Chapter 2
Stem Cells: The Holy Grail of Regenerative Medicine

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2.1 Introduction

Stem cells occupy a special position in cellular hierarchy during differentiation and development of any organism. These undifferentiated cells have the potential to form any other cell type with specialized function and are characterized by their ability to self-renew and to differentiate. Both these cellular properties are prime requisites for the success of current regenerative medicine.

Depending upon the potential to differentiate into a particular lineage, stem cells could be grouped into five types (Fig. 2.1).

1. **Totipotent**: A single cell capable of dividing and forming various differentiated cells including extraembryonic tissues is known as totipotent or omnipotent cell, e.g., a zygote.
2. **Pluripotent**: The pluripotent stem cells have the ability to differentiate into all cell types of the three germ layers, i.e., ectoderm, mesoderm, and endoderm. Inability to form extraembryonic tissues such as placenta is the only limitation that makes them inferior to totipotent stem cells, e.g., embryonic stem (ES) cells.
3. **Multipotent**: Stem cells that demonstrate a restricted pattern of differentiation toward few lineages are termed as multipotent cells such as hematopoietic stem cell (HSC), which can develop into various types of blood cells but not into brain or liver cells.

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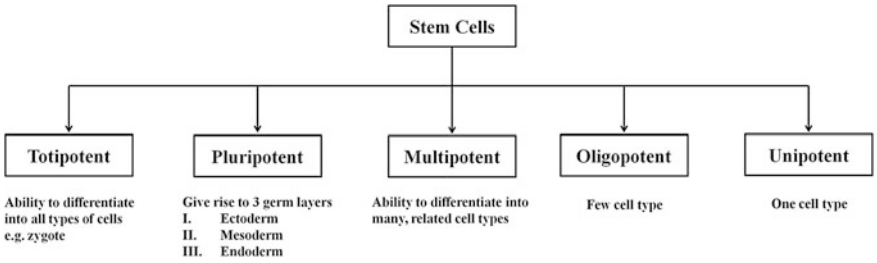

Fig. 2.1 Classification of stem cells: Depending upon the differentiation potential into different lineages, stem cells are categorized as totipotent, pluripotent, multipotent, oligopotent, and unipotent

4. **Oligopotent**: Oligopotent stem cells are able to differentiate into few cell types of specific lineages such as lymphoid stem cells that can be differentiated only into basophil, neutrophil, eosinophil, monocyte, and thrombocytes.
5. **Unipotent**: Unipotent stem cells can only differentiate into one particular cell type such as hepatoblasts forming hepatocytes.

Besides normal developmental functions in multicellular organisms, these mother cells are believed to be the holy grail of medical therapy with high promise for regenerative medicine.

2.2 Engineering of Stem Cells

Stem cells with their unique differentiation potential may act as therapeutic tool to cure diseases which are beyond treatment with routine drug therapy including genetic disorders. Since stem cells are able to generate functionally active healthy cells/tissues, ailments such as neurodegenerative diseases, cardiovascular diseases, liver failure, diabetes, and renal failure where unhealthy cells that are at fault may achieve significant alleviation by stem cell therapy (Fig. 2.2). Current medical practices have not adapted these new therapy regimes routinely as most of them are under clinical trials and not approved yet. The main challenges involve modulation of stem cells toward lineage-specific differentiation in vitro and in vivo, monitor the differentiation, and finally assess the success rate in clinic. Preliminary results appear to be quite promising, which are discussed in the following sections.

2.2.1 Human Embryonic Stem Cells

The unique potential of human ESCs (hESCs) to differentiate into three main germ layers and subsequent to any cell type of human body brought them into forefront of biomedical research with a caveat of forming teratoma in vivo during differentiation. However, recent progress in cell fate control, directed differentiation,

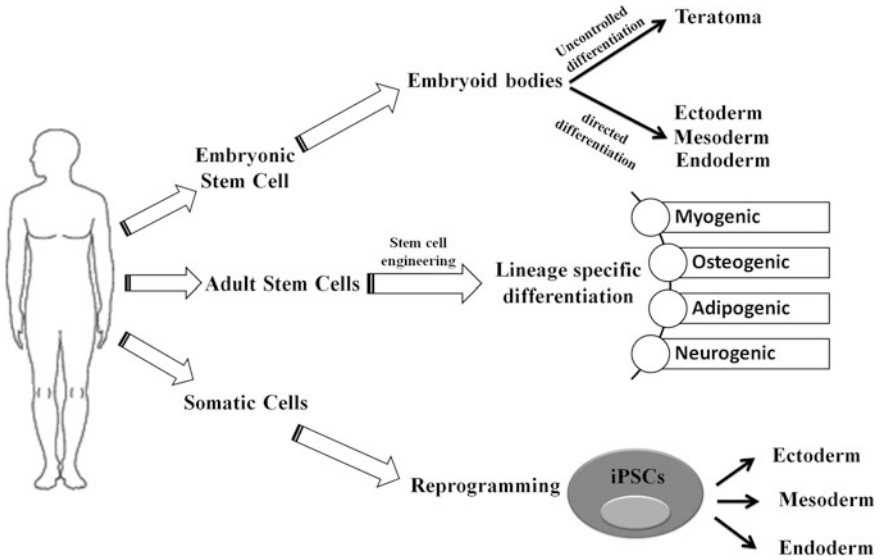

Fig. 2.2 Schematic representation of differentiation process of embryonic stem cell (ESC), adult stem cell (ASC), and induced pluripotent stem cells (iPSCs): While ESCs can generate three germ layers via directed differentiation, it may give rise to teratomas through uncontrolled differentiation in vivo. The ASCs have restricted potential to generate myogenic, osteogenic, adipogenic, and neurogenic lineages under specified conditions. In contrary to ESC and ASC that only differentiate toward specific lineage, iPSCs follow a dedifferentiation (from somatic cell) and then differentiation to germ-layer-specific cellular lineages

and tissue engineering crossed the boundaries of laboratory and extended to the arena of regenerative medicine. hESC lines are conventionally derived from the inner cell mass (ICM) of preimplantation-stage blastocysts, morula-stage embryos, or late-stage blastocysts and express pluripotency markers (transcription factors—Oct4, Sox2, Nanog; surface antigens—SSEA-4, SSEA-3; proteoglycans—TRA-1-60, TRA-1-81) [1]. To maintain the undifferentiated state, these cells are co-cultured with a support or feeder layer derived from mouse embryonic fibroblast (MEF) that provides all the essential growth factors [1]. The reported success rate for hESC derivation is highly variable, possibly due to variation in embryo quality and culture conditions. Major progress had happened in derivation, propagation, cryopreservation, and efficient passaging of hESCs. Since clinical application of hESCs critically depends on well-characterized growth and differentiation of stem cells, much effort was put in developing conditioned media that will enable feeder-free growth of ESC cells and eliminate animal products [2, 3]. The original culture system for the maintenance of hESC using MEF feeder cell layer support possesses risk of zoonosis transmitted by animal pathogens, potential activation of animal retroviruses, and possibility of immune rejection due to the presence of nonhuman sialic acid. Several approaches such as use of extracellular matrix (ECM) derived from MEF than living feeder cells, hESC-derived fibroblasts,

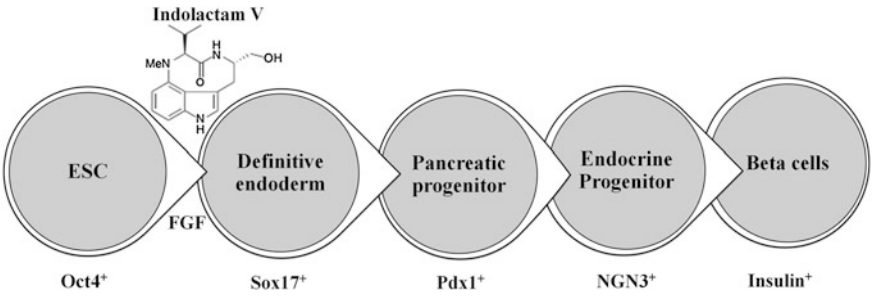

Fig. 2.3 In vitro differentiation of ESCs into insulin-producing beta-cells: Indolactam-V guides the differentiation of ESC into insulin-producing beta-cells via definitive endoderm, pancreatic progenitor, endocrine progenitor, and beta-cells

defined culture medium containing components solely derived from purified human material, MEF-conditioned Matrigel layer to establish and maintain clinical-grade hESCs cell lines are in progress [4]. However, these xeno-free and feeder-independent culture systems are costly and laborious and may lead to abnormal karyotype during long-term culture. Recently, Akopian et al. in conjunction with the Internal Stem Cell Initiative Consortium (ISCIC) compared several commercially available ESC culture media with Knockout Serum Replacer, FGF-2, and MEF cell layers for propagation of several hESC cell lines established in five different laboratories and showed that only mTeSR1 and STEMPRO were able to support most cell lines up to 10 passages [2].

The next major challenge for translational application of hESC is to direct their differentiation toward a specific cell lineage. The pioneer study by Itskovtz-eldor et al. [5] showed that hESC cells were capable of forming "embryoid bodies" (EB) comprised of three embryonic germ layers. In this study, hESCs were grown in suspension to induce their differentiation into EBs. Formation of in vitro EB required special cocktail of supplements and growth factors such as glutamine, beta-mercaptoethanol, nonessential amino acids, leukemia inhibitory factor (LIF), and basic fibroblast growth factor (bFGF). Under these conditions, majority of the cells remained in an undifferentiated state. For the formation of EBs, ES cells were transferred using either collagenase or trypsin/EDTA to plastic petri plates to allow aggregation and prevent adherence. About one million ES cells were plated in each of the 50-mm petri plates, and the hEBs were grown in the same culture medium without LIF and bFGF. The differentiation status of the human ES cells and EBs was determined by the expression pattern of several lineage-specific markers such as gamma-globin (hematopoietic cells), alpha-cardiac actin (myocardial cells), neurofilament (neuronal cells), and alpha-fetoprotein (endodermal cells).

Recently, Chen et al. performed a stepwise differentiation of ESCs to insulin-secreting functional beta-cells where ESCs first formed a definitive endoderm in the presence of indolactam-V and FGF, then Pdx1-expressing pancreatic progenitors, followed by the formation of endocrine progenitors and eventually insulin-producing beta-cells (Fig. 2.3) [6, 7].

Thus, strategic development of tissue-specific adult cells with fully functional potential from undifferentiated ES cells is possible and holds great promise for translational application.

Transplantation of hESC-derived cells into human patients:

The first clinical trial using hESC-derived retinal pigment epithelium (RPE) to establish the safety and tolerability in patients suffering from Stargardt's macular dystrophy and dry age-related macular degeneration was reported by Schwartz et al. [8]. They transplanted a low number of (5×10^4) RPE cells into subretinal space of patient's eye suffering from different forms of macular degeneration. Preoperative and postoperative ophthalmic examinations such as visual acuity, fluorescein angiography, optical coherence tomography, and visual field testing were performed for its validation (clinicaltrials.gov #NCT01345006 and #NCT01344993). This pilot study generated enthusiasm and hope for cell therapy trials in humans with ESCs, which was sidelined due to the adverse effect of generation of teratoma and ethical regulation. Since isolation of ESCs requires creation, treatment, and destruction of human embryos, hESC research always faces criticism and tight ethical regulation. The effort then moved toward using of adult stem cells, which in spite of limited differential potential has turned out to be a great source for cell therapy application.

2.2.2 Adult Stem Cells

Each adult organ in human body harbors a small population of stem cells that have the ability to maintain tissue homeostasis. These "adult stem cells" remain in quiescent or nondividing state until activated by any injury or disease, and they have limited ability to differentiate into organ/tissue-specific lineages. The common ones are HSCs, mesenchymal stem cells (MSCs), neuronal stem cells, umbilical stem cells, cardiac stem cells, retinal stem cells, and limbal stem cells that reside in their respective tissues. Unlike ES cells, adult stem cells do not require a feeder layer or supporting cells for their growth and thus easier to be engineered using different media, growth factors, and small molecules. They also do not pose a risk for developing teratoma and thus preferable in regenerative medicine and stem cell therapy. However, immune rejections of adult stem cells pose serious challenge in certain cases.

2.2.2.1 Hematopoietic Stem Cells

Pioneering studies in engineering of HSCs started in early 1990s at National Institute of Health for the treatment of patients suffering from adenosine deaminase (ADA) deficiency. These patients were treated with genetically modified CD34+ hematopoietic progenitors using retroviral vectors carrying different transgenes. Out of four successfully treated patients suffering from SCID, three

continued doing well up to 3.6 years after gene therapy, whereas one patient suffered serious adverse effect. During a routine checkup after 30 months of gene therapy, lymphocytosis consisting of a monoclonal population of $V_{\gamma}9/V\delta1$, γ/δ T cells of mature phenotype was detected. One pro-viral integration site was found on chromosome 11 within the LMO-2 locus. This insertion leads to an aberrant expression of the LMO-2 transcript in the monoclonal T-cell population (characteristics of acute lymphoblastic leukemia) [9].

These adverse events have resulted in discontinuation of the use of such long terminal repeat (LTR)-driven gamma-retroviral vectors for the genetic manipulations of HSCs, but at the same time, it provided a major thrust for developing novel approaches. New and modified types of retroviral vector such as a "self-inactivating" (SIN) vector [10], lentiviral vectors [11], and lineage-restricted vectors [12] are now entering the clinic. These vectors might reduce the risk of transactivation of proto oncogenes after semi random integrations.

Thrombocytopenia, a deficiency in blood platelets, is a major consequence of several hematological malignancies and chemotherapy [13]. In vitro platelet production from hematopoietic stem and progenitor cells (HSPCs)-derived megakaryocytes (Mks) could augment the supply and elude problems associated with bacterial and viral contamination, as well as immune rejection. Panuganti et al. [14] using HSPCs developed a three-stage strategy for ex vivo expansion of high-ploidy megakaryocytic cells for large-scale platelet production (Fig. 2.4). The $CD34^{+}$ HSPCs culture was started in a cytokine cocktail at 5 % O_2 (pH 7.2). At day 5, cells were shifted to 20 % O_2 (pH 7.4) and maintained in 1 of the 17 cytokine cocktails (identified using a 2^4 factorial design of experiment method to evaluate the effects of interleukin (IL)-3, IL-6, IL-9, and high- or low-dose stem cell factor (SCF) in conjunction with thrombopoietin (Tpo) and IL-11) for expansion of mature Mks from progenitors. The combination of Tpo, high-dose SCF, IL-3, IL-9, and IL-11 produced maximum Mk expansion. These Mks when cultured in IMDM + 20 % BIT 9,500 gave rise to platelets with functional activity similar to that of fresh platelets from normal donors, as validated by basal tubulin distribution and the expression of surface markers.

