficiency of the vector [132]. Such immune reactions have been found to be particularly more common against AAV2. Another impediment is the need for conversion of single-stranded DNA into double-stranded DNA before integration into the genome, which is a rate-limiting step. Therefore, there is a need to extensively investigate the molecular details of the AAV and host interaction biology and develop strategies to overcome the limitations and make use of the advantages that AAVs offer.

Adenovirus (AV)

Adenoviruses are double-stranded DNA viruses that lack an envelope. Non-pathogenicity is a major advantage in their use as vectors for gene transfer. There is no risk of insertional mutagenesis and the payload capacity of these vectors is high (~36Kb).

Several clinical studies have shown beneficial effects of MSCs modified with AVs. MSCs genetically engineered with bi-cistronic adenoviral vector expressing FGF2 and

PDGF-BB have shown to induce collateral vessel formation and angiogenesis in a hind limb ischemia model [133]. Human placenta-derived MSCs transduced with adenoviral vector encoding NK4, administered through tail vein, inhibited the development of lung metastases in C-26 lung metastasis model [134]. Human endometrial MSCs, engineered with adenoviral vector encoding soluble Flt-1, effectively regressed endometriotic lesions in NOD/SCID mice [135]. In order to develop a new treatment strategy for malignant brain tumors, umbilical cord-derived MSCs were transduced with a modified interleukin-12 (IL12M) encoding adenoviral vector. Intra-tumoral injection of the engineered MSC was shown to prolong the survival of glioma-bearing mouse. Further, when the tumor free mice were challenged again with tumor, they were found to be resistant to the ipsilateral and contralateral tumor, which is closely associated with tumor-specific longer T-cell immunity [136]. Another study has shown that intra-tumoral injection of human bone marrow-derived MSCs engineered with adenovirus encoding an immunotoxin, EphrinA1-PE38, inhibited tumor growth in malignant glioma tumor model [137].

Despite the well-documented usefulness, a major concern of using adenoviral vectors is their immunogenicity. Direct administration of Adenoviral vectors with a transgene into the host cells activates $CD4^+$, $CD8^+$ and antigen presenting cells. This activation of immune response caused by the expression of the transgene as well as the viral capsid proteins may sometimes lead to the elimination of the viral particle and silencing of the transgenes [138]. However, a few reports indicate that bone marrow MSCs transfected in-vitro and then transplanted into rat intravenously did not elicit any immune response [139]. Co-transfection with a second adenoviral vector encoding hemoxygenase 1 (an enzyme that prevents development of acute inflammation) is another approach to reduce the immune response [140]. Yet another drawback in the usage of adenoviral vectors is the transient expression of the transgene [141, 142]. Further, while some studies have reported that adenoviral vectors do not influence differentiation [143], some other studies have reported detrimental effects [144].

Baculovirus

Baculoviruses are a group of insect viruses among which *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) with a circular double-stranded DNA genome is the most extensively used. These viruses can infect a wide spectrum of mammalian cells, and express the transgenes provided and these are under the control of a promoter that is active in mammalian cells [145, 146]. Baculovirus are non-toxic to the mammalian cells and does not replicate inside the cells [146]. There is no evidence pointing to the integration of the viral genome into the host genome without selective pressure [147] and the viral DNA degrades over time [148]. An important advantage of these viruses is that they are non-pathogenic to humans. They are capable of transducing various stem cells including embryonic stem cells [149], induced pluripotent stem cells [150], neural stem cells [151], chondrocytes [152, 153] and bone marrow [154, 155] as well as adipose tissue-derived MSCs [156]. Bone marrow MSCs transduced with baculovirus encoding BMP-7 were shown to enhance postereo-lateral spinal fusion in rabbits [157]. Similarly, in another study, bone marrow MSCs transduced with baculovirus encoding BMP-7 showed a decrease in disk degeneration when transplanted in the rat tail [158]. Adipose-derived MSCs transduced with baculovirus expressing TGF-β3 effectively ameliorated chondrogenesis and formation of cartilage tissue. This approach provides a novel method for engineering cartilages [156]. In another study, rabbit MSCs engineered with baculovirus encoding BMP-2 and VEGF and seeded onto Tri-calcium phosphate (TCP) scaffolds showed enhanced postereo-lateral spine fusion in rabbit models [159]. MSCs transduced with baculovirus expressing herpes simplex virus thymidine kinase gene, when injected into mice pre-inoculated with human U87 glioma cells, showed inhibition of tumor growth and prolonged survival of the animals after ganciclovir injection [160].

Baculoviral vectors are being used for in-vivo applications as they do not replicate inside mammalian cells, but can efficiently deliver genes into many types of cells. However, there are disadvantages in using baculoviruses; One major disadvantage is the inactivation of the baculoviruses by the human complement system [146]. The other major disadvantage is with glycosylation; N-glycosylation pathway of the insects differs from the mammalian system such that the recombinant proteins will have a terminal paucimannose N-glycan instead of glycosylated and siaylated N-glycan. This is a major limitation as the N-glycans, especially sialic acid residues, are important for the function of the glycoprotein [161].

In addition to the number of vectors for transducing transgenes into MSCs described above, several other viral vectors are also being explored. For example, Herpex Simplex Virus & Vaccinia Virus (ds DNA viruses), Borna Disease Virus & Sendai viruses (negative strand RNA viruses). Some of the advantages of using these viral vectors include:

- Accommodating large fragments of foreign DNA [162]
- Attenuation of tumour toxicity through p53/p53-mediated activation of p21 [163] or vaccine immunogenicity [162]
- Selective delivery towards the tumor sites [164–167]
- Intra nuclear replication and transcription to express overlapping open reading frames [168–171]

- Long-term expression of transgenes in MSCs and iPSCs [172]

The above mentioned unique feature of these viruses could be exploited to improve the transfection efficacy. To date not many studies have been performed using these viral vectors. Considering the fact that half of the genes that are encoded for viral functions are non-essential [108], replacing them with genes of interest without losing the functionality of the vector could be a future strategy to use these vectors for clinical applications.

Currently, viral vector-based genetic modification of cells is being extensively exploited. However, several drawbacks have limited their use in clinical applications. One such factor is their low transduction efficiency. The low transduction efficiency of adenoviral and adeno-associated viral vectors can be enhanced by the following factors

- developing self-complementary AAV (sAAV) having an inverted repeat of the genome that can fold into dsDNA [173]
- altering the capsid fibers [174]
- brief exposure to UV light [175]
- creating mutations to surface receptors containing tyrosine residues [176]

Despite significant progress in the field, improving the transduction efficiency, controlling the immunomodulatory effects and preventing silencing of transgenes during differentiation need be be addressed for successful cell-based therapies.

Further, viral vector-based approaches to administer specific transcriptional factors to decide the fate of the cells into specific lineages have been seriously criticized for translational medical applications because of their inadequate validation [177, 178].

Factors to Improve Cell-Based Therapies

Cell-based therapies are likely to replace a large number of chemical-based therapies in course of time. However, many aspects need be addressed before cell-based therapies become routine clinical practices. The understanding of the molecular mechanisms of "tropism" is essential for successful cell-based therapies. Further studies on immune modulation, uncontrolled proliferation, intrinsic and extrinsic cues from the micro environment and post-transplantation teratoma formations are needed. MSCs are heterogeneous in nature and selection of pure MSC population is a major bottleneck for cell-based therapies. Further research should focus on the methods to isolate pure population of MSCs to transfect with desirable genes.

Suicidal genes have been incorporated to increase the purity and success of transplantation of MSCs and to control the long-term deleterious effects of donor cell population during the time of maturation, thus enabling an efficient control over the pre-differentiated pluripotent cells [179–181]. Adaptation of novel computational tools such as CellNet that calibrate and compare the primary cell lineages with the engineered cells by quantitating the gene expression profiles would be useful [182]. Despite the clinical improvement in infusion and transplantation of donor bone marrow-derived MSCs [183, 184], complete engraftment of the whole bone marrow is still doubtful. In a case study describing the graft versus host diseases (GVHD), transplantation of MSCs with hematopoietic stem cells reduced the toxicity and minimized the graft rejection [185]. However, the efficacy of engraftment was not validated [186, 187]. More detailed studies have to be carried out to increase the efficacy of MSCs by optimizing the prodrug dosages [188, 189] and anti angiogenic agents after transplantation [190]. Despite all the difficulties and impediments, there has been significant progress in the cell-based therapies using MSCs. The Fig. 1 shows the list and the number of cases in each disease that is attempted to treat using MSCs.