Later Eric Lagasse showed in vivo differentiation of purified HSCs into hepatocytes in a mouse model of a lethal hereditary liver disease. As few as 50 adult HSCs injected intravenously had the capacity to reconstitute hematopoiesis and produce hepatocytes [15].

2.2.2.2 Mesenchymal Stem Cells

MSCs comprised of the major portion of adult stem cells were first identified by Friedenstein from adult bone marrow [16]. These MSCs were shown to differentiate into osteoblasts, chondrocytes, adipocytes, and hematopoietic supporting stroma when a single colony-forming unit-fibroblast (CFU-F) was transplanted in vivo [17].

Fig. 2.4 Production of platelet forming MK cells from HSCs: Schematic representation of step-by-step differentiation of HSCs into MK progenitor, mature MK, polyploid MK, and proplatelet forming MK under a series of cytokine cocktails with increasing pH and pO_2

MSCs can easily be cultured on petri dishes, and their lineages are determined by specific cell markers or enzyme assays. Researchers have shown that MSC can differentiate toward adipocytes or osteocytes in vitro when cultured in adipogenic or osteogenic induction media. During adipogenic induction, the differentiated adipocyte cells stain oil red O-positive indicating a lipid-laden adipocyte phenotype. Similarly, differentiated osteocytes show calcification when stained with alizarin red for calcium deposits [18]. However, determination of the lineage identity in vivo is quite challenging and active research is going on to identify lineage/differentiation-specific biomarkers. A tabulated form of lineage-specific differentiation of MSCs is shown in Fig. 2.5. MSCs can be isolated during routine surgical procedures such as tooth extraction, baby delivery (from placenta and cord blood), or through some special isolation techniques from adipose tissues or bone marrow. These cells can be directed toward lineage-specific differentiation even toward bone using synthetic or natural scaffolds to attain the proper three-dimensional structures. A wide variety of natural and synthetic materials are being tested as scaffolds for bone regeneration. Natural proteins such as collagen [19–21], fibrin [22], silk [23–25], and polysaccharides such as hyaluronic acid and chitosan [26–29] are optimal choices as bone scaffolds. These materials have the advantages of biocompatibility and biodegradability with limited toxicity and may be molded to maintain mechanical flexibility of human bones [22]. Recently, Hassani et al. showed the potential of human endometrial stem cells (EnSCs) to form urinary bladder epithelial cells (urothelium) on nanofibrous silk–collagen scaffolds for construction of the urinary bladder wall [30].

A major thrust area for stem cell therapy is acute myocardial infarction where disruptions of blood supply to the heart muscle cells lead to myocardial infarction or death of cardiomyocytes. Attempts to use stem cells to reduce infarct size and enhance cardiac function in animal models and patients have been exponentially increased in the last decade [31]. Bone marrow and fat tissues serve as the major source of MSCs for cardiovascular disease [32]. Differentiation of mouse BM-MSC into myogenic lineage in vitro has been reported using culture medium supplemented with 5-azacytadine at a concentration of 3 μmol/L for 24 h [33]. The purified hMSCs from adult bone marrow engrafted in the myocardium appeared to differentiate into cardiomyocytes. The persistence of the engrafted hMSCs and their in situ differentiation in the small animal models paved the way to use these adult stem cells for human cellular cardiomyoplasty [34].

The enormous potential of various adult stem cells in curing diverse diseases encouraged researchers to explore their potential systematically either in vitro or

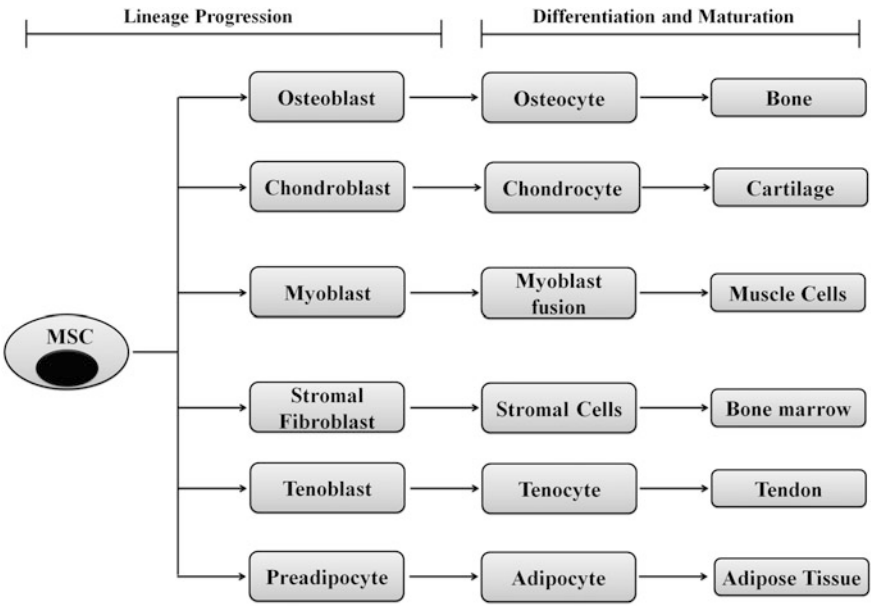

Fig. 2.5 Schematic representation showing differentiation of MSCs to various lineages: MSCs are like gold mines for the regenerative medicine since they could be directed toward any of the cell types through commitment, lineage progression, differentiation, and maturation. A single MSC can differentiate into bone, cartilage, muscle, bone marrow, tendon/ligament, adipose tissue, and connective tissue using appropriate developmental cues

in vivo condition. Some of these examples on MSC-based applications are mentioned in Table 2.1.

2.2.3 Induced Pluripotent Stem Cell

While stem cell therapy is emerging as a promising alternative for diseases and genetic disorders where drugs or gene therapy fail, it is limited by availability and stringent culture conditions. Exploiting epigenetic influence on phenotypic outcome, researchers have developed powerful genetic platforms for reversal of differentiated adult cells back to an embryonic state. Such reprogrammed cells are known as "induced pluripotent stem cells (iPSCs)," and the reprogramming strategies include "**therapeutic cloning**" and "**nuclear reprogramming.**" Both these strategies act through ectopic introduction of a small number of pluripotency-associated transcription factors into differentiated tissue-specific cells. iPSCs have the ability to differentiate into any of the three germ layers, ectoderm, mesoderm, and endoderm and the respective lineage-specific fully differentiated and functional cells/tissues.

Table 2.1 In vitro differentiation of adult stem cells to their respective lineages using small molecules, appropriate scaffold stiffness, cocktail of drugs and culture media

Cell type	Culture condition	Lineage	Reference
hESC	Ascorbic acid	Cardiac myocytes	[35]
BM-MSCs	Ascorbic acid, BMP2, dexamethasone, TGF-beta, and insulin	Osteogenic	[36]
	DMEM/10 % FBS + 0.5 μM dexamethasone, 0.5 mM isobutyl-l-methylxanthine, and 10 μg/ml insulin	Adipogenic	[37]
	DMEM/10 % FBS, 100 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μM ascorbic acid	Chondrogenic	[37]
	Laminin-1 without serum and differentiation growth factors, valproic acid, insulin, and butylated hydroxyanisole	Neurogenic	[36, 38]
Cardiac MSC	5 % FBS in 5 mM all-trans retinoic acid, 5 mM phenyl butyrate, and 200 mM diethylenetriamine/nitric oxide	Myogenic	[36, 39]
	Transferrin, IL3, IL6, and VEGF		
MSC	1. Osteogenic differentiation on stiffness (45–49 kPa)	Osteogenic	[40]
	2. Myogenic differentiation (13–17 kPa)	Myogenic	
Limbal stem cell	Human amniotic membrane + human corneal epithelial cell medium + autologous serum	Limbal epithelium	[41]

The concept of iPSCs demonstrated long back by Sir John Gurdon when he successfully cloned a frog using intact nuclei from intestinal epithelium cells of [42]. Later, he showed that even nuclei from terminally differentiated adult cells (e.g., blood cells, skeletal muscle, and kidney cells) could generate Xenopus larvae with nuclear transfer [42]. Decades later, in 2006, Takahashi et al. demonstrated the ability of adult mouse fibroblasts to reprogram themselves into pluripotent stem cells by introduction of four key transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) [43]. In November 2007, two independent studies were published simultaneously on successful transformation of differentiated human cells into pluripotent stem cells. While Takahashi et al. used retroviral delivery Oct3/4, Sox2, Klf4, and c-Myc combinations to induce pluripotency in human fibroblasts, Yu et al. delivered Oct4, Nanog, Sox2, and LIN28 by lentiviral transduction in hESC-derived mesenchymal cells to induce pluripotency [44, 45]. These groundbreaking experiments by Sir Gurdon and Yamanaka and his group were acknowledged by Nobel Prize award in 2010.

The most exciting and oversimplified part of iPSC generation is that a combination of only four transcription factors is able to reverse the differentiation process. To identify this main core of pluripotent factors, Yamanaka et al. (2006) evaluated twenty-four candidate genes and Thomson et al. (2007) screened sixteen transcription factors in an assay system in which the induction of the pluripotent state could be detected through the development of resistance to neomycin gene.

Both the groups identified Oct4, Sox2, and Nanog as the major pluripotency determinants. Though promising, the current iPSC reprogramming method experiences certain drawbacks such as

1. Requires host cell to be genetically engineered to express a drug resistance gene driven by a marker of pluripotency.
2. Requires viral-mediated integration of transgenes into the genome.
3. Reactivation of c-Myc in differentiated progeny of the induced ES-like cells is common and may result in tumor formation [46].

Thus, alternative approaches such as use of purified transcription factors, replacement of c-Myc, and strategy to avoid drug resistance selection method are being explored [47].

Their et al. [48] reported generation of TAT-modified cell permeate versions of recombinant Oct4 and Sox2 proteins (Oct4 TAT and Sox2 TAT), and later, Zhou et al. generated protein-piPSCs from murine embryonic fibroblasts. The deleterious effects of c-Myc could be circumvented by using *n-myc*, and host cell need not be drug resistant if using serum-free condition for iPSCs generation [49]. Recently, miRNA particularly the miR302/367 cluster was used to generate iPSCs from mouse and human somatic cells without adding the exogenous transcription factors. This miRNA-based reprogramming was found to be more efficient (twofold) than the standard Oct4/Sox2/Klf4/Myc-mediated reprogramming and ultimately overcome the deleterious effect of c-Myc reactivation [50].

Using the above-mentioned methods, one can now generate individual-specific iPS cell lines to derive patient-specific progenitor cells and eliminate immune rejection crisis. Moreover, iPSC-based technology will facilitate the production of cell line panels that closely reflect the genetic diversity of a population enabling the discovery, development, and validation of therapies tailored for each individual. Till today, iPSCs has been generated from ten different species mouse, human, rhesus monkey, rat, dog, rabbit, horse, and bird [43, 44, 51–58] and into various lineages as listed in Table 2.2.

However, it still would be a long way for iPSCs to reach the clinic, which requires stringent and systematic validation of lineage-specific differentiation.

2.3 Monitoring of Stem Cells

Success of regenerative medicine and stem cell therapy depends on efficient in vivo differentiation of stem cells into specific lineages. Monitoring of engineered stem cells in cell cultures and in vivo before and after transplantation is a prerequisite for any stem cell application. It is also necessary to perform such studies directly in living subjects in a longitudinal, reliable, and accurate manner. Various microscopic techniques are extensively used for detail visualization of growth, differentiation, and functional validation of stem cells and iPSCs in cultures. Some of these techniques are also utilized for monitoring of the stem cells in

Table 2.2 In vitro differentiations of species-specific iPSCs toward various lineages in their respective culture condition

iPSCs	Culture condition	Lineage	Species	Reference
Murine	Valproic acid, zonisamide, and estradiol	Neural	Rat model of ALS	[59]
	ES medium without LIF for the first 4 days and day 5 onwards in differentiating medium supplemented with RPE-conditioned medium	RPE	Mouse	[60]
Human	Serum-free embryoid-body-like aggregates	Dopaminergic neurons	Mouse	[61]
	Keratinocyte growth factor and fibroblast growth factor	Hepatocyte-like cell	Human	[62, 63]
	Embryoid bodies	Erythropoietin	Human	[64]
	EGF and bFGF	Oligodendrocyte progenitors	Rat model	[65]
Porcine	Activin A, bFGF, BMP-4, and oncostatin	Hepatic	Porcine	[66]

living subjects with high resolution. In parallel, macroscopic or noninvasive in vivo imaging modalities turn out to be indispensible for longitudinal monitoring of translational applications. Commonly used noninvasive imaging modalities for stem cell therapy are radioisotopic imaging (PET or positron emission tomography and SPECT or single-photon emission computed tomography), CT, ultrasound, magnetic resonance imaging (MRI), and optical imaging (bioluminescence and fluorescence). The next two sections will elaborate microscopic and macroscopic imaging of stem cells, an essential requirement for clinical application (Fig. 2.6).

2.3.1 Microscopic Techniques

For centuries, microscopy is an indispensable tool for visualizing dynamics of biomolecules in live cells. Optical microscopic techniques including conventional light (phase contrast) microscopy, fluorescence microscopy, confocal and multi-photon microscopy, intravital microscopy (IVM) have emerged as powerful tools for noninvasive monitoring and characterization of engineered stem cells and tissues [67].

2.3.1.1 Phase Contrast Microscopy

The age-old phase contrast microscopy is routinely required to monitor the culture conditions and kinetics of HSCs during their expansion for therapeutic use. Recently, these microscopes were improved with automated time-lapse system to

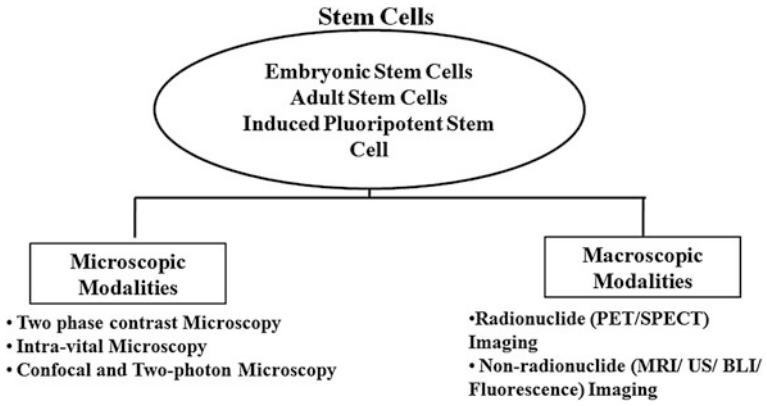

Fig. 2.6 Imaging modalities for in vitro and in vivo monitoring of stem cells: Naive or engineered stem cells in in vitro culture or transplanted in living subjects can be visualized noninvasively either by microscopic or by macroscopic imaging techniques. Microscopic modalities include phase contrast microscopy, intravital microscopy, confocal and two-photon microscopy, whereas macroscopic modalities include radionuclide-based (positron emission tomography or PET and single-photon emission computed tomography or SPECT) and nonradionuclide-based (MRI, ultrasonography imaging, bioluminescence, and fluorescence) imaging

capture the mitotic divisions of stem cells such as multipotent C3H10T1/2 mesenchymal and C2C12 myoblastic stem cells in real time [68, 69].

2.3.1.2 Fluorescence Microscopy

The complexity of the biological samples can be unraveled by labeling specimen with a fluorophore to achieve single-cell resolution when monitored with fluorescent microscopes. Stem cell labeling for fluorescence microscopy includes DNA binding dyes (such as Hoechst dye, BrdU, DAPI), nanoparticles and quantum dots, the later ones are also suitable for in vivo imaging. These labeling methods can only be used for short-term cell tracking due to loss of fluorophores through cell division. For long-term monitoring, tracking, engraftment and differentiation of stem cells, genetic manipulation with fluorescent proteins of different excitation and emission spectra (GFP and RFP and their mutants) is an ideal approach. These proteins can also be coupled to another protein to act as molecular reporters in living cells [70].

Tumbar et al. [71] developed a new strategy to identify multipotent epithelial stem cells (ESC) in their native environment by fluorescent labeling. These quiescent cells residing in the bulge of hair follicles can differentiate into various cell types upon stimulation. Transgenic mice expressing a H2B-GFP fusion protein under tetracycline-regulated keratinocyte-specific K5 promoter showed specific GFP expression only in rapidly dividing skin epithelium. Administration of doxycycline led to the loss of GFP expression in the keratinocytes but not in the

SCs due to their slow cycling and quiescent nature. The fluorescence microscopy of the sections from the keratinocytes clearly demonstrated the label-retaining capability of SC niche. This method was later used by many research groups to isolate and purify the label-retaining cells (LRCs) or SCs for further characterization [70, 71].

Fluorescent probes with emission wavelengths in the near-infrared (NIR) spectra ($\sim$700–800 nm) enhance the feasibility of tracking cells in vitro and in small animal models due to high depth penetration and lower absorption and scattering [72]. Several dyes including NIR fluorochrome DiD, a lipophilic dye that binds to the cell membrane, have proven effective for both in vitro labeling of MSCs and in vivo cell tracking with optical imaging [73, 74].

As described in Sect. 2.2.3, direct reprogramming of somatic cells into iPSCs can be achieved by overexpression of four reprogramming factors (RFs). A dynamic fluorescence microscopy study of iPSCs expressing Nanog-GFP suggests that the number of cell divisions is a key parameter of driving epigenetic reprogramming to pluripotency [75]. Similarly, fluorescence microscopy combined with long-term time-lapse imaging and single-cell tracking revealed the "birth" of pluripotent cells and early iPSC clusters from murine embryonic fibroblasts transduced with multicistronic lentiviral vectors carrying RFs (Klf4, Sox2, Oct4, Myc) tagged with different fluorescence proteins (GFP, RFP, YFP) [76].

2.3.1.3 Confocal Microscopy and Multiphoton Microscopy

A major drawback of fluorescence microscopy is that irrespective of the vertical focusing of the specimen, illumination causes the entire specimen to fluoresce and is unable to produce tomographic images. Confocal microscopy, multiphoton microscopy, and intravital microscopy are competent of generating tomographic imaging essential for localizing fluorescent targets in three-dimensional space [77–79].

In a recent study, transgenic ES cells co-expressing myristoylated RFP (labels plasma membrane) and histone H2B-GFP (labels active chromatin) fusions were introduced into a nontransgenic embryo and then dissected out of the maternal uterus at mid-gestation period and cultured ex utero on the stage of a confocal microscope. These labeled ES cells produced information on dynamic changes in morphology and chromatin distribution that occurred during mitotic progression [80]. The powerful confocal/two-photon hybrid microscopy can also track the clonal history of HSPCs expressing various fluorescence proteins noninvasively in intact tissues, including bone marrow with long-term monitoring after transplantation [81].

2.3.1.4 Intravital Microscopy

Intravital microscope (IVM) can be referred as "microscope for living subjects," which enables single-cell imaging in thin sections of live tissues [82]. In a remarkable study, Rompolas et al. [83] monitored the regeneration of hair follicles temporarily in a transgenic mouse expressing H2B-GFP driven by keratin 14 promoter. They demonstrated that stem cells are quiescent during initial stages of hair regeneration and their progeny is actively dividing within follicular organization [83].

In another elegant study, Takayama et al. [84, 85] imaged the functioning of human iPSC-derived platelets during thrombus formation by intravital microscopy in live mice. The study demonstrated that transient expression of c-Myc was critical for efficient platelet generation from human iPSCs, which were capable of mediating hemostasis and thrombosis in a laser-induced vessel wall injury.

2.3.2 Macroscopic Imaging Modalities

Though microscopic imaging techniques can generate critical information, they are restricted at cellular level and do not depict the kinetics, distribution, and location of in vivo differentiation of stem cells in a living subject. Even IVM requires an artificially created "window chamber" or "tissue flap" at the body surface and is not applicable for deep tissue imaging. Recent developments in macroscopic or in vivo imaging modalities enable the "visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems." Along with other applications, these modalities are now widely used for imaging stem cell delivery, migration and localization, cell viability, and therapeutic effects in various diseases [86–88].

The Six commonly used modalities for imaging stem cell therapy are ultrasound, CT, SPECT, PET, MRI, and optical. For many instances, multimodality approaches that can collectively assess different parameters specific for each modality are gaining popularity. For imaging the stem cells in vivo, it is required to label the cells with an appropriate probe. The two important methods for labeling cells are as follows:

(a) Direct labeling such as with magnetic resonance contrast agents, radionuclides, fluorophores, and nanoparticles.
(b) Indirect labeling with reporter genes.

Direct labeling of cells poses limitation to long-term monitoring since the signal gets diminished by dilution or loss of labeling agents via cell division or differentiation. This limitation can be overcome with "indirect-labeling strategy" where cells are exogenously labeled with reporter genes (bioluminescence, fluorescence, PET, SPECT, or MR reporters) and then implanted for imaging. Indirect labeling requires genetic manipulation of the cells that are often not possible to follow in

Table 2.3 Stem cell therapy studies using PET imaging approach in human

Stem cells	Radionuclides	Applications	Reference
HSCs	^{18}F-FDG	Homing and tissue distribution of intracoronary injected peripheral HSCs	[106]
MSCs	^{18}F-FDG	Functional efficacy of MSC therapy in patients with multiple system atrophy	[107]
Cord blood stem cells (CBSC)	^{18}F-FDG	CBSC transplant in patients for local engraftment and reconstitution of haematopoiesis	[108]
CD33 + bone marrow stem cells	^{13}N-ammonia and ^{18}F-FDG	Improvement in the myocardial perfusion in patients AMI	[109]
HSCs	^{18}F-FDG	HSC therapy for osteosarcoma	[110]

clinics [86–90]. However, both the strategies have own advantages and disadvantages and should be implemented by experimental need.

2.3.2.1 Radionuclide Imaging

Majority of the radionuclide imaging techniques follow direct-labeling strategies and are extensively used in human studies. Both radionuclide imaging techniques, i.e., PET and SPECT, have picomolar sensitivity and high tissue penetration with least attenuation and are tomographic in nature.

Positron Emission Tomography (PET)

In PET, two 180° apart high-energy (511 keV) gamma rays produced by the annihilation of a positron (from the radioactive atom) with a neighboring tissue electron are captured by detectors and produce a three-dimensional image of functional processes in living subjects. The commonly used positron-emitting isotopes are ^{11}C, ^{13}N, ^{15}O, ^{124}I, ^{64}Cu, and ^{18}F. The widely used PET tracers in clinic are 2-deoxy-2-^{18}F fluoro-D-glucose (^{18}F-FDG, which images glucose metabolism) and 3-deoxy-3-^{18}F fluorothymidine (^{18}F-FLT, which images cell proliferation). Several studies have elaborated the use of PET imaging approach for the stem cells therapy in various human disorders [91–100] (Table 2.3). Some studies have also used small animal models for studying the role of human stem cells in homing, engraftment, and survival through PET imaging [101–105].

Herpes simplex virus type 1 thymidine kinase (HSV-tk) is the most widely used reporter gene for PET imaging for preclinical as well as clinical studies [90]. ^{18}Fluorine-labeled FIAU (2′-fluoro-2′-deoxy-β-D-arabinofuranosyl-5-iodouracil) and FHBG (9-(4-fluoro-3-hydroxy-methyl-butyl) guanine) are the two common reporter probes used for HSV1-tk. This reporter gene–reporter probe approach has been used to monitor viability of stem cells after transplantation in myocardium,

tracking, and survival of autologous MSCs in pig myocardium and tumor stroma [111, 112].

Recently, human sodium iodide symporter (hNIS) gene has emerged as an important PET reporter gene that could be used for SPECT imaging as well. Uptake of ^{124}Iodine was seen in areas deficit of myocardial perfusion in rat myocardium injected with MSCs expressing hNIS gene by PET imaging [113]. NIS-mediated ^{124}I PET imaging was also used to monitor the delivery and survival of endothelial progenitor cells (EPCs) after transplantation into the rat heart [114]. Some other promising PET reporter genes for stem cell therapy are dopamine 2-like receptor (D2R), human somatostatin receptor subtype 2 (hSSTr2), human norepinephrine transporter (hNET), neurotensin receptors, and cytosine deaminase [115].

Stem cell therapy is often benefited from multimodality imaging approaches. In an elegant study, Cao et al. [116] monitored the survival, proliferation, and migration of ESCs expressing a triple fusion (TF) reporter comprised of a fluorescence (mrfp), a bioluminescence (fluc), and a PET reporter (ttk) gene after transplantation into rat myocardium for 40 days (Fig. 2.7). Some studies have used bifusion reporters such as tk-GFP to monitor neuronal stem cell, therapy in treatment of malignant glioma [117].

Single-Photon Emission Computed Tomography (SPECT)

In contrast to PET imaging, SPECT imaging is a log-order less sensitive technique due to the presence of collimators (to restrict detection of nonspecific random gamma rays) between the detectors. The widely used SPECT radionuclides are ^{99m}Tc, ^{111}In, and ^{123}I. SPECT imaging probes are extensively applied to image in vivo trafficking and biodistribution of MSCs in myocardial injuries in large animal models [118–120]. Some human clinical studies have also used ^{111}In-oxine to track bone marrow stem cells or pro-angiogenic progenitor cells in acute and chronic myocardial injuries. Goussetis et al. [121] performed SPECT imaging with ^{99m}Tc hexamethylpropyleneamine-oxime-labeled autologous CD133^{-}CD34^{+} bone marrow progenitor cells transplanted in patients with ischemic cardiomyopathy. Higher uptake of radioactivity was observed in the infracted area of the heart, suggesting preferential migration and retention of stem cells in the chronic ischemic myocardium.

Both HSV1-tk and hNIS genes can serve as SPECT reporter genes in combination with probes labeled with SPECT isotopes (^{123}I/^{125}I-labeled probes FIAU or ^{99m}Tc) [113, 122]. Table 2.4 summarizes various studies involved in stem cell visualization by SPECT in human. Studies related to small animal models can be found in other articles [123–125].

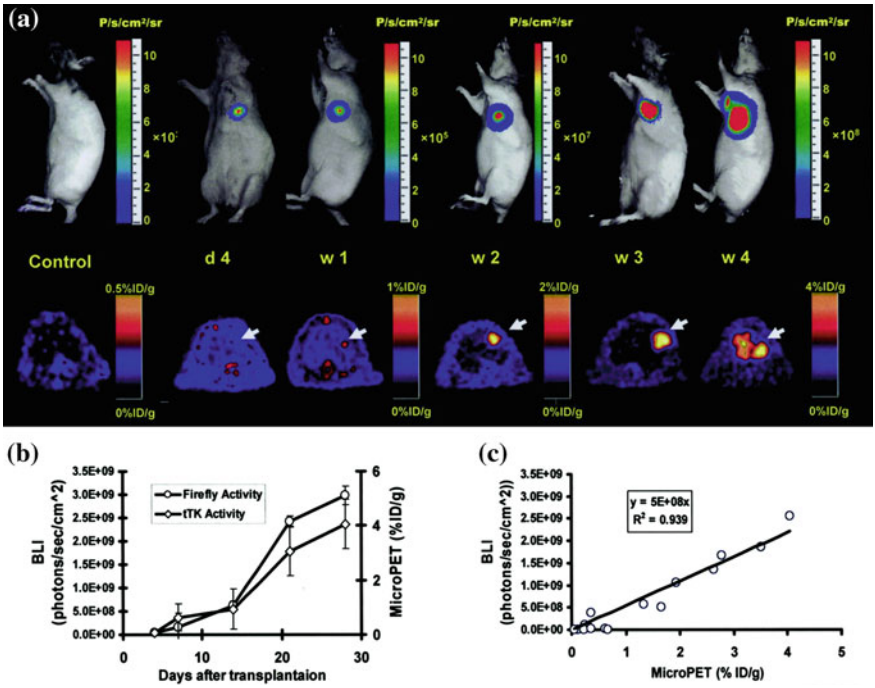

Fig. 2.7 Multimodality imaging for long-term monitoring of mouse embryonic stem cells expressing a triple fusion reporter (ES-TF): **a** Representative images of animal injected with ES-TF in infracted rat heart showed significant increase in bioluminescence (*top*) and PET (*bottom*) signals from day 4, week 1, week 2, week 3, and week 4. **b** Quantification of imaging signals showed a drastic increase in luciferase and thymidine kinase activities from week 2 to week 4. **c** Quantification of cell signals showed a robust in vivo correlation between bioluminescence and PET imaging ($r^2 = 0.92$). (Reprinted from Cao et al. [116] with permission)

2.3.2.2 Optical Imaging

Optical imaging has emerged as an established tool to assess efficacy and treatment outcomes for cell-based therapeutics in preclinical models. Two optical imaging methods, bioluminescence, and fluorescence are extensively used in stem cell research.