In addition, well-defined guidelines for general safety in terms of sterility of MSCs and monitoring of proliferative potency from donor cells, close monitoring of the genetic integrity of the donor cell population are required. There should be a mechanism to monitor the strict adherence to the prescribed guidelines.

Future Prospects

Over the decades, there has been significant progress in the translational efforts using stem cells for various pathological conditions. Most of these are now at different stages of pre-clinical and clinical studies. In the coming years, many of these studies can be expected to lead to better stem cell therapies.

The advent of several new approaches and technologies such as CRISPR/Cas, Optogenetics and Pharmacogenomics are likely to influence and catalyze the cell-based translational approaches (Fig. 2).

Genome editing has been facilitated by the development of programmable sequence-specific DNA nuclease technologies that allow targeted modification of endogenous sequences with high efficiency [191]. RNA-guided Cas9 nucleases from the CRISPR/Cas system can be employed to engineer targeted double-stranded breaks in eukaryotic cells. A 20 nucleotide guide sequence within single-guide RNA is used to direct the *Streptococcus pyrogens* Cas9 nuclease to the desired genomic target that is followed by a 5′NGG proto spacer adjacent motif via Watson–Crick base-pairing [192]. This enables the targeting of a desired genomic locus

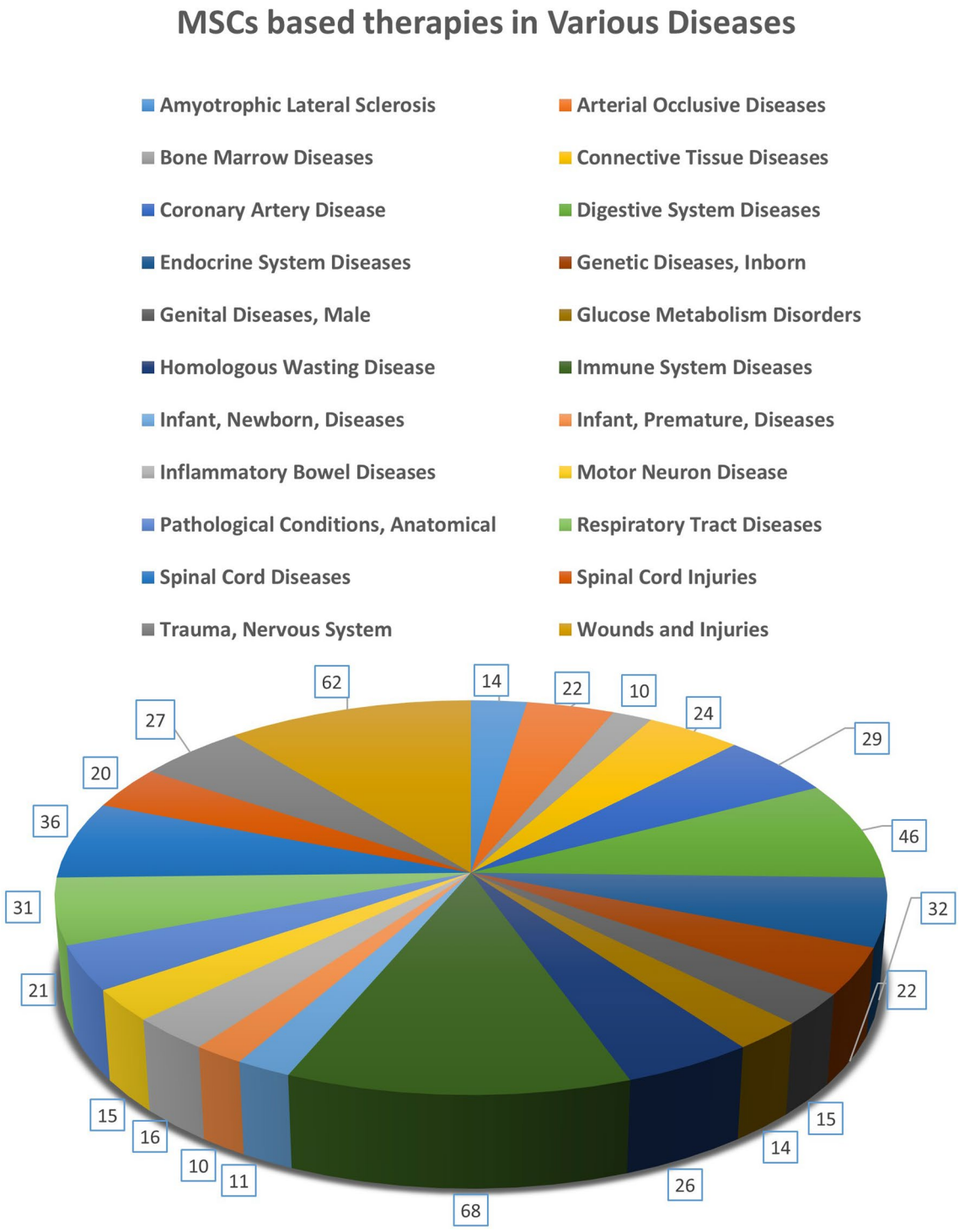

Fig. 1 The numbers in the pie diagram indicates the number of subjects recruited for the case study in MSC transplantation. (Data Source: ClinicalTrials.gov)

with defined alterations. The cellular non-homologous end joining or homology-directed repair pathways can then be used to induce defined alterations [193]. This strategy could be exploited to introduce the genes of interest in MSCs with high efficiency.

A recent study has shown that CRISPR/Cas9 system coupled with iPSC technology improved the therapeutic potential for correcting Recessive Dystrophic Epidermolysis Bullosa (RDEB) [194]. The ease and design of Cas9-based therapeutics could be exploited beyond the

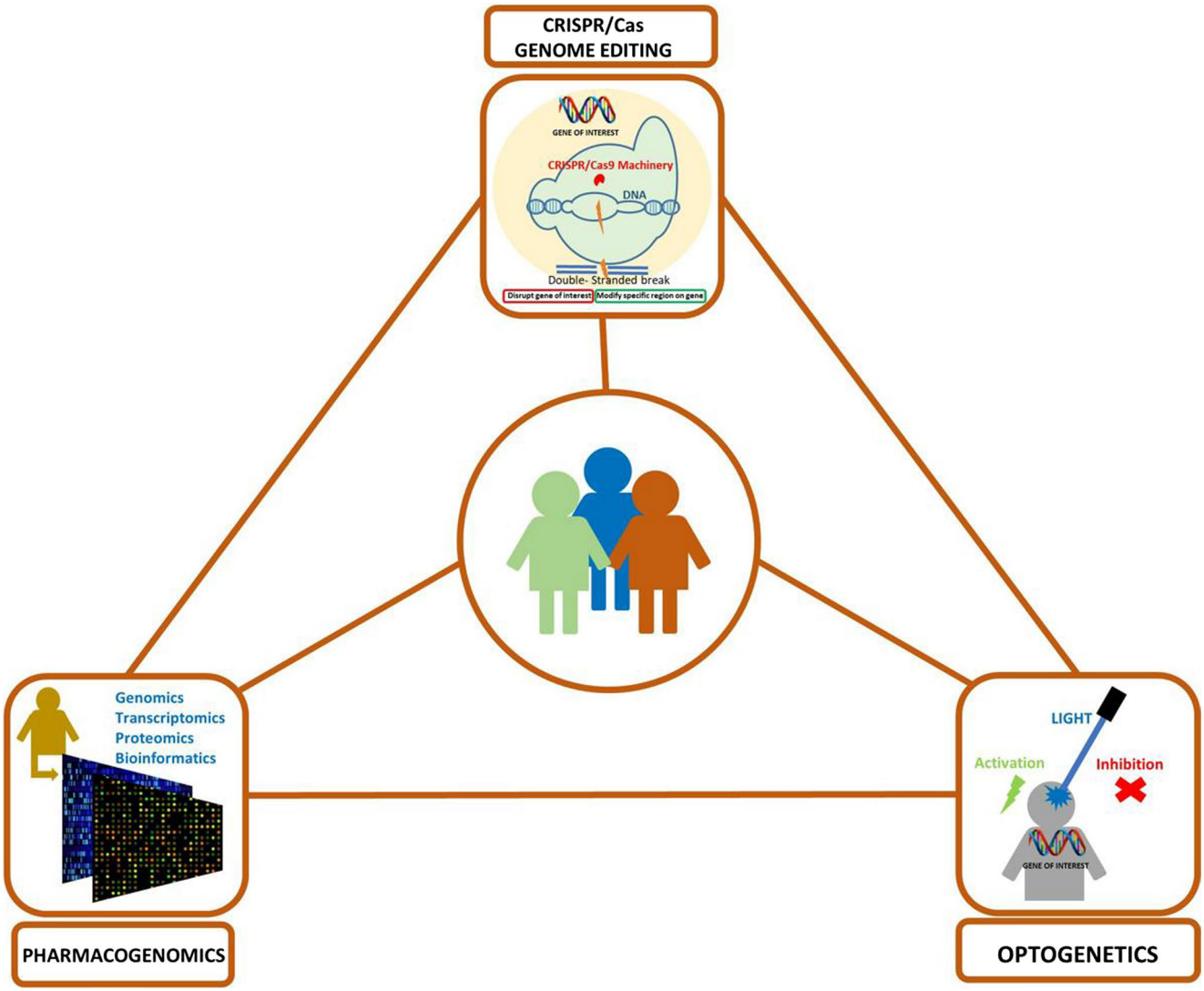

Fig. 2 The figure shows three emerging technologies that can revolutionize stem cell-based therapy (MSCs). CRISPR/Cas technology enables specific targeting of loci in genes of interest. Optogenetics provides handle to regulate the activation/inactivation of genes using light sensitive switches. Pharmacogenomics identifies the changes in gene/protein expression. The unification of CRISPR/Cas, Optogenetics and Pharmacogenomic tools for e targeting of cell types in humans. The figure shows the combined application of these technologies for identifying candidate genes/proteins and effective, specific gene delivery and regulated gene expression for personalized stem cell therapy

direct genome modification of somatic tissue. A recent report described the use of *S.pyrogenes* Cas9 (SpCas9) and RNase iii (SpRNase) along with attached nuclear localization signals (NLSs) to ensure efficient transformation of the desired expression in mammalian cells [195]. The efficient use of this precise toolbox has been carried out extensively in cancer research, in the case of sarcoma to target genes such as TET2, APC, KRAS, SMAD4, PTEN and EML4 [196]. The CRISPR/Cas9 method has been proved to be a better alternative than Cre-Lox, zincfingers (ZFN) and transcription activator-like effector nucleases (TALENs) in terms of target design simplicity, efficiency and introducing multiplexed mutations.

The major challenges in using CRISPR/Cas9 system include

- Off-target effects, which are the consequence of the non-specific activity of the Cas nuclease in the genome [197, 198]
- Engineering Cas enzymes with higher fidelity and specificity [199–201], and
- The necessity to develop complex algorithms to design gRNAs [201]

However, the use of recombinant Cas9 protein and specific design of gRNAs (Cas9 RNP) would probably be

preferential compared with traditional plasmid or mRNA delivery methods, as it has been shown to achieve lower rates of off-target effects [202–204].

Optogenetic approaches can be exploited to control the expression of gene of interest within the host by light sensitive pumps or channels such as Opsins. Exposure of these channels to light for milliseconds can activate or silence the gene expression in in-vivo as well as in-vitro models. Using optogenetics, it was possible to modify the mouse embryonic stem cells with light-sensitive switch (short and long pulses of light) to control the gene expression to determine the developmental cues for efficient differentiation and control over the stem cell development [205]. In another study optogenetic stimulation of rodent hippocampus region transplanted with human stem cell-derived neurons enabled neurons to integrate into existing brain circuits for efficient neural activity in an ischemic model, suggesting that shining light can integrate the neurons into existing brain circuits and fire patterns that are critical for neural network activity [206]. It has been suggested that turning on the light of different wavelengths could potentially allow stem cells to create complex three-dimensional tissues/organ which could be used for transplantation [205]. Developing strategies to exploit light-activated gene expression could be an interesting area for further research. Exploring opsin and non-opsin natural light-sensitive proteins would help to control the gene expression within the cells and also to understand different biological pathways [206].

This strategy could be adopted to manipulate stem cells to secrete specific proteins that drive a specific purpose in the target tissues that need to be treated. Optogenetics is thus a valuable tool that could help in identifying the lesser known mechanisms behind molecular pathways. There is an immediate need for further studies to demonstrate the potential of optogenetics in regenerative medicine.

However, there are a few limitations/drawbacks in using optogenetics to alter gene expression which are mentioned below

- Indiscriminately drives all cells within a genetically defined targeted population [207, 208]
- Expression of opsin proteins has the potential to alter the function of intrinsic cellular machinery, and insertion of large numbers of foreign ion channels or pumps in cell membrane could lead to physiological differences [209]
- Poor penetration capacity of visible light in the biological tissues [210]. This limitation could probably be overcome by replacing visible light with multichannel micro LEDs with different frequencies in the light spectrum that could enhance the light penetration inside the tissues and control the specific expression of opsin proteins.

Pharmacogenomics involves systemic identification of all human genes combined with changes in gene and protein expressions to enable personalized assessment of individuals towards health and diseases [211]. There is a growing concern regrading the substantial person-to-person variations in the outcome of stem cell therapy. Pharmacogenomics should help optimize patient-specific therapy (drug/cell based) with respect to the patient genotype by identifying specific genetic variations associated with the disease. It would facilitate tailored treatment with maximum efficacy and limited adverse effects. This approach requires genomic (high-density microarrays) [212], proteomic and bio-informatic tools to integrate with stem cell therapies. The validation of recipient and donor genotypes and selecting the appropriate cell types could increase the success of stem cell therapy.

Pharmacogenomics has the limitations as follows

- Pharmacogenomic analysis of individual genomic network (both intrinsic and extrinsic pathways) and identifying specific targets at the genome level [213]
- Predicting the impact of specific drug toxicity on miRNA and cell lineage mechanisms [214]
- Lack of large scale cell line libraries, and databases for the development of algorithms in relation to the computational quantitative systems for effective selection of drugs to specific cell types [215].

Pharmacogenomic tools like Single-cell RNA sequencing may be one such technology that could identify targeted cells through a changed mRNA sequence and relate that to potential transcriptional changes.

Conclusions

The therapeutic potential of MSCs with beneficial genes using viral and non-viral methods are being tested in several disease models, albeit with some limitations. However, unequivocal in-vivo evidence supporting the true differentiation and regenerative potential of MSCs still needs further investigations. CRISPR/Cas and optogenetics are emerging as future technologies due to the rapidity and specificity of gene delivery using these techniques. Another potential future avenue is to adopt next generation sequencing and genotypic techniques (pharmacogenomics) as a new paradigm to target the specific cell types for personalized medicine. Development of these techniques is likely to revolutionize stem cell-based therapy in general and MSC based therapy in particular.

Acknowledgements Authors acknowledge Dr. T, Ramakrishna for critical reading of the manuscript and suggestions. Kiran Kumar

acknowledge CSIR network project mIND (BSC-0115) for financial assistance. Ch. Mohan Rao acknowledges the Department of Science and Technology, Government of India for the Sir JC Bose National Fellowship.

Compliance with Ethical Standards

Conflict of Interest The authors confirm that this article content has no conflict of interest.