Fluorescence Imaging (FLI)

Fluorescence imaging is the only modality that directly translates the finding/observation from live cell to live animals. However, due to inherent autofluorescence and signal attenuation issues, whole-body FLI has not been extensively used for stem cell therapy. Tzukerman et al. [133] applied fluorescence imaging to demonstrate the growth and invasiveness of cancer cells in the niche of teratomas

Table 2.4 Stem cell therapy studies using SPECT imaging approach in human

Stem cell types	Radionuclides	Applications	Reference
MSCs	^{111}In-tropolone	Delivery, tracking, and differentiation of stem cells	[126]
BM-MSCs	^{111}In-oxide	Monitoring blood flow and bone metabolism after cell transplantation	[127]
CD34^{+} stem cells	^{99m}Tc hexamethylpropyleneamine oxime	5-year follow-up of CD34^{+} stem cell in nonischemic cardiomyopathy patients	[128]
MSCs	^{99m}Tc-pertechnetate	Demonstrate the feasibility of MSCs as virus carriers to ovarian tumors in phase I clinical trails	[129]
BM-MSCs	^{99m}Tc-sestamibi	Improvement in cardiac function after BMMSCs transplantation in patients with acute myocardial infarction	[130]
Autologous CD34^{+} cells	^{99m}Tc-exametazime	Homing of transplanted cells to injured myocardium	[131]
BM-HSCs	^{99m}Tc	Repair efficacy for ischemic heart	[132]

derived from hESCs. Tao et al. [134] reported that e-GFP is a better probe than DS-Red protein for long-term monitoring of HSCs.

Other than reporter gene strategy, FLI can be performed with exogenous contrast agents such as quantum dots, nanoparticles, and fluorescent dyes that emit light in the visible, red, and near-infrared region. Inorganic fluorescent semiconductor nanocrystals (quantum dots, QDs) are rapidly replacing organic fluorophores like indocyanine green with NIR emission due to their ability for multiplex imaging [135]. Lin et al. [136] for the first time demonstrated the in vivo multiplex imaging of mouse ES cells labeled with six quantum dots (QDs). The six emission spectra from all the six QDs were recorded with a single excitation light source. These QDs are emerging as a promising tool for tracking stem cells within deep tissues noninvasively in vivo. Human ESC-derived cardiomyocytes (hESCs-CM) were also labeled with indocyanine green for noninvasive tracking by fluorescent imaging [137].

Bioluminescence Imaging (BLI)

Among all the functional imaging modalities, BLI acts only through reporter gene–reporter probe strategy and has been extensively used for monitoring stem cell therapy. Though restricted to small animals, bioluminescence imaging

generates essential clues on differentiation, behavior, and viability of stem cells after transplanted in small animals, assisting to predict the behavior of stem cell therapy in humans. A glimpse of the large number of BLI-based studies is summarized in Table 2.5, and a few important studies are discussed.

Tsuji et al. [138], using iPSC-derived neurospheres in a mouse model of spinal cord injury, demonstrated that iPSCs can "safely" promote locomotor function recovery in injured mouse models with BLI. They observed that these cells can even differentiate into trilineage neural cells in the injured spinal cord. In another study, Daadi et al. [139] investigated the efficacy of human neural stem cells (hNSCs) derived from human ES cells to repair brain injury. In this study, rats with neonatal HI (hypoxic-ischemic) brain injury were implanted with hNSCs expressing luciferase and their survival was monitored using BLI. The study suggests that hNSCs transplants are able to enhance brain injury repair in response to HI brain injury and that the location and survival can be monitored noninvasively. Further, the deleterious effect of ESC differentiation on teratomas was spatially and temporally monitored by Cao et al. [116] with high sensitivity, which was not possible with other imaging modalities (Fig. 2.7).

2.3.2.3 Magnetic Resonance Imaging

MRI is the sole imaging modality that generates both functional information and anatomical information. Among all the other noninvasive imaging techniques, MRI has the highest spatial resolution (~ 100 μm) and thus is the most preferred strategy for stem cell imaging. Since endogenous molecules (such as H_2 atoms) do not generate enough contrast to achieve that high resolution, supra-paramagnetic iron oxide (SPIOs) and paramagnetic nanoparticles are often used to label the cells to enhance image contrast. However, SPIO-based MRI is not well suited for long-term monitoring since SPIOs get diluted with cell proliferation and are often engulfed by macrophages [86]. Chelated gadolinium (Gd^{3+}), manganese (Mn^{2+}), and iron (Fe^{3+}) could also act as contrast agents. Recently, certain metal-ion-based enzymes namely metalloproteinase, transferrin, ferritin, tyrosinase are being evaluated as reporter genes in MR imaging [87].

In contrast to optical imaging where smaller animals such as mouse and rat are preferred as model systems, MR-based stem cell imaging is tested both in smaller and larger animals and in humans. The first autologous transplantation of iron oxide-labeled iPSCs reprogrammed from canine adipose stromal cells and fibroblasts showed repair of infracted myocardium and hindlimb ischemia by MR imaging in adult mongrel dogs [158]. Similarly, an enhanced effect of combining human cardiac stem cells and bone marrow MSCs to reduce infarct size and restore cardiac function after myocardial infarction was followed by MR imaging in a Yorkshire swine model [159].

To overcome the shortcoming of the contrast agents, reporter-gene-based strategies are also being employed in stem cell imaging by MR. Liu et al. [160] showed that engraftment of transgenic mouse ESCs expressing human ferritin

Table 2.5 Studies involving BLI approach for stem cell therapy

Stem cell types	Origin	Applications	Reference
HSCs	Human	Longitudinal monitoring of human HSC engraftment	[140]
Neural progenitor cells	Murine	Migratory capability of NPCs and their preferential accumulation in brain tumors on CNS	[141]
Embryonic rat H9C2 cardiomyoblast	Rat	Location, magnitude, and survival duration of embryonic cardiomyoblast	[142]
ESC-derived insulin-producing cells (IPCs)	Murine	Novel source of unlimited cells for transplantation to treat type 1	[143]
ESCs	Murine	Longitudinal monitoring and tumorigenic potential of ESCs	[144]
	Human	Longitudinal monitoring of differentiation in the tumors	[145]
	Human	Preferential differentiation of hESC-derived $CD34^{+}$ cells into endothelial cells	[146]
	Mouse	Longitudinal monitoring of implanted ESCs in rat corpus cavernosum	[147]
	Human	Serial imaging of human embryonic stem cell engraftment and teratoma formation in murine model	[122]
Neural stem cells	Mouse	Improved engraftment of neural stem cells	[148]
MSCs	Mouse	Migration and engraftment of transplanted cell into primary breast tumor sites	[149]
		Monitoring survival of transplanted MSCs injected intramyocardially	[150]
		Homing to kidneys in mice with ischemia- and reperfusion-induced acute kidney injury (AKI)	[151]
MSCs expressing bone morphogenetic protein 2 (BMP2)	Human	Bone and cartilage repair in articular fractures	[152]
Umbilical cord blood HSCs	Human	Cell engraftment of HSCs after bone marrow transplantation in nonobese diabetic/SCID mice	[153]
BM-MSCs	Human	Monitoring inhibition and eradication of glioma with BM-MSCs labeled with Delta-24-RGD	[154]
MSCs	Murine	Localization, survival, proliferation, and differentiation of MSCs to osteoblasts and adipocytes	[155]
iPSCs	Human and Murine	Long-term tracking of iPSCs in the gastrocnemius muscle of recipient mice monitored	[156]
ESCs-derived cardiomyocytes (CM)	Murine	Tracking of immature (ESCs-CM) demonstrate longer survival than the mature CM	[157]

heavy chain (FTH) resulted in increased cellular iron uptake and MRI contrast and did not interfere with stem cell pluripotency, neural differentiation, and teratoma formation.

Stem cell therapy has immense potential to treat neurodegenerative diseases, traumatic injury, and stroke. However, risk is associated with intracranial surgery used to deliver the cells to the brain. In some studies, MRI was combined with ultrasound modality to obtain higher sensitivity and resolution. For targeted delivery of neural stem cells to brain, Burgess et al. [161] employed MRI-guided focused ultrasound (MRIgFUS) imaging to monitor noninvasive delivery of stem cells from the blood to the brain by opening the blood–brain barrier at specific regions (striatum and hippocampus) in rat brain. Entry of cells crossing the BBB to brain was verified by MRI. The study also demonstrated that these stem cells started expressing double cortin, a marker of immature neurons, indicating occurrence of in vivo differentiation. An excellent review by Qiu et al. [162] described many such MRI-based studies for stem cell therapy such as migration and homing of hematopoietic stem–progenitor cells to injured arteries and atherosclerosis, stem–progenitor-cell-mediated vascular gene therapy, and several novel techniques for magnetic labeling of stem or progenitor cells. Table 2.6 describes some of the MRI-based stem cell tracking studies in living subjects.

2.3.2.4 Ultrasound Imaging

Ultrasound imaging (US) utilizes the interaction of sound waves with living tissue to produce an anatomical image. Since US imaging is the only modality that generates real-time images during scanning, several investigators are using this technology for longitudinal monitoring of stem-cell-mediated tissue repair and vessel formation. One such application was shown by Watts et al. [173] where equine fetal-derived embryonic-like stem cells (fdESCs) expressing Oct 4, Nanog, SSEA-4, TRA-1-60, TRA-1-81 stem cell markers and telomerase were implanted in equine flexor tendonitis model through intralesional injection. Thoroughbred horses ($n = 8$) were induced with tendon injury in the mid-metacarpal region of the superficial digital flexor tendon and injected with fdESCs, and serial ultrasound examinations were performed. After 8 weeks, significant improvement in tissue architecture, tendon size, tendon lesion size, and tendon linear fiber pattern was found by US imaging, which was further corroborated by tissue histology.

To enhance the contrast of signal intensity of US imaging, microbubbles (MBs) tagged with nanoparticles, antibodies, or other signatures are being developed. Such an approach was demonstrated by Leng et al. [174] where biocompatible polymer MBs were internalized by human bone-marrow-derived MSCs (MB-MSCs) and used for US imaging. Nude mice injected with MSCs and MB-MSCs in the hindlimb region were temporally imaged by ultrasound, which showed that the MB-MSCs are acoustically active in vivo and can be imaged for at least 4 h from the time of injection [174].

Table 2.6 MRI-based stem cell tracking studies in living subjects

Stem cells	Contrast agents (CAs)/reporter genes	Applications	Reference
hESCs	SPIO MnC_{12}	Comparison between two CAs to monitor stem cell therapy for failing heart	[163]
hESCs	SPIO	Long-term monitoring of transplanted cells in mouse myocardial infraction model	[164]
hESC-derived neural stem cells	SPIO	Long-term monitoring of differentiation in rat brain injury model	[165]
Rat MSCs	Gadolinium–diethylene triamine penta-acetic acid (Gd-DTPA)	Transplanted cells are used to track spinal cord injury	[166]
ESCs/MSCs	Iron oxide nanoparticles	Evaluation of migration and fate of transplanted cells in rat central nervous system (CNS)	[167]
Pig cardiospheres (Cs): clusters of cardiac stem cells	Ferritin	In vivo tracking of stem cells in rat model of myocardial infarction	[168]
BM-MSCs	Gd-DTPA	Improvement in cardiac function in swine myocardial infarct model	[169]
Canine adipose-derived MSCs	FeO	Engraftment and migration of MSCs	[170]
Canine-MSCs	SPIO	Tracking of MSCs delivered intraarterially	[171]
Swine BM-derived stem progenitor cells	0.032-inch MR imaging-guidewire (MRIG)	Monitoring cell-based arterial repair	[172]

2.3.2.5 Computed Tomography

X-ray CT is a purely anatomical imaging modality, which generates high-resolution three-dimensional anatomical images. CT imaging, however, can be applied to monitor degree of differentiation of embryonic or adult stem cells. Arpornmaeklong et al. [175] demonstrated that undifferentiated hESCs can be cultivated in osteogenic medium to increase the quantity of osteoblast-like cells (hESCs-OS). These hESCs-OS when transplanted in mice with calvarial defects showed limited mineralization of tissue in central region, margin of defect, and calvarial bone adjacent to the defect site by micro-CT imaging. Image analysis revealed that bone mineral density of the new bone in the cranial defect generated by the transplanted cells at passage 5 was significantly higher than that in the controls (without cell implantation). However, CT being purely anatomical imaging modality has limited use in measurement and monitoring of stem cell differentiation. Integrated PET-CT and SPECT-CT are better approaches for such evaluations.

2.4 Applications of Stem Cells

The previous two sections describe the advances in stem cell research and monitoring the outcome in live cells as well as in live animals. The most challenging phase is to get "biological solutions to biological diseases" with an aim to achieve success in curing patients. To estimate the extent of success, a large number of clinical trials are ongoing with stem cell transplantation in various pathological conditions.

In this section, we will provide an overview of these studies categorized by the disease types based on the information obtained from trials mostly initiated by the National Institute of Health (Figs. 2.8 and 2.9). Due to overwhelming number of animal studies, we have limited this section only to human trials. The animal studies can be found in other excellent reviews [176, 177].

2.4.1 Stem Cells and Neurodegenerative Diseases

Two percent of worldwide death is contributed by neurodegenerative diseases such as Alzheimer's and Parkinson's, and medical science is still unable to cure these diseases. Recent progress in stem cell science showed that functional neuronal replacement is possible, raising the hope for ultimate cure for these dreadful diseases. The idea of stem-cell-based therapy for neurodegenerative diseases is not new. The first neuronal transplantation was reported in 1890 by Thompson who made a very bold attempt to transplant cortical tissue from a cat into the brain of a dog [178]. This encouraged scientists to consider cell transplantation to treat deadly neurodegenerative diseases such as Parkinson's and Alzheimer's.