References

1. Avasthi, S., Srivastava, R., Singh, A., et al. (2008). Stem cell: past, present and future–a review article. *Internet Journal of Medical Update, 3*(1), 22–31.
2. Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science, 282*(5391), 1145–1147.
3. Nadig, R. R. (2009). Stem cell therapy-hype or hope? A review. *Journal of Conservative Dentistry, 12*(4), 131.
4. Ullah, I., Subbarao, R. B., & Rho, G. J. (2015). Human mesenchymal stem cells-current trends and future prospective. *Bioscience Reports, 35*(2), e00191.
5. Lo, B., & Parham, L. (2009). Ethical issues in stem cell research. *Endocrine Reviews, 30*(3), 204–213.
6. Correia, A. S., Anisimov, S. V., Li, J. Y., et al. (2005). Stem cell-based therapy for Parkinson's disease. *Annals of Medicine, 37*(7), 487–498.
7. Zhang, J., Huang, X., Wang, H., et al. (2015). The challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy. *Stem Cell Research & Therapy, 6*(1), 234.
8. Aiuti, A., Slavin, S., Aker, M., et al. (2002). Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science, 296*(5577), 2410–2413.
9. Sun, Q., Zhang, Z., & Sun, Z. (2014). The potential and challenges of using stem cells for cardiovascular repair and regeneration. *Genes & Diseases, 1*(1), 113–119.
10. Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., et al. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature, 418*(6893), 41–49.
11. Mackay, A., Beck, S., Jaiswal, R., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science, 284*(5411), 143–147.
12. Tremain, N., Korkko, J., Ibberson, D., et al. (2001). MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. *Stem Cells, 19*(5), 408–418.
13. Petersen, B., Bowen, W., Patrene, K., et al. (1999). Bone marrow as a potential source of hepatic oval cells. *Science, 284*(5417), 1168–1170.
14. Schwartz, R. E., Reyes, M., Koodie, L., et al. (2002). Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *The Journal of Clinical Investigation, 109*(10), 1291–1302.
15. Rose, R. A., Keating, A., & Backx, P. H. (2008). Do mesenchymal stromal cells transdifferentiate into functional cardiomyocytes? *Circulation Research, 103*(9), 120.
16. Pijnappels, D. A., Schalij, M. J., Ramkisoensing, A. A., et al. (2008). Forced alignment of mesenchymal stem cells undergoing cardiomyogenic differentiation affects functional integration with cardiomyocyte cultures. *Circulation Research, 103*(2), 167–176.
17. Tropel, P., Platet, N., Platel, J. C., et al. (2006). Functional neuronal differentiation of bone marrow-derived mesenchymal stem cells. *Stem Cells, 24*(12), 2868–2876.
18. Cogle, C. R., Yachnis, A. T., Laywell, E. D., et al. (2004). Bone marrow transdifferentiation in brain after transplantation: a retrospective study. *The Lancet, 363*(9419), 1432–1437.
19. Lindvall, O., Kokaia, Z., & Martinez-Serrano, A. (2004). Stem cell therapy for human neurodegenerative disorders–how to make it work. *Nature Medicine, 10*, 42–50.
20. Lindvall, O., & Kokaia, Z. (2006). Stem cells for the treatment of neurological disorders. *Nature, 441*(7097), 1094–1096.
21. Amariglio, N., Hirshberg, A., Scheithauer, B. W., et al. (2009). Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Medicine, 6*(2), e1000029.
22. Weiden, P. L., Flournoy, N., Thomas, E. D., et al. (1979). Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *New England Journal of Medicine, 300*(19), 1068–1073.
23. Slavin, S., Or, R., Naparstek, E., et al. (1988). Cellular-mediated immunotherapy of leukemia in conjunction with autologous and allogeneic bone marrow transplantation in experimental animals and man. *Blood, 72*(suppl 1), 407.
24. Kolb, H., Mittermuller, J., Clemm, C., et al. (1990). Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood, 76*(12), 2462–2465.
25. Van Besien, K., De Lima, M., Giralt, S., et al. (1997). Management of lymphoma recurrence after allogeneic transplantation: the relevance of graft-versus-lymphoma effect. *Bone Marrow Transplantation, 19*(10), 977–982.
26. Afessa, B., Litzow, M., & Tefferi, A. (2001). Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplantation, 28*(5), 425.
27. Qin, Y., Guan, J., & Zhang, C. (2014). Mesenchymal stem cells: mechanisms and role in bone regeneration. *Postgraduate Medical Journal, 90*(1069), 643–647.
28. Studeny, M., Marini, F. C., Dembinski, J. L., et al. (2004). Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *Journal of the National Cancer Institute, 96*(21), 1593–1603.
29. Stagg, J., Pommey, S., Eliopoulos, N., et al. (2006). Interferon-γ-stimulated marrow stromal cells: a new type of nonhematopoietic antigen-presenting cell. *Blood, 107*(6), 2570–2577.
30. Ye, Z., Wang, Y., Xie, H.-Y., et al. (2008). Immunosuppressive effects of rat mesenchymal stem cells: involvement of CD4+ CD25+ regulatory T cells. *Hepatobiliary & Pancreatic Diseases International, 7*(6), 608–614.
31. Di Ianni, M., Del Papa, B., De Ioanni, M., et al. (2008). Mesenchymal cells recruit and regulate T regulatory cells. *Experimental Hematology, 36*(3), 309–318.
32. Joo, S.-Y., Cho, K.-A., Jung, Y.-J., et al. (2010). Mesenchymal stromal cells inhibit graft-versus-host disease of mice in a dose-dependent manner. *Cytotherapy, 12*(3), 361–370.
33. Madec, A., Mallone, R., Afonso, G., et al. (2009). Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. *Diabetologia, 52*(7), 1391–1399.
34. Keating, A. (2008). How do mesenchymal stromal cells suppress T cells? *Cell Stem Cell, 2*(2), 106–108.
35. Di Nicola, M., Carlo-Stella, C., Magni, M., et al. (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood, 99*(10), 3838–3843.

36. Ren, G., Zhang, L., Zhao, X., et al. (2008). Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell, 2*(2), 141–150.
37. Meisel, R., Zibert, A., Laryea, M., et al. (2004). Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2, 3-dioxygenase–mediated tryptophan degradation. *Blood, 103*(12), 4619–4621.
38. Marigo, I., & Dazzi, F. (2011). The immunomodulatory properties of mesenchymal stem cells. In *Seminars in immunopathology*. Springer. 593.
39. Dazzi, F., & Marelli-Berg., F. M. (2008). Mesenchymal stem cells for graft-versus-host disease: close encounters with T cells. *European Journal of Immunology, 38*(6), 1479–1482.
40. Krampera, M., Cosmi, L., Angeli, R., et al. (2006). Role for interferon-γ in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells, 24*(2), 386–398.
41. Barry, F. P., & Murphy, J. M. (2004). Mesenchymal stem cells: clinical applications and biological characterization. *The International Journal of Biochemistry & Cell Biology, 36*(4), 568–584.
42. Mahmood, A., Lu, D., Lu, M., et al. (2003). Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. *Neurosurgery, 53*(3), 697–703.
43. Ryan, J., Barry, F., Murphy, J., et al. (2007). Interferon-γ does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. *Clinical & Experimental Immunology, 149*(2), 353–363.
44. Ryan, J. M., Barry, F. P., Murphy, J. M., et al. (2005). Mesenchymal stem cells avoid allogeneic rejection. *Journal of Inflammation, 2*(1), 8.
45. George, J. C. (2010). Stem cell therapy in acute myocardial infarction: a review of clinical trials. *Translational Research, 155*(1), 10–19.
46. Harris, D. T. (2014). Stem cell banking for regenerative and personalized medicine. *Biomedicines, 2*(1), 50–79.
47. Bang, O. Y., Lee, J. S., Lee, P. H., et al. (2005). Autologous mesenchymal stem cell transplantation in stroke patients. *Annals of Neurology, 57*(6), 874–882.
48. Prockop, D. J. (2017). The exciting prospects of new therapies with mesenchymal stromal cells. *Cytotherapy, 19*(1), 1–8.
49. Bang, O. Y., Kim, E. H., Cha, J. M., et al. (2016). Adult stem cell therapy for stroke: challenges and progress. *Journal of Stroke, 18*(3), 256.
50. Steinbeck, J. A., & Studer, L. (2015). Moving stem cells to the clinic: potential and limitations for brain repair. *Neuron, 86*(1), 187–206.
51. Kim, E. H., Kim, D. H., Kim, H. R., et al. (2016). Stroke serum priming modulates characteristics of mesenchymal stromal cells by controlling the expression miRNA-20a. *Cell Transplantation, 25*(8), 1489–1499.
52. Choi, Y. J., Li, W. Y., Moon, G. J., et al. (2010). Enhancing trophic support of mesenchymal stem cells by ex vivo treatment with trophic factors. *Journal of the Neurological Sciences, 298*(1), 28–34.
53. Efimenko, A. Y., Kochegura, T. N., Akopyan, Z. A., et al. (2015). Autologous stem cell therapy: how aging and chronic diseases affect stem and progenitor cells. *BioResearch Open Access, 4*(1), 26–38.
54. Hu, F., Wang, X., Liang, G., et al. (2013). Effects of epidermal growth factor and basic fibroblast growth factor on the proliferation and osteogenic and neural differentiation of adipose-derived stem cells. *Cellular Reprogramming, 15*(3), 224–232.
55. Mylotte, L. A., Duffy, A. M., Murphy, M., et al. (2008). Metabolic flexibility permits mesenchymal stem cell survival in an ischemic environment. *Stem Cells, 26*(5), 1325–1336.
56. McGinley, L., McMahon, J., Strappe, P., et al. (2011). Lentiviral vector mediated modification of mesenchymal stem cells & enhanced survival in an in vitro model of ischaemia. *Stem Cell Research & Therapy, 2*(2), 12.
57. Kumar Bokara, K., Suresh Oggu, G., Josyula Vidyasagar, A., et al. (2014). Modulation of stem cell differentiation by the influence of nanobiomaterials/carriers. *Current Stem Cell Research & Therapy, 9*(6), 458–468.
58. Meisel, R., Brockers, S., Heseler, K., et al. (2011). Human but not murine multipotent mesenchymal stromal cells exhibit broad-spectrum antimicrobial effector function mediated by indoleamine 2, 3-dioxygenase. *Leukemia, 25*(4), 648–654.
59. Ljungman, P., De La Camara, R., Cordonnier, C., et al. (2008). Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. *Bone Marrow Transplantation, 42*(4), 227–240.
60. Tan, J., Wu, W., Xu, X., et al. (2012). Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA, 307*(11), 1169–1177.
61. Khera, M., Albersen, M., & Mulhall, J. P. (2015). Mesenchymal stem cell therapy for the treatment of erectile dysfunction. *The Journal of Sexual Medicine, 12*(5), 1105–1106.
62. Gehl, J. (2003). Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. *Acta Physiologica, 177*(4), 437–447.
63. Gresch, O., Engel, F. B., Nesic, D., et al. (2004). New non-viral method for gene transfer into primary cells. *Methods, 33*(2), 151–163.
64. Otani, K., Yamahara, K., Ohnishi, S., et al. (2009). Nonviral delivery of siRNA into mesenchymal stem cells by a combination of ultrasound and microbubbles. *Journal of Controlled Release, 133*(2), 146–153.
65. Sanchez-Antequera, Y., Mykhaylyk, O., van Til, N. P., et al. (2011). Magselectofection: an integrated method of nanomagnetic separation and genetic modification of target cells. *Blood, 117*(16), e171-e181.
66. Kim, H.-J., & Im, G.-I. (2011). Electroporation-mediated transfer of SOX trio genes (SOX-5, SOX-6, and SOX-9) to enhance the chondrogenesis of mesenchymal stem cells. *Stem Cells and Development, 20*(12), 2103–2114.
67. Ferreira, E., Potier, E., Vaudin, P., et al. (2012). Sustained and promoter dependent bone morphogenetic protein expression by rat mesenchymal stem cells after BMP-2 transgene electrotransfer. *European Cells & Materials, 24*(1), 18–28.
68. Park, S. A., Ryu, C. H., Kim, S. M., et al. (2011). CXCR4-transfected human umbilical cord blood-derived mesenchymal stem cells exhibit enhanced migratory capacity toward gliomas. *International Journal of Oncology, 38*(1), 97.
69. Ryser, M. F., Ugarte, F., Thieme, S., et al. (2008). mRNA transfection of CXCR4-GFP fusion—simply generated by PCR—results in efficient migration of primary human mesenchymal stem cells. *Tissue Engineering Part C: Methods, 14*(3), 179–184.
70. Sheyn, D., Pelled, G., Zilberman, Y., et al. (2008). Nonvirally engineered porcine adipose tissue-derived stem cells: use in posterior spinal fusion. *Stem Cells, 26*(4), 1056–1064.
71. Rome, C., Deckers, R., & Moonen, C. T. (2008). The use of ultrasound in transfection and transgene expression. *Molecular imaging II, hand book of experimental pharmacology*. Springer.
72. Nakashima, M., Tachibana, K., Iohara, K., et al. (2003). Induction of reparative dentin formation by ultrasound-mediated gene delivery of growth/differentiation factor 11. *Human Gene Therapy, 14*(6), 591–597.

73. Li, W., Ma, N., Ong, L. L., et al. (2008). Enhanced thoracic gene delivery by magnetic nanobead-mediated vector. *The Journal of Gene Medicine, 10*(8), 897–909.
74. Li, W., Nesselmann, C., Zhou, Z., et al. (2007). Gene delivery to the heart by magnetic nanobeads. *Journal of Magnetism and Magnetic Materials, 311*(1), 336–341.
75. Bharali, D. J., Klejbor, I., Stachowiak, E. K., et al. (2005). Organically modified silica nanoparticles: a nonviral vector for in vivo gene delivery and expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America, 102*(32), 11539–11544.
76. Gao, L., Nie, L., Wang, T., et al. (2006). Carbon nanotube delivery of the GFP gene into mammalian cells. *ChemBioChem, 7*(2), 239–242.
77. Guo, S., Huang, Y., Jiang, Q., et al. (2010). Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. *ACS Nano, 4*(9), 5505–5511.
78. Tan, W. B., Jiang, S., & Zhang, Y. (2007). Quantum-dot based nanoparticles for targeted silencing of HER2/neu gene via RNA interference. *Biomaterials, 28*(8), 1565–1571.
79. Wu, H. C., Wang, T. W., Bohn, M. C., et al. (2010). Novel magnetic hydroxyapatite nanoparticles as non-viral vectors for the glial cell line-derived neurotrophic factor gene. *Advanced Functional Materials, 20*(1), 67–77.
80. Kim, T. H., Kim, M., Eltohamy, M., et al. (2013). Efficacy of mesoporous silica nanoparticles in delivering BMP-2 plasmid DNA for in vitro osteogenic stimulation of mesenchymal stem cells. *Journal of Biomedical Materials Research Part A, 101*(6), 1651–1660.
81. Lin, C., Wang, Y., Lai, Y., et al. (2011). Incorporation of carboxylation multiwalled carbon nanotubes into biodegradable poly (lactic-co-glycolic acid) for bone tissue engineering. *Colloids and Surfaces B: Biointerfaces, 83*(2), 367–375.
82. Baik, K. Y., Park, S. Y., Heo, K., et al. (2011). Carbon nanotube monolayer cues for osteogenesis of mesenchymal stem cells. *Small, 7*(6), 741–745.
83. Bhattacharya, M., Wutticharoenmongkol-Thitiwongsawet, P., Hamamoto, D. T., et al. (2011). Bone formation on carbon nanotube composite. *Journal of Biomedical Materials Research Part A, 96*(1), 75–82.
84. Cellot, G., Toma, F. M., Varley, Z. K., et al. (2011). Carbon nanotube scaffolds tune synaptic strength in cultured neural circuits: novel frontiers in nanomaterial–tissue interactions. *Journal of Neuroscience, 31*(36), 12945–12953.
85. Fabbro, A., Villari, A., Laishram, J., et al. (2012). Spinal cord explants use carbon nanotube interfaces to enhance neurite outgrowth and to fortify synaptic inputs. *ACS Nano, 6*(3), 2041–2055.
86. Joydeep, D., Choi, Y.-J., Yasuda, H., et al. (2016). Efficient delivery of C/EBP beta gene into human mesenchymal stem cells via polyethylenimine-coated gold nanoparticles enhances adipogenic differentiation. *Scientific Reports, 6*, 37480.
87. Cao, X., Deng, W., Wei, Y., et al. (2011). Encapsulation of plasmid DNA in calcium phosphate nanoparticles: stem cell uptake and gene transfer efficiency. *International Journal of NanoMedicine, 6*, 3335–3349.
88. Cho, J. W., Lee, C. Y., & Ko, Y. (2012). Therapeutic potential of mesenchymal stem cells overexpressing human forkhead box A2 gene in the regeneration of damaged liver tissues. *Journal of Gastroenterology and Hepatology, 27*(8), 1362–1370.
89. Jeon, S. Y., Park, J. S., Yang, H. N., et al. (2012). Co-delivery of SOX9 genes and anti-Cbfa-1 siRNA coated onto PLGA nanoparticles for chondrogenesis of human MSCs. *Biomaterials, 33*(17), 4413–4423.
90. Kim, N. Y., Choi, Y. B., Kang, C. I., et al. (2010). An hydrophobically modified arginine peptide vector system effectively delivers DNA into human mesenchymal stem cells and maintains transgene expression with differentiation. *The Journal of Gene Medicine, 12*(9), 779–789.
91. Thakor, D. K., Teng, Y. D., Obata, H., et al. (2010). Nontoxic genetic engineering of mesenchymal stem cells using serum-compatible pullulan-spermine/DNA anioplexes. *Tissue Engineering Part C: Methods, 17*(2), 131–144.
92. Jordan, M., & Wurm, F. (2004). Transfection of adherent and suspended cells by calcium phosphate. *Methods, 33*(2), 136–143.
93. Masotti, A., Mossa, G., Cametti, C., et al. (2009). Comparison of different commercially available cationic liposome–DNA lipoplexes: parameters influencing toxicity and transfection efficiency. *Colloids and Surfaces B: Biointerfaces, 68*(2), 136–144.
94. Ruponen, M., Rönkkö, S., Honkakoski, P., et al. (2001). Extracellular glycosaminoglycans modify cellular trafficking of lipoplexes and polyplexes. *Journal of Biological Chemistry, 276*(36), 33875–33880.
95. Rejman, J., Conese, M., & Hoekstra, D. (2006). Gene transfer by means of lipo-and polyplexes: role of clathrin and caveolae-mediated endocytosis. *Journal of Liposome Research, 16*(3), 237–247.
96. Simões, S., Slepushkin, V., Pires, P., et al. (1999). Mechanisms of gene transfer mediated by lipoplexes associated with targeting ligands or pH-sensitive peptides. *Gene Therapy, 6*(11), 1798–1807.
97. Wen, Y., Guo, Z., Du, Z., et al. (2012). Serum tolerance and endosomal escape capacity of histidine-modified pDNA-loaded complexes based on polyamidoamine dendrimer derivatives. *Biomaterials, 33*(32), 8111–8121.
98. Li, W., Ma, N., Ong, L. L., et al. (2007). Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells, 25*(8), 2118–2127.
99. Kim, H. H., Choi, H. S., Yang, J. M., et al. (2007). Characterization of gene delivery in vitro and in vivo by the arginine peptide system. *International Journal of Pharmaceutics, 335*(1), 70–78.
100. Bachrach, U. (2005). Naturally occurring polyamines: interaction with macromolecules. *Current Protein and Peptide Science, 6*(6), 559–566.
101. Azzam, T., Raskin, A., Makovitzki, A., et al. (2002). Cationic polysaccharides for gene delivery. *Macromolecules, 35*(27), 9947–9953.
102. Han, S.-W., Nakamura, C., Kotobuki, N., et al. (2008). High-efficiency DNA injection into a single human mesenchymal stem cell using a nanoneedle and atomic force microscopy. *Nanomedicine: Nanotechnology, Biology and Medicine, 4*(3), 215–225.
103. Subramanian, A., Ranganathan, P., & Diamond, S. L. (1999). Nuclear targeting peptide scaffolds for lipofection of nondividing mammalian cells. *Nature Biotechnology, 17*(9), 873–877.
104. Vallhov, H., Gabrielsson, S., Strømme, M., et al. (2007). Mesoporous silica particles induce size dependent effects on human dendritic cells. *Nano Letters, 7*(12), 3576–3582.
105. Simões, S., Filipe, A., Faneca, H., et al. (2005). Cationic liposomes for gene delivery. *Expert Opinion on Drug Delivery, 2*(2), 237–254.
106. Wang, W., Xu, X., Li, Z., et al. (2014). Genetic engineering of mesenchymal stem cells by non-viral gene delivery. *Clinical Hemorheology and Microcirculation, 58*(1), 19–48.
107. Nowakowski, A., Andrzejewska, A., Janowski, M., et al. (2013). Genetic engineering of stem cells for enhanced therapy. *Acta Neurobiologiae Experimentalis, 73*(1), 1–18.
108. Dahlberg, J. (1987). An overview of retrovirus replication and classification. *Advances in Veterinary Science and Comparative Medicine, 32*, 1–35.
109. Aiuti, A., Cattaneo, F., Galimberti, S., et al. (2009). Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *New England Journal of Medicine, 360*(5), 447–458.