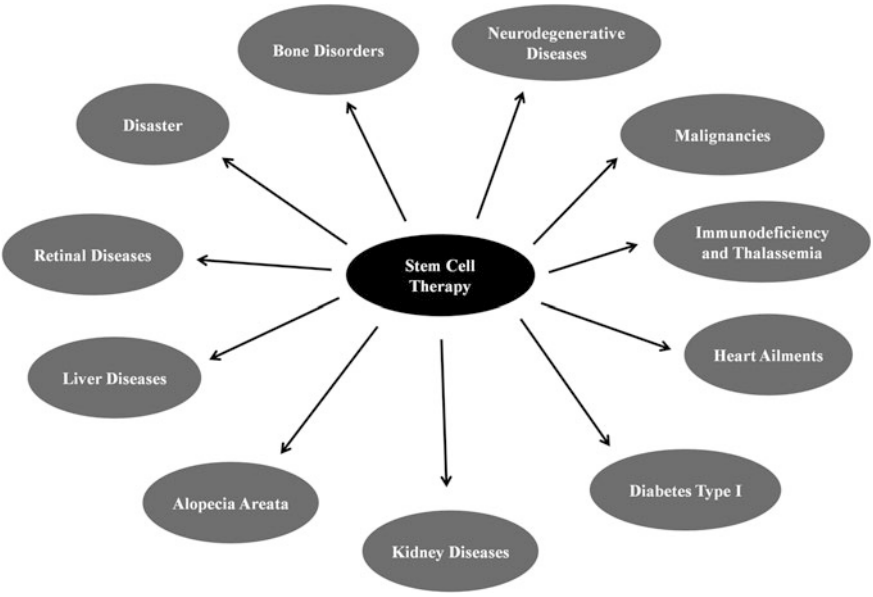

Fig. 2.8 Diseases treated with stem cells: Stem cells are emerging as an alternate or sole option for treating a wide spectrum of diseases such as neurodegenerative diseases, immune disorders, diabetes type 1, cardiac aliments, kidney disease, malignancies, liver disease, bone and retinal disorders. At certain times, health damages caused by disasters could also be managed with stem cell therapy

Earlier, the Parkinson's disease (PD) patients received a transplant of the adrenal medullary tissue, which did not result in significant improvement. Later, transplantation with fetal tissue and human embryonic neural tissue for cell-based therapy was also explored. However, serious ethical issues with these tissue replacements led to search for the alternate cell source. One such cell source was found to be the neural stem cells [179]. Clinical trials with transplantation of human fetal mesencephalic tissues rich in neuronal stem cells have produced satisfactory results. In a trial by Freed et al. [180], 40 patients between the age of 35–75 years were divided into transplantation group (mesencephalic tissue was implanted bilaterally into the putamen) and sham surgery group (tissue implanted by drilling a hole into the brain but not disturbing the dura mater) and received mesencephalic tissue transplantation. At the end of the surgery, younger patients in the transplantation group showed better signs of improvement as compared to the sham surgery, while significant improvement was seen in the group of the older patients. A small trial by Venketaramana et al. [181] generated a lot of hope for the PD patients. In this pilot study, seven advanced-stage PD patients between the age of 22 and 65 years were subjected to bone-marrow-derived MSC transplantation extracted from the iliac crest. Patients were reported to show improvement and a reduction in the levels of L-DOPA. Spinal muscular dystrophy is a dreadful

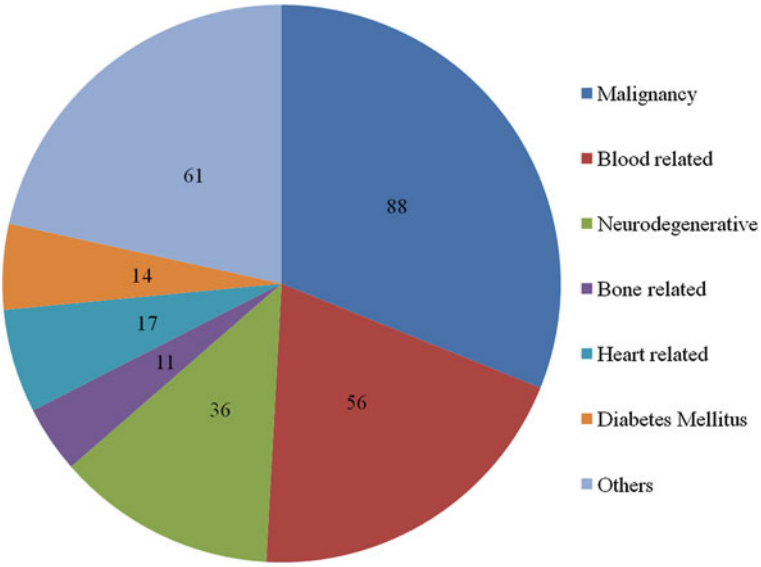

Fig. 2.9 Clinical trials with stem cells: A graphical representation of various disease-specific trials undertaken by US government around the world involving stem-cell-mediated therapy ($n = 283$). (*Source* Clinicaltrials.gov, accessed: 28/3/2013)

neurodegenerative disease characterized by the loss of motor neurons in the spinal cord leading to overall muscle weakness. Remarkable results were obtained with stem cells in animal models, but clinical trials are yet to be initiated [182].

Like PD, MSCs are also used as a powerful therapy regime against Huntington's disease as they can enhance tissue repair by secreting various neurotrophic factors leading to neural growth, inhibition of apoptosis, and regulation of inflammation. Bachoud-Lévi AC et al. (2006) have shown that 3 out of 5 patients showed some degree of improvement with fetal neural grafts, but the other two had no signs of benefit. This trial proved that such transplantations provide relief for a short period of time but offers no permanent cure [183]. Many of the clinical trials for Huntington's disease have shown mixed results. Gaura et al. [184] transplanted fetal striatal neuroblasts (progenitor cells derived from the neural stem cells) as allograft in five Huntington's disease patients. The patients were assessed based on the neural hypometabolism and glucose metabolism rate in the brain. While two patients showed drastic improvement, patient 3 actually showed a slight decline in condition. Patient 4 and patient 5 deteriorated during course of the experiment [184].

In amyotrophic lateral sclerosis (ALS), upper and lower motor neurons gradually degenerate and lead to muscular atrophy and severe weakness. In a clinical trial by Mazzini et al. [185, 186], autologous MSCs were transplanted into the spinal cord of seven ALS patients. In the follow-up of 2 years, no permanent clinical side effects or change in the spinal cord was reported as assessed by MRI.

A relatively slow linear decline of the forced vital capacity was seen in four out of seven patients, indicating that MSCs might have the ability to repair tissue damage and prolong the survival of the patients. A similar exercise was performed by Mazzini et al. [187] with 10 patients. The results obtained indicated that stem cell therapy could be extremely beneficial for ALS patients. Thus, stem cell therapy upon proper guidance and validation is finally bringing hope for patients with neurodegenerative diseases such as ALS, chronic spinal injuries, advanced PD [188].

2.4.2 Stem Cells and Kidney Diseases

Both human ES cells and allogenic/autologous HSC transplantations are used to correct kidney disorders in clinics. Trivedi et al. [189, 190] was the first group to report their clinical experience with 24 patients. In their experiment, they introduced unfractionated HSCs into the thymus and bone marrow before surgery and infused them peripherally after transplantation. The aim of the study was to design strategy to enhance tolerance to cadaver renal transplantation and thus prevent graft rejection. Patients were divided into two groups of which group-A received infusion of the concentrated marrow before and after surgery and group-B underwent direct transplantation. Since this procedure did not yield any graft rejection, the study continued, and by 2011, more than 1,000 transplantations were performed with further modifications.

2.4.3 Stem Cells in Immunodeficiency and Thalassemia

Life-threatening immunodeficiency can be induced either by drugs or by malfunction of immune system for which cell-based gene transfer is a good treatment option. Unfortunately, several such trials are reported with little benefit. Thrasher et al. [191, 192] reported a trial in which engineered $CD34^+$ bone marrow cells failed to produce any effect in the subjects. Gaspar et al. [192] treated 10 patients with autologous $CD34^+$HSPCs transduced with (gamma c) γc retroviral vectors. Follow-up of 80 months showed all patients to be alive with functional T cells, though mild pulmonary infections in most patients and development of acute T-cell lymphoblastic leukemia in one patient were seen. Another strikingly similar attempt was made by Hacein-Bey-Abina et al. [193] where $CD34^+$ cells isolated from five X-SCID patients were transduced with a retroviral vector expressing gamma c(γc) transgene and then transplanted back in patients. The follow-up data for four out of five patients were quite promising. Their T cells and the natural killer cells carrying the transduced gene appeared normal in number, in phenotype, and in proliferative response for at least 2 years after therapy.

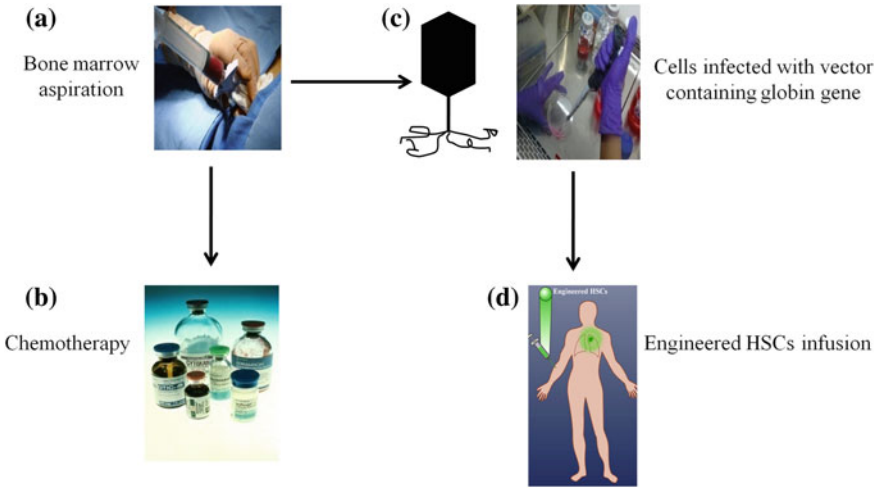

Fig. 2.10 Schematic representation of the trial conducted by Cavazzana-Calvo et al.: HSCs are isolated from patient by bone marrow aspiration (**a**) followed by patients treated with chemotherapy (**b**) cells mobilized in culture subjected to retroviral transduction with appropriate gene (**c**), and finally, these engineered $CD34^+$ HSC cells are infused into the body of the patient (**d**)

Stem-cell-mediated gene therapy was attempted to tackle thalassemia, a blood-related disorder that affects 7 % of population worldwide. The first trial involving transfer of β-globin gene into the $CD34^+$ bone marrow cells using lentiviral vectors was performed by Cavazzana-Calvo et al. Among three patients, the first one failed to engraft. Fortunately, the second patient accepted the graft and was continuously followed up for the next 5 years. The third patient was engrafted after a few months without complications. In a detailed report, Cavazzana-Calvo et al. [176, 194] have discussed the health of the second patient who had become transfusion independent for a period of 21 months after transplantation. The process of the trial is diagrammatically represented in Fig. 2.10.

In the last few years, a few trials were done on diseases such as ADA, gaucher, X-SCID using engineered HSCs with limited success [195].

2.4.4 Stem Cells and Type 1 Diabetes

Type 1 diabetes is an autoimmune disease associated with T-cell-mediated destruction of insulin-producing cells, which results in a lifelong dependence on insulin. Several attempts such as pancreas transplantation and islet transplantation have been attempted by scientists and clinicians to improve the quality of life of the patients [196].

Autologous and allogenic transplantations of hematopoietic cells were found to rescue patients from their diabetic symptoms. Recently, Gu et al. [197] published a clinical trial in which 28 patients who were having type 1 diabetes, antiglutamine decarboxylase antibody, and devoid of conditions such as cardiorespiratory insufficiency, renal or kidney failure or chronic and acute infection enrolled for autologous HSC transplantation between the years 2007 and 2010. After the administration of cyclophosphamide and rabbit antithymocyte globulin, autologous HSCs were infused. The daily requirement of insulin started decreasing in these patients within a month, and the decrease was found to be significant during the course of next 3 months and remained stable for the next 24 months. A similar observation was made in a group of patients with diabetes ketoacidosis (DKA) in which 20 out of 23 patients showed a remarkable insulin-free state. Twelve out of the twenty patients maintained the state of complete remission (CR) for 31 months, and the rest came back with the disease [197].

The umbilical cord blood rich in T regulatory cells and stem cells preserved at the time of birth also proves to be beneficial to combat such disease conditions. In a clinical study, Haller et al. [198] reported the results of infusion (about 100 ml cord blood/year) of own stored cord blood in 23 patients with diabetes type 1. No significant adverse effects were observed in 15 of these patients who were a part of the follow-up regime. In fact, the study illustrated the process of cord blood infusion in young to be feasible. However, the infusion failed to preserve the C-peptide levels in children.

Human placenta being a rich source of pluripotent and multipotent stem cells has become an attractive resource to the translational researchers. In one such trial performed, Hou et al. [199, 200] have shown that human amnion epithelial cells can be differentiated into insulin-producing cells of the pancreas and can reverse the state of hyperglycemia in C57 diabetic mouse. Unfortunately, no such trial has been initiated in patients.

2.4.5 Stem Cells and Malignancies

Stem cell transplantation has become a standard of care for the hematological cancers but yet to reach clinic for all types of solid tumors. HSC transplantation is a process in which HSCs are injected into the patients receiving bone-marrow-toxic drugs with or without whole-body radiation therapy. HSC transplantation could be of two types: allogenic transplantation when the stem cells come from another person with matched immune profile and autologous transplantation where the stem cells come from own body.

Allogenic transplantation is used to treat acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), and non-Hodgkin's lymphoma. CLL accounts for 25 % of all leukemia in which

allogenic stem cell transplantation is a good option of treatment. However, due to the lack of sufficient matching donors, this transplantation method is rarely followed. A trial by Michallet et al. (2011) enrolled 223 patients between the age of 31–65 years and in a stage of CR who were divided into two groups, 112 for transplantation arm or ACST arm and 111 for observation arm. Eighty of the 112 patients were subjected to transplantation of autologous stem cells, and the rest were spared due to collection failure, refusal, or secondary malignancies. According to the results published in 2011, no significant difference was found between ASCT and observation arm with respect to mortality without relapse even after 43.7 months of follow-up. However, a statistically significantly improved event-free survival was obtained in the ASCT arm. Only occurrence of myelodysplastic syndrome was reported. Thus, the transplantation study proved to be beneficial for CLL patients in CR. In contrary to the above-mentioned trial, a study by Sutton et al. [203] did not show beneficial effects of ASCT over combined chemotherapy of fludarabine and cyclophosphamide in patients aged between 18 and 65 years with Binet stage B or C CLL who were not treated before [201–203].

A few more randomized clinical trials were performed with allogenic HSC transplantation. The main focus of these trials was to overcome the chances of developing chronic graft versus host disease (GVHD), which is a very common complication encountered by clinicians after allogenic stem cell transplantation. Socie et al. [201, 204] performed a randomized trial that assessed prophylactic treatment regime with ATGs. Administration of corticosteroids or antithymocyte globulin along with drugs such as methotrexate and tacrolimus was shown to reduce the chances of GVHD.

Apart from trials on hematological malignancies, few very interesting clinical trials have been performed on solid tumors such as neuroblastoma, Wilms' tumor, retinoblastoma. In an attempt by George et al. 205, high-risk neuroblastoma patients enrolled between the years 1999 and 2002 were initially subjected to five cycles of standard chemotherapy and peripheral blood stem cell transplantation was performed after recovery from the second or the third cycle of chemotherapy. Out of the selected 97 patients, 51 died, and in the remaining 46, patient's progression-free survival was estimated to be around 47 and 45 % at the end of 5 and 7 years, respectively. The overall survival rate was found to be around 60 and 53 % at the end of 5 and 7 years, respectively. Thus, a combination of high-dose therapy with autologous stem cell rescue proved to be beneficial for high-risk neuroblastoma patients. An identical study was performed by Berthold et al. [206] with increased number of high-risk neuroblastoma patients. Patients subjected to high-dose chemotherapy with autologous stem cell transplantation were shown to have an extended 3-year event-free survival as compared to the batch of subjects given a maintenance therapy.