110. Laker, C., Meyer, J., Schopen, A., et al. (1998). Host cis-mediated extinction of a retrovirus permissive for expression in embryonal stem cells during differentiation. *Journal of Virology, 72*(1), 339–348.
111. Challita, P.-M., & Kohn, D. B. (1994). Lack of expression from a retroviral vector after transduction of murine hematopoietic stem cells is associated with methylation in vivo. *Proceedings of the National Academy of Sciences, 91*(7), 2567–2571.
112. Hacein-Bey-Abina, S., Hauer, J., Lim, A., et al. (2010). Efficacy of gene therapy for X-linked severe combined immunodeficiency. *New England Journal of Medicine, 363*(4), 355–364.
113. Huang, S., & Terstappen, L. (1994). Lymphoid and myeloid differentiation of single human CD34+, HLA-DR+, CD38-hematopoietic stem cells. *Blood, 83*(6), 1515–1526.
114. Naldini, L., Blomer, U., Gallay, P., et al. (1996). In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science, 272*(5259), 263.
115. Hu, J., Lang, Y., Zhang, T., et al. (2016). Lentivirus-mediated PGC-1α overexpression protects against traumatic spinal cord injury in rats. *Neuroscience, 328*, 40–49.
116. Xiang, Q., Hong, D., Liao, Y., et al. (2016). Overexpression of gremlin1 in mesenchymal stem cells improves hindlimb ischemia in mice by enhancing cell survival. *Journal of Cellular Physiology, 232*(5), 996–1007.
117. De Melo, S. M., Bittencourt, S., Ferrazoli, E. G., et al. (2015). The anti-tumor effects of adipose tissue mesenchymal stem cell transduced with HSV-Tk gene on U-87-driven brain tumor. *PLoS One, 10*(6), e0128922.
118. Cartier, N., & Aubourg, P. (2010). Hematopoietic stem cell transplantation and hematopoietic stem cell gene therapy in X-Linked adrenoleukodystrophy. *Brain Pathology, 20*(4), 857–862.
119. Cavazzana-Calvo, M., Payen, E., Negre, O., et al. (2010). Transfusion independence and HMGA2 activation after gene therapy of human [bgr]-thalassaemia. *Nature, 467*(7313), 318–322.
120. Aiuti, A., Biasco, L., Scaramuzza, S., et al. (2013). Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science, 341*(6148), 1233151.
121. Biffi, A., Montini, E., Lorioli, L., et al. (2013). Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science, 341*(6148), 1233158.
122. Tomás, H. A., Rodrigues, A. F., Alves, P. M., et al. (2013). *Lentiviral gene therapy vectors: Challenges and future directions*. InTech.
123. Ke, J., Zheng, L., & Cheung, L. (2011). Orthopaedic gene therapy using recombinant adeno-associated virus vectors. *Archives of Oral Biology, 56*(7), 619–628.
124. Zaiss, A.-K., Liu, Q., Bowen, G. P., et al. (2002). Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors. *Journal of Virology, 76*(9), 4580–4590.
125. Wright, J. F. (2009). Transient transfection methods for clinical adeno-associated viral vector production. *Human Gene Therapy, 20*(7), 698–706.
126. Frisch, J., Venkatesan, J. K., Rey-Rico, A., et al. (2014). Influence of insulin-like growth factor I overexpression via recombinant adeno-associated vector gene transfer upon the biological activities and differentiation potential of human bone marrow-derived mesenchymal stem cells. *Stem Cell Research & Therapy, 5*(4), 103.
127. Frisch, J., Venkatesan, J. K., Rey-Rico, A., et al. (2014). Determination of the chondrogenic differentiation processes in human bone marrow-derived mesenchymal stem cells genetically modified to overexpress transforming growth factor-β via recombinant adeno-associated viral vectors. *Human Gene Therapy, 25*(12), 1050–1060.
128. Venkatesan, J. K., Ekici, M., Madry, H., et al. (2012). SOX9 gene transfer via safe, stable, replication-defective recombinant adeno-associated virus vectors as a novel, powerful tool to enhance the chondrogenic potential of human mesenchymal stem cells. *Stem Cell Research & Therapy, 3*(3), 22.
129. Tao, K., Frisch, J., Rey-Rico, A., et al. (2016). Co-overexpression of TGF-β and SOX9 via rAAV gene transfer modulates the metabolic and chondrogenic activities of human bone marrow-derived mesenchymal stem cells. *Stem Cell Research & Therapy, 7*(1), 20.
130. Zanotti, L., Angioni, R., Calì, B., et al. (2016). Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1. *Leukemia, 30*, 1143–1154.
131. Liu, Z., Wang, C., Wang, X., et al. (2015). Therapeutic effects of transplantation of as-mir-937-expressing mesenchymal stem cells in murine model of alzheimer's disease. *Cellular Physiology and Biochemistry, 37*(1), 321–330.
132. Nayak, S., & Herzog, R. W. (2010). Progress and prospects: immune responses to viral vectors. *Gene Therapy, 17*(3), 295–304.
133. Yin, T., He, S., Su, C., et al. (2015). Genetically modified human placenta-derived mesenchymal stem cells with FGF-2 and PDGF-BB enhance neovascularization in a model of hindlimb ischemia. *Molecular Medicine Reports, 12*(4), 5093–5099.
134. Kanehira, M., Xin, H., Hoshino, K., et al. (2007). Targeted delivery of NK4 to multiple lung tumors by bone marrow-derived mesenchymal stem cells. *Cancer Gene Therapy, 14*(11), 894–903.
135. Gopalakrishnan, A. K., Pandit, H., Metkari, S., et al. (2016). Adenoviral vector encoding soluble Flt-1 engineered human endometrial mesenchymal stem cells effectively regress endometriotic lesions in NOD/SCID mice. *Gene Therapy, 23*(7), 580–591.
136. Ryu, C. H., Park, S.-H., Park, S. A., et al. (2011). Gene therapy of intracranial glioma using interleukin 12–secreting human umbilical cord blood–derived mesenchymal stem cells. *Human Gene Therapy, 22*(6), 733–743.
137. Sun, X. L., Xu, Z. M., Ke, Y. Q., et al. (2011). Molecular targeting of malignant glioma cells with an EphA2-specific immunotoxin delivered by human bone marrow-derived mesenchymal stem cells. *Cancer Letters, 312*(2), 168–177.
138. Yang, Y., Nunes, F. A., Berencsi, K., et al. (1994). Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proceedings of the National Academy of Sciences, 91*(10), 4407–4411.
139. Treacy, O., Ryan, A. E., Heinzl, T., et al. (2012). Adenoviral transduction of mesenchymal stem cells: in vitro responses and in vivo immune responses after cell transplantation. *PLoS One, 7*(8), e42662.
140. McCarter, S., Scott, J., Lee, P., et al. (2003). Cotransfection of heme oxygenase-1 prevents the acute inflammation elicited by a second adenovirus. *Gene Therapy, 10*(19), 1629–1635.
141. Alba, R., Bosch, A., & Chillon, M. (2005). Gutless adenovirus: last-generation adenovirus for gene therapy. *Gene Therapy, 12*, S18-S27.
142. Lamb, A. (1996). Paramyxoviridae: the virus and their replication. *Fields Virology*.
143. Knaän-Shanzer, S., van de Watering, M. J., van der Velde, I., et al. (2005). Endowing human adenovirus serotype 5 vectors with fiber domains of species B greatly enhances gene transfer into human mesenchymal stem cells. *Stem Cells, 23*(10), 1598–1607.
144. Zaldumbide, A., Carlotti, F., Gonçalves, M. A., et al. (2012). Adenoviral vectors stimulate glucagon transcription in human mesenchymal stem cells expressing pancreatic transcription factors. *PLoS One, 7*(10), e48093.
145. Hu, Y. C. (2006). Baculovirus vectors for gene therapy. *Advances in Virus Research, 68*, 287–320.