2.4.6 Stem Cells and Heart Ailments

Heart ailments such as myocardial infarction and ischemic heart failure lead to drastic loss of cardiomyocytes, interstitial cells, and vascular cells, which make recovery slow and difficult. A number of clinical trials are being conducted with mononuclear bone marrow cells rich in MSCs transplantation and are summarized in Table 2.7 [207].

In a recent report, Vrtovec and his colleagues (2013) discussed a clinical trial registered under NIH (NCT01350310) performed on 110 dilated cardiomyopathy patients. In phase I, the patients received doses of granulocyte-stimulating factor, and in phase II, 55 out of 110 patients were given an infusion of bone-marrow-derived autologous $CD34^+$ cells. During the 5-year follow-up, patients were assessed by echocardiography and walking test. However, the most significant feature of the trial was the use of 99 m Tc hexamethylpropyleneamine oxime tracer accumulation method, which was used to determine the homing of the infused $CD34^+$ cells. The stem cell transplantation was hence seen to be well associated with longtime survival of the patients [128].

2.4.7 Stem Cells and Retinal Diseases

Age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy are the three most common causes of retinal degeneration, visual impairment, and complete blindness. In the recent years, a number of studies have been performed on animal models that have explored the potential of mouse or human adult bone-marrow-derived stem cells containing endothelial precursor to stabilize and rescue retinal blood vessels in experimental retinal dystrophies. Fewer numbers of clinical studies were done. Jonas et al. [177, 216, 217] produced case reports of trials performed in three patients with end-stage macular degeneration and glaucoma. Bone marrow was harvested, and mononuclear cells were separated by Ficoll density gradient sedimentation. The cells were then injected into the intraocular space. After the procedure was completed, a regular follow-up was done till 12 months. In spite of the trial being technically feasible, no significant improvement was obtained.

2.4.8 Stem Cells and Alopecia Areata

Alopecia areata is a common T-cell-mediated autoimmune disease leading to chronic and recurrent hair loss. The incidence of the disease is 0.1–0.2 % worldwide. Cell therapy is extremely limited and discouraging for alopecia areata. A clinical trial was initiated in August 2012 using the technique of "stem cell educator." This technique is extremely useful in treating autoimmune diseases

Table 2.7 Trials on the various cardiac diseases

Disease	Trial	Patients	Cell type used	Result
Myocardial infraction	Boost Trial [207]	60	Nucleated BMC	Effect prominent in 6 months but diminished between 18 and 61 months
	Leuven AMI trial [208, 209]	67	Mononucleated BMC	Fast recovery
	Astami trial [210]	100	Mononucleated BMC	No significant effect observed after 6 and 12 months
	Fincell trial [211]	80	Thrombolytic therapy	Improvement seen before and after 6 months
	Regent trial [212]	200	Mononucleated BMC	Specific marker expressing cell population associated with the observed effects
	HEBE Trial [213]	20	Mononucleated BMC and standard therapy as control	Subjects showed either enhanced ventricular recovery or remodeling. Results need further validation
Chronic myocardial ischemia	[214]	24	$CD34^+$ cells collected from peripheral blood	Satisfactory results

such as alopecia areata and type 1 diabetes. The principle of this technique is to change the behavior of the immune cells of the body so that they do not function against self-immune system. This phase I/II trial was performed with an objective to see how the stem cells modulate autoimmunity. The date of completion of the trial is expected to be in July 2013 [218].

2.4.9 Stem Cells and Disasters

Disasters are always unpredictable and could be either natural or man-made. At times, human errors can lead to accidents such as radiation exposure, road mishaps that may cause irreversible damages. Strategies such as isolating cells from an autologous healthy source and transplanting them into the damaged sites for generating functional cells with desired characteristics are adopted to cope up with such events. A case study was published by Lataillade et al. [219] in which a 27-year-old Chilean man overexposed to gammagraphy radioactive source was treated using dosimetry-guided lesion excision and mesenchymal stem cell therapy. Autologous total bone marrow cells were obtained from unexposed iliac crest by aspiration and were grown in culture using medium supplemented with 8 % platelet lysate. MSCs characterized by surface markers, clonogenic assays, and telomerase activity were infused into the patient body at day 90 and day 99. Within

five and a half months postradiation, the patient showed signs of improvement with complete healing. After a series of successful animal experiments, a phase I/II clinical trial was initiated in the year 2011 (NCT01298830). The trial was designed based on the use of encapsulated and engineered human bone-marrow-derived MSCs to cure traumatic brain injury. In spite of obtaining some positive results from 11 patients, the trial was terminated in 2013 [220].

2.4.10 Stem Cells and Bone Disorders

Bone loss has always been a challenge for the clinicians. Marcacci et al. [221] published a trial report that they conducted on four subjects of whom two patients had an affected ulna and the other two had defective tibia and humerus. As mentioned in Sect. 2.2.3, a porous bioceramics was used as the scaffold in this study. Bone marrow stem cells isolated from the patients were cultured with minor modifications and seeded on the scaffold within 36 h from harvest. During the follow-up course, no complications in terms of pain, swelling, or infections were observed. Even after 6–7 years of postsurgery, a good integration of implants was observed in patients 1 and 3. Angiographic evaluation performed even after 6.5 years postsurgery indicated vascularization of the grafted zone. Pre/postoperative radiography and 2D and 3D CT scans were used to monitor bone development.

Apart from the above-mentioned bone disease, osteogenesis imperfecta is a very painful genetic disorder caused by a defect in the collagen type I encoding gene. The disease is characterized by bony deformities, fragility, fractures, and short stature. Two identical clinical trials were performed by Horwitz et al. (1999 and 2001). Analysis was conducted using techniques such as dual-energy X-ray absorptiometry and bone histologic examination from bone biopsy samples taken before and after transplantation using the technique of microscopy as explained previously. In the first trial, three patients were infused with unmanipulated bone marrow from HLA-matched identical siblings. In the second trial, a similar approach was adopted for the three patients, while the two patients in the control arm were not given any specific therapy. Improvement in bone histology and a significant increase in the number of osteoblasts were observed. Unlike the second trial, the first one also accounted for a significant increase in bone mineral content. However, complications such as sepsis, pulmonary insufficiency, and bifrontal hygroma were commonly seen in both trials. In spite of the aftereffects as mentioned above, these trials indeed generated a lot of hope in patients suffering from this tragic genetic defect [222, 223].

2.4.11 Stem Cells and Liver Diseases

Liver diseases such as liver cirrhosis and hepatitis B are considered to be extremely life threatening. No effective treatment regime has been designed for almost a decade now. Hence, intensive research is carried out using HSCs due to their promising results in other fatal diseases. A phase I trial was designed and published by Gordon et al. and Levicar et al. [224, 225] in which $CD34^+$ HSCs in granulocyte-stimulating-factor-mobilized blood were infused into the portal vein of 5 patients with chronic liver diseases. Further, the data collected during the follow-up period of 12–18 months were discussed in the report published in 2008. During the follow-up period of 12–18 months, patients were assessed on the basis of CT and levels of bilirubin and alpha-fetoproteins in serum and they showed no side effects. The study implicated that the treatment with stem cells lasts for about 12 months. Terai et al. [226] in 2006 conducted autologous bone marrow transplantation in patients with liver cirrhosis. One of the successful trials reported by Gasbarrini et al. [227–229] showed a reversal of drug-induced liver failure by transplanting $CD34^+$ BMSCs. Zang et al. [230] discussed a trial on infusion of umbilical cord MSCs in 30 patients with 15 controls (only saline). Reduction in the amount of ascites, improvement in liver function with increased serum albumin, and decrease in serum bilirubin levels were achieved. Another remarkable clinical trial on end-stage liver disease was reported by Kharaziha [231] in which autologous MSCs were used. The cells were isolated from the iliac crest and grown in vitro to get a count of 30 to 50 millions. Posttransplantation follow-ups were regularly done in the 1st, 2nd, 4th, 8th, and 24th weeks. The transplantation was well tolerated as seen in the improved liver function test results that were reflected in the levels of serum creatinine, serum albumin, and serum bilirubin.

2.4.12 Stem Cells and Pharmaceutical Companies

Cell therapy by using stem cells has been a subject of interest for clinicians and scientists for almost a decade. The recent years have seen many pharmaceutical companies participating in stem cell research, therefore making regenerative medicine a vibrant industry. The first FDA-approved trial for patients with spinal cord injury with ESC-derived oligodendrocyte progenitor cells (GRNOPC1) (#NCT01217008) was conducted by Geron, which is currently on hold. Recently, Genzyme reported a quality control program conducted on 303 patients subjected to autologous transplantation of cultured chondrocytes to repair knee damages. The follow-up of these patients produced positive results [232]. Many companies are also involved in iPSC research for future like Progenitor Labs involved in stem cell engineering [233, 234]. Some company-sponsored trials are listed in Table 2.8.

Table 2.8 Companies and their contribution in the stem-cell-therapy-based trials

Companies	Trials
TCA Cellular Therapy	NCT00518401; NCT00643981; NCT00721006; NCT00790764; NCT00548613
Shenzhen Beike Biotechnology Co. Ltd	NCT01360164; NCT01343511; NCT01610440; NCT01742533; NCT01443689
Manipal Acunova Ltd	NCT01501773
Banc de Sang i Teixits	NCT01227694
NCIC Clinical Trial Group	NCT00003032
TECAM Group	NCT00984178
Cellonis Biotechnology Co. Ltd	NCT01143168; NCT01142050
Osiris Therapeutics	NCT00482092; NCT01233960
International Stem Cell Service Ltd	NCT01152125

Table 2.9 describes ongoing and completed trials categorized on the basis of the different types of stem cells used for transplantation.

2.5 Conclusion and Future Prospect

Self-renewal and multilineage differentiation properties of stem cells are keys to the lifelong homeostatic maintenance of tissues and organs. These properties could also make the cells as potential therapeutic tool for many incurable diseases where traditional "chemical solution to biological diseases" fails to work. Last few decades of biomedical research have seen extensive experimentation on stem cell therapy for regenerative medicine. Most early work was carried out with pluripotent ES cells derived from the inner mass of blastocyst embryo that can differentiate into any type of cells belong to all the three germ layers. However, significant challenge was met in successfully culturing the hESCs on MEF cells and induction of directed differentiation toward a specific cell lineage. An ongoing effort is going to establish feeder-independent culture system under the supervision of International Stem Cell Initiative Consortium. Various combinations of growth factors, chemicals, cell densities, and matrices are being tested to achieve proper differentiation of hESCs. A remarkable success was reported by Chen et al. [6] who were able to differentiate hESCs to functionally active insulin-secreting beta-cells in a stepwise manner. However, application of hESCs in regenerative medicine is still limited due to the formation of teratoma in vivo and ethical issues. A potential future solution for overcoming teratoma formation would be implantation of hESC-derived progenitor cells with specific lineage memory instead of implantation of undifferentiated hESC.

The next and most widely accepted choice for cell therapy after hESC is the adult stem cells, which beat the major limitations of hESCs. Adult stem cells, majority of which are known as MSCs, reside in various organs and are potential

Table 2.9 Ongoing or completed clinical trials on various diseases using stem cells

Stem Cells	Disease	Trials completed/in progress
Autologous HSCs transplantation	Severe systemic lupus erythematosus	NCT00076752
	Retinoblastoma	NCT00554788
	Type 1 diabetes	NCT01285934; NCT01341899
Peripheral blood stem cell transplantation	Neuroblastoma	NCT00004188; NCT01526603
	Ewing's tumor	NCT00003081
Allogenic HSCs transplantation	Hematological malignancies	NCT00152139; NCT00176930
	Ewing's tumor	NCT00357396
Autologous transplantation of $CD34^+$ cells	Coronary artery disease, angina, myocardial infarction, and chronic ischemia	NCT00081913; NCT00221182
Bone-marrow-derived mononuclear cell transplantation	Retinitis pigmentosa	NCT01068561
	Liver cirrhosis	NCT01120925; NCT01724697
	Wilms' tumor	NCT00025103
HLA-matched bone-marrow-derived stem cells	Alopecia areata	NCT01758510
Mesenchymal stem cell	Amyotrophic lateral sclerosis	NCT01609283
	Osteonecrosis of the femoral head	NCT01605383
Umbilical cord-derived MSCs	Liver cirrhosis and liver failure	NCT01573923; NCT01724398; NCT01728727
	Hereditary ataxia	NCT01360164
	Diabetes type 1	NCT01374854
Autologous bone marrow mononuclear cells and umbilical cord mesenchymal stem cell transplantation	Diabetes type 1	NCT01143168
Cord blood stem cells (educator therapy)	Diabetes type 1	NCT01350219
	Alopecia areata	NCT01673789
Autologous adipose-tissue-derived stem cells	Diabetes type 1	NCT00703599
Development of iPSCs from cell culture established from skin biopsies	Neurodegenerative diseases	NCT00874783
Combined stem cell therapy	Severe leg ischemia	NCT00721006

to form different types of cells through proper differentiation. An extensive overview of MSC and other adult stem cell (such as HSC)-based applications are discussed in the main sections. The wide application of MSCs in preclinical and clinical trials with many of them displaying effective prevention and control of a diversity of diseases brings them to forefront of stem cell therapy research. A number of clinical trials are currently going on with MSCs to treat

neurodegenerative diseases, thalassemia, diabetes, cardiovascular diseases, and malignancies and even in disaster-related injuries. However, success rate is still low and more number of trials would be required to assess the real potential of stem cell therapy.

The low abundance and need of large-scale culture of adult stem cells for clinical application can overcome by iPSCs, which shows great promise for regenerative medicine. However, application of iPSCs is still limited in preclinical stages and expected to reach clinic soon.

Finally, monitoring the efficacy of stem cell therapy is an extremely important component and a large number of microscopic and macroscopic techniques are being implemented to check for in vitro differentiation, in vivo differentiation, and functional output. Each of these cell based and preclinical imaging techniques has own merits and demerits and is often used in combination to achieve a multimodal approach. Since stem cell imaging requires high resolution, MRI despite having lower sensitivity is probably the most suitable technology. A large number of preclinical applications are using bioluminescence imaging as well. These imaging techniques are also important in understanding the interactions of local microenvironment with stem cells. Overall, the excitement and hope for effective treatment for many incurable diseases by stem cells are quite promising but require a thorough and long-term monitoring at preclinical level, followed by practical protocols. It is highly expected that detailed investigations of stem cell biology and clinical applicability will result in revolutions in medical technologies.

References

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147
2. Akopian V, Andrews PW, Beil S, Benvenisty N, Brehm J, Christie M, Ford A, Fox V, Gokhale PJ, Healy L, Holm F, Hovatta O, Knowles BB, Ludwig TE, McKay RD, Miyazaki T, Nakatsuji N, Oh SK, Pera MF, Rossant J, Stacey GN, Suemori H (2010) Comparison of defined culture systems for feeder cell free propagation of human embryonic stem cells. Vitro Cell Dev Biol Anim 46(3–4):247–258. doi:10.1007/s11626-010-9297-z
3. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, Carpenter MK (2001) Feeder-free growth of undifferentiated human embryonic stem cells. Nat Biotechnol 19(10):971–974. doi:10.1038/nbt1001-971
4. Lei T, Jacob S, Ajil-Zaraa I, Dubuisson JB, Irion O, Jaconi M, Feki A (2007) Xeno-free derivation and culture of human embryonic stem cells: current status, problems and challenges. Cell Res 17(8):682–688. doi:10.1038/cr.2007.61
5. Itskovitz-Eldor J, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, Benvenisty N (2000) Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med 6(2):88–95
6. Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D (2009) A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 5(4):258–265. doi:10.1038/nchembio.154

7. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26(4):443–452. doi:10.1038/nbt1393
8. Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, Mickunas E, Gay R, Klimanskaya I, Lanza R (2012) Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 379(9817):713–720. doi:10.1016/S0140-6736(12)60028-2
9. Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, Radford I, Villeval JL, Fraser CC, Cavazzana-Calvo M, Fischer A (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348(3):255–256. doi:10.1056/NEJM200301163480314
10. Yu SF, von Ruden T, Kantoff PW, Garber C, Seiberg M, Ruther U, Anderson WF, Wagner EF, Gilboa E (1986) Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. Proc Natl Acad of Sci USA. 83(10):3194–3198
11. Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272(5259):263–267
12. May C, Rivella S, Callegari J, Heller G, Gaensler KM, Luzzatto L, Sadelain M (2000) Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 406(6791):82–86. doi:10.1038/35017565
13. Vadhan-Raj S, Patel S, Bueso-Ramos C, Folloder J, Papadopolous N, Burgess A, Broemeling LD, Broxmeyer HE, Benjamin RS (2003) Importance of predosing of recombinant human thrombopoietin to reduce chemotherapy-induced early thrombocytopenia. J Clinc Oncol 21(16):3158–3167. doi:10.1200/JCO.2003.08.003
14. Panuganti S, Schlinker AC, Lindholm PF, Papoutsakis ET, Miller WM (2013) Three-stage ex vivo expansion of high-ploidy megakaryocytic cells: toward large-scale platelet production. Tissue Eng Part A 19(7–8):998–1014. doi:10.1089/ten.TEA 2011.0111
15. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 6(11):1229–1234. doi:10.1038/81326
16. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA (1974) Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol 2(2):83–92
17. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV (1974) Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 17(4):331–340
18. Polisetty N, Fatima A, Madhira SL, Sangwan VS, Vemuganti GK (2008) Mesenchymal cells from limbal stroma of human eye. Mol Vis 14:431–442
19. Chan BP, Hui TY, Wong MY, Yip KH, Chan GC (2010) Mesenchymal stem cell-encapsulated collagen microspheres for bone tissue engineering. Tissue Eng Part C Methods 16(2):225–235. doi:10.1089/ten.tec.2008.0709
20. Sumanasinghe RD, Bernacki SH, Loboa EG (2006) Osteogenic differentiation of human mesenchymal stem cells in collagen matrices: effect of uniaxial cyclic tensile strain on bone morphogenetic protein (BMP-2) mRNA expression. Tissue Eng 12(12):3459–3465. doi:10.1089/ten.2006.12.3459
21. Sumanasinghe RD, Osborne JA, Loboa EG (2009) Mesenchymal stem cell-seeded collagen matrices for bone repair: effects of cyclic tensile strain, cell density, and media conditions on matrix contraction in vitro. J Biomed Mater Res A 88(3):778–786. doi:10.1002/jbm.a.31913
22. Ahmed TA, Dare EV, Hincke M (2008) Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng Part B Rev 14(2):199–215. doi:10.1089/ten.teb2007.0435

23. Kim HJ, Kim UJ, Kim HS, Li C, Wada M, Leisk GG, Kaplan DL (2008) Bone tissue engineering with premineralized silk scaffolds. Bone 42(6):1226–1234. doi:10.1016/j.bone.2008.02.007
24. Li C, Vepari C, Jin HJ, Kim HJ, Kaplan DL (2006) Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials 27(16):3115–3124. doi:10.1016/j.biomaterials.2006.01.022
25. Mieszawska AJ, Fourligas N, Georgakoudi I, Ouhib NM, Belton DJ, Perry CC, Kaplan DL (2010) Osteoinductive silk-silica composite biomaterials for bone regeneration. Biomaterials 31(34):8902–8910. doi:10.1016/j.biomaterials.2010.07.109
26. Patterson J, Siew R, Herring SW, Lin AS, Guldberg R, Stayton PS (2010) Hyaluronic acid hydrogels with controlled degradation properties for oriented bone regeneration. Biomaterials 31(26):6772–6781. doi:10.1016/j.biomaterials.2010.05.047
27. Kim J, Kim IS, Cho TH, Kim HC, Yoon SJ, Choi J, Park Y, Sun K, Hwang SJ (2010) In vivo evaluation of MMP sensitive high-molecular weight HA-based hydrogels for bone tissue engineering. J Biomed Mater Res A 95(3):673–681. doi:10.1002/jbm.a.32884
28. Kim J, Kim IS, Cho TH, Lee KB, Hwang SJ, Tae G, Noh I, Lee SH, Park Y, Sun K (2007) Bone regeneration using hyaluronic acid-based hydrogel with bone morphogenic protein-2 and human mesenchymal stem cells. Biomaterials 28(10):1830–1837. doi:10.1016/j.biomaterials.2006.11.050
29. Venkatesan J, Kim SK (2010) Chitosan composites for bone tissue engineering–an overview. Mar Drugs 8(8):2252–2266. doi:10.3390/md8082252
30. Shoae-Hassani A, Mortazavi-Tabatabaei SA, Sharif S, Seifalian AM, Azimi A, Samadikuchaksaraei A, Verdi J (2013) Differentiation of human endometrial stem cells into urothelial cells on a three-dimensional nanofibrous silk-collagen scaffold: an autologous cell resource for reconstruction of the urinary bladder wall. J Tissue Eng Regen Med. doi:10.1002/term.1632
31. Mummery CL, Davis RP, Krieger JE (2010) Challenges in using stem cells for cardiac repair. Sci Transl Med 2(27):27ps17. doi:10.1126/scitranslmed.3000558
32. Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, Bae YC, Jung JS (2004) Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem 14(4–6):311–324. doi:10.1159/000080341
33. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S (1999) Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103(5):697–705. doi:10.1172/JCI5298
34. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD (2002) Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105(1):93–98
35. Takahashi T, Lord B, Schulze PC, Fryer RM, Sarang SS, Gullans SR, Lee RT (2003) Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. Circulation 107(14):1912–1916. doi:10.1161/01.CIR.0000064899.53876.A3
36. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317. doi:10.1080/14653240600855905
37. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147
38. Mruthyunjaya S, Manchanda R, Godbole R, Pujari R, Shiras A, Shastry P (2010) Laminin-1 induces neurite outgrowth in human mesenchymal stem cells in serum/differentiation factors-free conditions through activation of FAK-MEK/ERK signaling pathways. Biochem Biophys Res Commun 391(1):43–48. doi:10.1016/j.bbrc.2009.10.158
39. Vecellio M, Meraviglia V, Nanni S, Barbuti A, Scavone A, DiFrancesco D, Farsetti A, Pompilio G, Colombo GI, Capogrossi MC, Gaetano C, Rossini A (2012) In vitro epigenetic

reprogramming of human cardiac mesenchymal stromal cells into functionally competent cardiovascular precursors. PLoS ONE 7(12):e51694. doi:10.1371/journal.pone.0051694
40. Zouani OF, Kalisky J, Ibarboure E, Durrieu MC (2013) Effect of BMP-2 from matrices of different stiffnesses for the modulation of stem cell fate. Biomaterials 34(9):2157–2166. doi:10.1016/j.biomaterials.2012.12.007
41. Fatima A, Sangwan VS, Iftekhar G, Reddy P, Matalia H, Balasubramanian D, Vemuganti GK (2006) Technique of cultivating limbal derived corneal epithelium on human amniotic membrane for clinical transplantation. J Postgrad Med 52(4):257–261
42. Gurdon JB (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J Embryol Exp Morphol 10:622–640
43. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676. doi:10.1016/j.cell.2006.07.024
44. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872. doi:10.1016/j.cell.2007.11.019
45. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858):1917–1920. doi:10.1126/science.1151526
46. Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448(7151):313–317. doi:10.1038/nature05934
47. Kawai H, Yamashita T, Ohta Y, Deguchi K, Nagotani S, Zhang X, Ikeda Y, Matsuura T, Abe K (2010) Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain. J Cereb Blood Flow Metab 30(8):1487–1493. doi:10.1038/jcbfm.2010.32
48. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Scholer HR, Duan L, Ding S (2009) Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4(5):381–384. doi:10.1016/j.stem.2009.04.005
49. Blelloch R, Venere M, Yen J, Ramalho-Santos M (2007) Generation of induced pluripotent stem cells in the absence of drug selection. Cell Stem Cell 1(3):245–247. doi:10.1016/j.stem.2007.08.008
50. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, Zhang Y, Yang W, Gruber PJ, Epstein JA, Morrisey EE (2011) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8(4):376–388. doi:10.1016/j.stem.2011.03.001
51. Liu H, Zhu F, Yong J, Zhang P, Hou P, Li H, Jiang W, Cai J, Liu M, Cui K, Qu X, Xiang T, Lu D, Chi X, Gao G, Ji W, Ding M, Deng H (2008) Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 3(6):587–590. doi:10.1016/j.stem.2008.10.014
52. Liao J, Cui C, Chen S, Ren J, Chen J, Gao Y, Li H, Jia N, Cheng L, Xiao H, Xiao L (2009) Generation of induced pluripotent stem cell lines from adult rat cells. Cell Stem Cell 4(1):11–15. doi:10.1016/j.stem.2008.11.013
53. Ezashi T, Telugu BP, Alexenko AP, Sachdev S, Sinha S, Roberts RM (2009) Derivation of induced pluripotent stem cells from pig somatic cells. Proc Natl Acad of Sci USA 106(27):10993–10998. doi:10.1073/pnas.0905284106
54. Shimada H, Nakada A, Hashimoto Y, Shigeno K, Shionoya Y, Nakamura T (2010) Generation of canine induced pluripotent stem cells by retroviral transduction and chemical inhibitors. Mol Reprod Dev 77(1):2. doi:10.1002/mrd.21117
55. Wu Y, Zhang Y, Mishra A, Tardif SD, Hornsby PJ (2010) Generation of induced pluripotent stem cells from newborn marmoset skin fibroblasts. Stem Cell Res 4(3):180–188. doi:10.1016/j.scr.2010.02.003
56. Honda A, Hirose M, Hatori M, Matoba S, Miyoshi H, Inoue K, Ogura A (2010) Generation of induced pluripotent stem cells in rabbits: potential experimental models for human generative medicine. J Biol Chem 285(41):31362–31369. doi:10.1074/jbc.M110.150540

57. Nagy K, Sung HK, Zhang P, Laflamme S, Vincent P, Agha-Mohammadi S, Woltjen K, Monetti C, Michael IP, Smith LC, Nagy A (2011) Induced pluripotent stem cell lines derived from equine fibroblasts. Stem Cell Rev 7(3):693–702. doi:10.1007/s12015-011-9239-5
58. Lu Y, West FD, Jordan BJ, Mumaw JL, Jordan ET, Gallegos-Cardenas A, Beckstead RB, Stice SL (2012) Avian-induced pluripotent stem cells derived using human reprogramming factors. Stem Cells Dev 21(3):394–403. doi:10.1089/scd2011.0499
59. Yoshikawa T, Samata B, Ogura A, Miyamoto S, Takahashi J (2013) Systemic administration of valproic acid and zonisamide promotes differentiation of induced pluripotent stem cell-derived dopaminergic neurons. Front Cell Neurosci 7:11. doi:10.3389/fncel.2013.00011
60. Mekala SR, Vauhini V, Nagarajan U, Maddileti S, Gaddipati S, Mariappan I (2013) Derivation, characterization and retinal differentiation of induced pluripotent stem cells. J Biosci 38(1):123–134
61. Nishimura K, Takahashi J (2013) Therapeutic application of stem cell technology toward the treatment of Parkinson's disease. Biol Pharma Bull. 36(2):171–175
62. Song Z, Cai J, Liu Y, Zhao D, Yong J, Duo S, Song X, Guo Y, Zhao Y, Qin H, Yin X, Wu C, Che J, Lu S, Ding M, Deng H (2009) Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Res 19(11):1233–1242. doi:10.1038/cr.2009.107
63. Sullivan GJ, Hay DC, Park IH, Fletcher J, Hannoun Z, Payne CM, Dalgetty D, Black JR, Ross JA, Samuel K, Wang G, Daley GQ, Lee JH, Church GM, Forbes SJ, Iredale JP, Wilmut I (2010) Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology 51(1):329–335. doi:10.1002/hep.23335
64. Kobari L, Yates F, Oudrhiri N, Francina A, Kiger L, Mazurier C, Rouzbeh S, El-Nemer W, Hebert N, Giarratana MC, Francois S, Chapel A, Lapillonne H, Luton D, Bennaceur-Griscelli A, Douay L (2012) Human induced pluripotent stem cells can reach complete terminal maturation: in vivo and in vitro evidence in the erythropoietic differentiation model. Haematologica 97(12):1795–1803. doi:10.3324/haematol.2011.055566
65. Pouya A, Satarian L, Kiani S, Javan M, Baharvand H (2011) Human induced pluripotent stem cells differentiation into oligodendrocyte progenitors and transplantation in a rat model of optic chiasm demyelination. PLoS ONE 6(11):e27925. doi:10.1371/journal.pone.0027925
66. Aravalli RN, Cressman EN, Steer CJ (2012) Hepatic differentiation of porcine induced pluripotent stem cells in vitro. Vet J 194(3):369–374. doi:10.1016/j.tvjl.2012.05.013
67. Georgakoudi I, Rice WL, Hronik-Tupaj M, Kaplan DL (2008) Optical spectroscopy and imaging for the noninvasive evaluation of engineered tissues. Tissue Eng Part B Rev 14(4):321–340. doi:10.1089/ten.teb2008.0248
68. Huh S, Ker DF, Bise R, Chen M, Kanade T (2011) Automated mitosis detection of stem cell populations in phase-contrast microscopy images. IEEE Trans Med Imaging 30(3):586–596. doi:10.1109/TMI.2010.2089384
69. S-iH Sungeun Eom, Ker Dai Fei Elmer, Bise Ryoma, Kanade Takeo (2007) Tracking of hematopoietic stem cells in microscopy images for lineage determination. J Latex Class Files 6(01–09):9
70. Jang YY, Ye Z, Cheng L (2011) Molecular imaging and stem cell research. Mol Imagings 10(2):111–122
71. Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E (2004) Defining the epithelial stem cell niche in skin. Science 303(5656):359–363. doi:10.1126/science.1092436
72. Giepmans BN, Adams SR, Ellisman MH, Tsien RY (2006) The fluorescent toolbox for assessing protein location and function. Science 312(5771):217–224. doi:312/5771/217
73. Sutton EJ, Henning TD, Pichler BJ, Bremer C, Daldrup-Link HE (2008) Cell tracking with optical imaging. Eur Radiol 18(10):2021–2032. doi:10.1007/s00330-008-0984-z