146. Kost, T. A., Condreay, J. P., & Jarvis, D. L. (2005). Baculovirus as versatile vectors for protein expression in insect and mammalian cells. *Nature Biotechnology, 23*(5), 567–575.
147. Merrihew, R. V., Clay, W. C., Condreay, J. P., et al. (2001). Chromosomal integration of transduced recombinant baculovirus DNA in mammalian cells. *Journal of Virology, 75*(2), 903–909.
148. Wang, K. C., Wu, J. C., Chung, Y. C., et al. (2005). Baculovirus as a highly efficient gene delivery vector for the expression of hepatitis delta virus antigens in mammalian cells. *Biotechnology and Bioengineering, 89*(4), 464–473.
149. Zeng, J., Du, J., Lin, J., et al. (2009). High-efficiency transient transduction of human embryonic stem cell–derived neurons with baculoviral vectors. *Molecular Therapy, 17*(9), 1585–1593.
150. Chen, G. Y., Pang, D. P., Hwang, S. M., et al. (2012). A graphene-based platform for induced pluripotent stem cells culture and differentiation. *Biomaterials, 33*(2), 418–427.
151. Lee, E. X., Lam, D. H., Wu, C., et al. (2011). Glioma gene therapy using induced pluripotent stem cell derived neural stem cells. *Molecular Pharmaceutics, 8*(5), 1515–1524.
152. Chen, H. C., Chang, Y. H., Chuang, C. K., et al. (2009). The repair of osteochondral defects using baculovirus-mediated gene transfer with de-differentiated chondrocytes in bioreactor culture. *Biomaterials, 30*(4), 674–681.
153. Sung, L. Y., Lo, W. H., Chiu, H. Y., et al. (2007). Modulation of chondrocyte phenotype via baculovirus-mediated growth factor expression. *Biomaterials, 28*(23), 3437–3447.
154. Bak, X. Y., Dang, H. L., Yang, J., et al. (2011). Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for prodrug gene therapy of glioblastoma. *Human Gene Therapy, 22*(11), 1365–1377.
155. Chuang, C. K., Lin, K. J., Lin, C. Y., et al. (2009). Xenotransplantation of human mesenchymal stem cells into immunocompetent rats for calvarial bone repair. *Tissue Engineering Part A, 16*(2), 479–488.
156. Lu, C. H., Lin, K. J., Chiu, H. Y., et al. (2012). Improved chondrogenesis and engineered cartilage formation from TGF-β3-expressing adipose-derived stem cells cultured in the rotating-shaft bioreactor. *Tissue Engineering Part A, 18*(19–20), 2114–2124.
157. Liao, J.-C. (2016). Bone marrow mesenchymal stem cells expressing baculovirus-engineered bone morphogenetic protein-7 enhance rabbit posterolateral fusion. *International Journal of Molecular Sciences, 17*(7), 1073.
158. Liao, J.-C. (2016). Cell therapy using bone marrow-derived stem cell overexpressing BMP-7 for degenerative discs in a rat tail disc model. *International Journal of Molecular Sciences, 17*(2), 147.
159. Fu, T.-S., Chang, Y.-H., Wong, C.-B., et al. (2015). Mesenchymal stem cells expressing baculovirus-engineered BMP-2 and VEGF enhance posterolateral spine fusion in a rabbit model. *The Spine Journal, 15*(9), 2036–2044.
160. Bak, X., Yang, J., & Wang, S. (2010). Baculovirus-transduced bone marrow mesenchymal stem cells for systemic cancer therapy. *Cancer Gene Therapy, 17*(10), 721–729.
161. Harrison, R. L., & Jarvis, D. L. (2006). Protein N-glycosylation in the baculovirus–insect cell expression system and engineering of insect cells to produce "Mammalianized" recombinant glycoproteins. *Advances in Virus Research, 68*, 159–191.
162. Manservigi, R., Argnani, R., & Marconi, P. (2010). HSV recombinant vectors for gene therapy. *The Open Virology Journal, 4*(1), 123–156.
163. Zhang, G., Kobayashi, T., Kamitani, W., et al. (2003). Borna disease virus phosphoprotein represses p53-mediated transcriptional activity by interference with HMGB1. *Journal of Virology, 77*(22), 12243–12251.
164. Leoni, V., Gatta, V., Palladini, A., et al. (2015). Systemic delivery of HER2-retargeted oncolytic-HSV by mesenchymal stromal cells protects from lung and brain metastases. *Oncotarget, 6*(33), 34774.
165. Breitbach, C. J., Burke, J., Jonker, D., et al. (2011). Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. *Nature, 477*(7362), 99–102.
166. Heo, J., Reid, T., Ruo, L., et al. (2013). Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nature Medicine, 19*(3), 329–336.
167. Park, B. H., Hwang, T., Liu, T. C., et al. (2008). Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. *The Lancet Oncology, 9*(6), 533–542.
168. De la Torre, J. (1994). Molecular biology of borna disease virus: prototype of a new group of animal viruses. *Journal of Virology, 68*(12), 7669.
169. Briese, T., de La Torre, J. C., Lewis, A., et al. (1992). Borna disease virus, a negative-strand RNA virus, transcribes in the nucleus of infected cells. *Proceedings of the National Academy of Sciences, 89*(23), 11486–11489.
170. Briese, T., Schneemann, A., Lewis, A. J., et al. (1994). Genomic organization of Borna disease virus. *Proceedings of the National Academy of Sciences, 91*(10), 4362–4366.
171. Schneemann, A., Schneider, P. A., Lamb, R. A., et al. (1995). The remarkable coding strategy of Borna disease virus: a new member of the nonsegmented negative strand RNA viruses. *Virology, 210*(1), 1–8.
172. Ikeda, Y., Makino, A., Matchett, W. E., et al. (2016). A novel intranuclear RNA vector system for long-term stem cell modification. *Gene Therapy, 23*(3), 256–262.
173. McCarty, D. M. (2008). Self-complementary AAV vectors; advances and applications. *Molecular Therapy, 16*(10), 1648–1656.
174. Gall, J., Kass-Eisler, A., Leinwand, L., et al. (1996). Adenovirus type 5 and 7 capsid chimera: fiber replacement alters receptor tropism without affecting primary immune neutralization epitopes. *Journal of Virology, 70*(4), 2116–2123.
175. Ito, H., Goater, J., Tiyapatanaputi, P., et al. (2004). Light-activated gene transduction of recombinant adeno-associated virus in human mesenchymal stem cells. *Gene Therapy, 11*(1), 34–41.
176. Li, M., Jayandharan, G. R., Li, B., et al. (2010). High-efficiency transduction of fibroblasts and mesenchymal stem cells by tyrosine-mutant AAV2 vectors for their potential use in cellular therapy. *Human Gene Therapy, 21*(11), 1527–1543.
177. Pang, Z. P., Yang, N., Vierbuchen, T., et al. (2011). Induction of human neuronal cells by defined transcription factors. *Nature, 476*(7359), 220–223.
178. Graf, T., & Enver, T. (2009). Forcing cells to change lineages. *Nature, 462*(7273), 587–594.
179. Lee, A. S., Tang, C., Rao, M. S., et al. (2013). Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nature Medicine, 19*(8), 998–1004.
180. Zhang, Y., Pak, C., Han, Y., et al. (2013). Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron, 78*(5), 785–798.
181. Ramos, C. A., Asgari, Z., Liu, E., et al. (2010). An inducible caspase 9 suicide gene to improve the safety of mesenchymal stromal cell therapies. *Stem Cells, 28*(6), 1107–1115.
182. Morris, S. A., Cahan, P., Li, H., et al. (2014). Dissecting engineered cell types and enhancing cell fate conversion via CellNet. *Cell, 158*(4), 889–902.
183. Rombouts, W., & Ploemacher, R. (2003). Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. *Leukemia, 17*(1), 160–170.
184. Banfi, A., Muraglia, A., Dozin, B., et al. (2000). Proliferation kinetics and differentiation potential of ex vivo expanded human

bone marrow stromal cells: implications for their use in cell therapy. *Experimental Hematology, 28*(6), 707–715.
185. Baron, F., Lechanteur, C., Willems, E., et al. (2010). Cotransplantation of mesenchymal stem cells might prevent death from graft-versus-host disease (GVHD) without abrogating graft-versus-tumor effects after HLA-mismatched allogeneic transplantation following nonmyeloablative conditioning. *Biology of Blood and Marrow Transplantation, 16*(6), 838–847.
186. Zhou, H., Guo, M., Bian, C., et al. (2010). Efficacy of bone marrow-derived mesenchymal stem cells in the treatment of sclerodermatous chronic graft-versus-host disease: clinical report. *Biology of Blood and Marrow Transplantation, 16*(3), 403–412.
187. Kuzmina, L. A., Petinati, N. A., Parovichnikova, E. N., et al. (2011). Multipotent mesenchymal stromal cells for the prophylaxis of acute graft-versus-host disease—a phase II study. *Stem Cells International, 2012(2012)*, 8.
188. Miletic, H., Fischer, Y., Litwak, S., et al. (2007). Bystander killing of malignant glioma by bone marrow–derived tumor-infiltrating progenitor cells expressing a suicide gene. *Molecular Therapy, 15*(7), 1373–1381.
189. Cavarretta, I. T., Altanerova, V., Matuskova, M., et al. (2010). Adipose tissue–derived mesenchymal stem cells expressing prodrug-converting enzyme inhibit human prostate tumor growth. *Molecular Therapy, 18*(1), 223–231.
190. Van Eekelen, M., Sasportas, L., Kasmieh, R., et al. (2010). Human stem cells expressing novel TSP-1 variant have anti-angiogenic effect on brain tumors. *Oncogene, 29*(22), 3185–3195.
191. Scholefield, J., & Weinberg, M. S. (2016). The application of CRISPR/Cas9 technologies and therapies in stem cells. *Current Stem Cell Reports, 2*(2), 95–103.
192. Ran, F. A., Hsu, P. D., Wright, J., et al. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature Protocols, 8*(11), 2281–2308.
193. Ran, F. A., Hsu, P. D., Lin, C.-Y., et al. (2013). Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell, 154*(6), 1380–1389.
194. Webber, B. R., Osborn, M. J., McElroy, A. N., et al. (2016). CRISPR/Cas9-based genetic correction for recessive dystrophic epidermolysis bullosa. *NPJ Regenerative Medicine, 1*, 16014.
195. Cong, L., Ran, F. A., Cox, D., et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*(6121), 819–823.
196. Liu, T., Shen, J. K., Li, Z., et al. (2016). Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma. *Cancer Letters, 373*(1), 109–118.
197. Cho, S. W., Kim, S., Kim, Y., et al. (2014). Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Research, 24*(1), 132–141.
198. Pattanayak, V., Lin, S., Guilinger, J. P., et al. (2013). High-throughput profiling of off-target DNA cleavage reveals RNA-programmed Cas9 nuclease specificity. *Nature Biotechnology, 31*(9), 839–843.
199. Kleinstiver, B. P., Pattanayak, V., Prew, M. S., et al. (2016). High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. *Nature, 529*(7587), 490–495.
200. Slaymaker, I. M., Gao, L., Zetsche, B., et al. (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science, 351*(6268), 84–88.
201. Albitar, A., Rohani, B., Will, B., et al. (2017). The application of CRISPR/Cas technology to efficiently model complex cancer genomes in stem cells. *Journal of Cellular Biochemistry*.
202. Blitz, I. L., Biesinger, J., Xie, X., et al. (2013). Biallelic genome modification in F0 Xenopus tropicalis embryos using the CRISPR/Cas system. *Genesis*, 51(12), 827–834.
203. Jao, L.E., Wente, S. R., & Chen, W. (2013). Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. *Proceedings of the National Academy of Sciences, 110*(34), 13904–13909.
204. Wang, H., Yang, H., Shivalila, C. S., et al. (2013). One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell, 153*(4), 910–918.
205. Sokolik, C., Liu, Y., Bauer, D., et al. (2015). Transcription factor competition allows embryonic stem cells to distinguish authentic signals from noise. *Cell Systems, 1*(2), 117–129.
206. Delavar, H. M., Karamzadeh, A., & Pahlavanneshan, S. (2016). Shining light on the sprout of life: optogenetics applications in stem cell research and therapy. *The Journal of Membrane Biology, 249*(3), 215–220.
207. Fenno, L. E., Mattis, J., Ramakrishnan, C., et al. (2014). Targeting cells with single vectors using multiple-feature Boolean logic. *Nature Methods, 11*(7), 763–772.
208. Liu, X., Ramirez, S., Pang, P. T., et al. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature, 484*(7394), 381–385.
209. Guru, A., Post, R. J., Ho, Y. Y., et al. (2015). Making sense of optogenetics. *International Journal of Neuropsychopharmacology, 18*(11), 79.
210. Pliss, A., Ohulchanskyy, T. Y., Chen, G., et al. (2017). Subcellular optogenetics enacted by targeted nanotransformers of near-infrared light. *ACS Photonics, 4*(4), 806–814.
211. Meyer, U. A. (2002). Introduction to pharmacogenomics: promises, opportunities, and limitations. *Pharmacogenomics: The search for individualized therapies*, pp. 1–8.
212. Langman, L. J., Nesher, L., Shah, D. P., et al. (2016). Challenges in determining genotypes for pharmacogenetics in allogeneic hematopoietic cell transplant recipients. *The Journal of Molecular Diagnostics, 18*(5), 638–642.
213. Licinio, J., & Wong, M. L. (2009). *Pharmacogenomics: The search for individualized therapies*. Wiley.
214. Al-Mahayri, Z. N., & Patrinos, G. P., & Ali, R. (2017). Pharmacogenomics in pediatric acute lymphoblastic leukemia: promises and limitations. *The Journal of Molecular Diagnostics, 18*(7), 687–699.
215. Van Hassselt, J. C., & Iyengar, R. (2017). Systems pharmacology-based identification of pharmacogenomic determinants of adverse drug reactions using human iPSC-derived cell lines. *Current Opinion in Systems Biology, 4*, 9–15.