74. Sutton EJ, Boddington SE, Nedopil AJ, Henning TD, Demos SG, Baehner R, Sennino B, Lu Y, Daldrup-Link HE (2009) An optical imaging method to monitor stem cell migration in a model of immune-mediated arthritis. Opt Express 17(26):24403–24413. doi:194171
75. Hanna J, Saha K, Pando B, van Zon J, Lengner CJ, Creyghton MP, van Oudenaarden A, Jaenisch R (2009) Direct cell reprogramming is a stochastic process amenable to acceleration. Nature 462(7273):595–601. doi:10.1038/nature08592
76. Warlich E, Kuehle J, Cantz T, Brugman MH, Maetzig T, Galla M, Filipczyk AA, Halle S, Klump H, Scholer HR, Baum C, Schroeder T, Schambach A (2011) Lentiviral vector design and imaging approaches to visualize the early stages of cellular reprogramming. Mol Ther 19(4):782–789. doi:10.1038/mt.2010.314
77. Conchello JA, Lichtman JW (2005) Optical sectioning microscopy. Nat Methods 2(12):920–931. doi:10.1038/nmeth815
78. Helmchen F, Denk W (2005) Deep tissue two-photon microscopy. Nat Methods 2(12):932–940. doi:10.1038/nmeth818
79. Rice WL, Kaplan DL, Georgakoudi I (2010) Two-photon microscopy for non-invasive, quantitative monitoring of stem cell differentiation. PLoS ONE 5(4):e10075. doi:10.1371/journal.pone.0010075
80. Jérôme Artus A-KH (2007) Live imaging genetically-encoded fluorescent proteins in embryonic stem cells using confocal microscopy. In: Méndez-Vilas A, Díaz J (eds) Modern research and educational topics in microscopy. Sloan-Kettering Institute, New York
81. Malide D, Metais JY, Dunbar CE (2012) Dynamic clonal analysis of murine hematopoietic stem and progenitor cells marked by 5 fluorescent proteins using confocal and multiphoton microscopy. Blood 120(26):e105–e116. doi:blood-2012-06-440636
82. Ntziachristos V (2010) Going deeper than microscopy: the optical imaging frontier in biology. Nat Methods 7(8):603–614. doi:10.1038/nmeth.1483
83. Rompolas P, Deschene ER, Zito G, Gonzalez DG, Saotome I, Haberman AM, Greco V (2012) Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. Nature 487(7408):496–499. doi:10.1038/nature11218
84. Takayama N, Eto K (2012) Pluripotent stem cells reveal the developmental biology of human megakaryocytes and provide a source of platelets for clinical application. Cell Mol Life Sci 69(20):3419–3428. doi:10.1007/s00018-012-0995-4
85. Takayama N, Nishimura S, Nakamura S, Shimizu T, Ohnishi R, Endo H, Yamaguchi T, Otsu M, Nishimura K, Nakanishi M, Sawaguchi A, Nagai R, Takahashi K, Yamanaka S, Nakauchi H, Eto K (2010) Transient activation of c-MYC expression is critical for efficient platelet generation from human induced pluripotent stem cells. J Exp Med 207(13):2817–2830. doi:10.1084/jem.20100844
86. Rodriguez-Porcel M, Wu JC, Gambhir SS (2008) Molecular imaging of stem cells. Stem Book, Cambridge
87. Shah K, Jacobs A, Breakefield XO, Weissleder R (2004) Molecular imaging of gene therapy for cancer. Gene Ther 11(15):1175–1187. doi:10.1038/sj.gt.3302278
88. Nguyen PK, Lan F, Wang Y, Wu JC (2011) Imaging: guiding the clinical translation of cardiac stem cell therapy. Circ Res 109(8):962–979. doi:10.1161/CIRCRESAHA.111.242909
89. Beeres SL, Bengel FM, Bartunek J, Atsma DE, Hill JM, Vanderheyden M, Penicka M, Schalij MJ, Wijns W, Bax JJ (2007) Role of imaging in cardiac stem cell therapy. J Am Col Cardiol 49(11):1137–1148. doi:10.1016/j.jacc.2006.10.072
90. Rodriguez-Porcel M (2010) In vivo imaging and monitoring of transplanted stem cells: clinical applications. Curr Cardiol Rep 12(1):51–58. doi:10.1007/s11886-009-0073-1
91. Chan AT, Abraham MR (2012) SPECT and PET to optimize cardiac stem cell therapy. J Nucl Cardiol 19(1):118–125. doi:10.1007/s12350-011-9485-6
92. Dimayuga VM, Rodriguez-Porcel M (2011) Molecular imaging of cell therapy for gastroenterologic applications. Pancreatology 11(4):414–427. doi:10.1159/000327395
93. Kircher MF, Gambhir SS, Grimm J (2011) Noninvasive cell-tracking methods. Nat Rev Clin Oncol 8(11):677–688. doi:10.1038/nrclinonc.2011.141

94. Manley NC, Steinberg GK (2012) Tracking stem cells for cellular therapy in stroke. Curr Pharm Des 18(25):3685–3693
95. Sandu N, Schaller B (2010) Stem cell transplantation in brain tumors: a new field for molecular imaging? Mol Med 16(9–10):433–437. doi:10.2119/molmed.2010.00035
96. Shah P, Choi BG, Mazhari R (2011) Positron emission tomography for the evaluation and treatment of cardiomyopathy. Ann New York Acad Sci 1228:137–149. doi:10.1111/j.1749-6632.2011.06017.x
97. Wang J, Tian M, Zhang H (2011) PET molecular imaging in stem cell therapy for neurological diseases. Eur J Nucl Med Mol Imaging 38(10):1926–1938. doi:10.1007/s00259-011-1860-7
98. Wu C, Ma G, Li J, Zheng K, Dang Y, Shi X, Sun Y, Li F, Zhu Z (2013) In vivo cell tracking via (1)(8)F-fluorodeoxyglucose labeling: a review of the preclinical and clinical applications in cell-based diagnosis and therapy. Clin Imaging 37(1):28–36. doi:10.1016/j.clinimag.2012.02.023
99. Zhou R, Acton PD, Ferrari VA (2006) Imaging stem cells implanted in infarcted myocardium. J Am Col Cardiol 48(10):2094–2106. doi:10.1016/j.jacc.2006.08.026
100. Bengel FM (2011) Noninvasive stem cell tracking. J Nucl Cardiol 18(5):966–973. doi:10.1007/s12350-011-9436-2
101. Cao F, Li Z, Lee A, Liu Z, Chen K, Wang H, Cai W, Chen X, Wu JC (2009) Noninvasive de novo imaging of human embryonic stem cell-derived teratoma formation. Cancer Res 69(7):2709–2713. doi:10.1158/0008-5472.CAN-08-4122
102. Martinez-Quintanilla J, Bhere D, Heidari P, He D, Mahmood U, Shah K (2013) In vivo Imaging of the therapeutic efficacy and fate of bimodal engineered stem cells in malignant brain tumors. Stem Cells. doi:10.1002/stem.1355
103. Rueger MA, Backes H, Walberer M, Neumaier B, Ullrich R, Simard ML, Emig B, Fink GR, Hoehn M, Graf R, Schroeter M (2010) Noninvasive imaging of endogenous neural stem cell mobilization in vivo using positron emission tomography. J Neurosci 18:6454–6460. doi:10.1523/JNEUROSCI.6092-09.2010
104. Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, Obradovich JE, Muzik O, Mangner TJ (1998) Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. Nat Med 4(11):1334–1336. doi:10.1038/3337
105. Tarantal AF, Lee CC, Batchelder CA, Christensen JE, Prater D, Cherry SR (2012) Radiolabeling and in vivo imaging of transplanted renal lineages differentiated from human embryonic stem cells in fetal rhesus monkeys. Mol Imaging Biol 14(2):197–204. doi:10.1007/s11307-011-0487-1
106. Kang WJ, Kang HJ, Kim HS, Chung JK, Lee MC, Lee DS (2006) Tissue distribution of 18F-FDG-labeled peripheral hematopoietic stem cells after intracoronary administration in patients with myocardial infarction. J Nucl Med 47(8):1295–1301
107. Lee PH, Kim JW, Bang OY, Ahn YH, Joo IS, Huh K (2008) Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin Pharmcol Ther 83(5):723–730. doi:10.1038/sj.clpt.6100386
108. Marini C, Podesta M, Massollo M, Capitanio S, Fiz F, Morbelli S, Brignone M, Bacigalupo A, Piana M, Frassoni F, Sambuceti G (2012) Intrabone transplant of cord blood stem cells establishes a local engraftment store: a functional PET/FDG study. J Biomed Biotechnol 2012:767369. doi:10.1155/2012/767369
109. Castellani M, Colombo A, Giordano R, Pusineri E, Canzi C, Longari V, Piccaluga E, Palatresi S, Dellavedova L, Soligo D, Rebulla P, Gerundini P (2010) The role of PET with 13 N-ammonia and 18F-FDG in the assessment of myocardial perfusion and metabolism in patients with recent AMI and intracoronary stem cell injection. J Nucl Med 51(12):1908–1916. doi:10.2967/jnumed.110.078469
110. Loeb DM, Hobbs RF, Okoli A, Chen AR, Cho S, Srinivasan S, Sgouros G, Shokek O, Wharam MD Jr, Scott T, Schwartz CL (2010) Tandem dosing of samarium-153 ethylenediamine tetramethylene phosphoric acid with stem cell support for patients with high-risk osteosarcoma. Cancer 116(23):5470–5478. doi:10.1002/cncr.25518

111. Li Z, Wu JC, Sheikh AY, Kraft D, Cao F, Xie X, Patel M, Gambhir SS, Robbins RC, Cooke JP (2007) Differentiation, survival, and function of embryonic stem cell derived endothelial cells for ischemic heart disease. Circulation 116(11 Suppl):I46–I54. doi:10.1161/CIRCULATIONAHA.106.680561
112. Hung SC, Deng WP, Yang WK, Liu RS, Lee CC, Su TC, Lin RJ, Yang DM, Chang CW, Chen WH, Wei HJ, Gelovani JG (2005) Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. Clin Cancer Res 11(21):7749–7756. doi:10.1158/1078-0432.CCR-05-0876
113. Terrovitis J, Kwok KF, Lautamaki R, Engles JM, Barth AS, Kizana E, Miake J, Leppo MK, Fox J, Seidel J, Pomper M, Wahl RL, Tsui B, Bengel F, Marban E, Abraham MR (2008) Ectopic expression of the sodium-iodide symporter enables imaging of transplanted cardiac stem cells in vivo by single-photon emission computed tomography or positron emission tomography. J Am Col Cardiol. 52(20):1652–1660. doi:10.1016/j.jacc.2008.06.051
114. Higuchi T, Anton M, Saraste A, Dumler K, Pelisek J, Nekolla SG, Bengel FM, Schwaiger M (2009) Reporter gene PET for monitoring survival of transplanted endothelial progenitor cells in the rat heart after pretreatment with VEGF and atorvastatin. J Nucl Med 50(11):1881–1886. doi:10.2967/jnumed.109.067801
115. Yaghoubi SS, Campbell DO, Radu CG, Czernin J (2012) Positron emission tomography reporter genes and reporter probes: gene and cell therapy applications. Theranostics 2(4):374–391. doi:10.7150/thno.3677
116. Cao F, Lin S, Xie X, Ray P, Patel M, Zhang X, Drukker M, Dylla SJ, Connolly AJ, Chen X, Weissman IL, Gambhir SS, Wu JC (2006) In vivo visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. Circulation 113(7):1005–1014. doi:10.1161/CIRCULATIONAHA.105.588954
117. Rath P, Shi H, Maruniak JA, Litofsky NS, Maria BL, Kirk MD (2009) Stem cells as vectors to deliver HSV/tk gene therapy for malignant gliomas. Curr Stem Cell Res Ther 4(1):44–49
118. Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen HH, Fritzges D, Izbudak I, Young RG, Marcelino M, Pittenger MF, Solaiyappan M, Boston RC, Tsui BM, Wahl RL, Bulte JW (2005) Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. Circulation 112(10):1451–1461. doi:10.1161/CIRCULATIONAHA.105.537480
119. Blackwood KJ, Lewden B, Wells RG, Sykes J, Stodilka RZ, Wisenberg G, Prato FS (2009) In vivo SPECT quantification of transplanted cell survival after engraftment using (111)In-tropolone in infarcted canine myocardium. J Nucl Med 50(6):927–935. doi:10.2967/jnumed.108.058966
120. Gu E, Chen WY, Gu J, Burridge P, Wu JC (2012) Molecular imaging of stem cells: tracking survival, biodistribution, tumorigenicity, and immunogenicity. Theranostics 2(4):335–345. doi:10.7150/thno.3666
121. Goussetis E, Manginas A, Koutelou M, Peristeri I, Theodosaki M, Kollaros N, Leontiadis E, Theodorakos A, Paterakis G, Karatasakis G, Cokkinos DV, Graphakos S (2006) Intracoronary infusion of CD133+ and CD133-CD34+ selected autologous bone marrow progenitor cells in patients with chronic ischemic cardiomyopathy: cell isolation, adherence to the infarcted area, and body distribution. Stem Cells 24(10):2279–2283. doi:10.1634/stemcells.2005-0589
122. Pomper MG, Hammond H, Yu X, Ye Z, Foss CA, Lin DD, Fox JJ, Cheng L (2009) Serial imaging of human embryonic stem-cell engraftment and teratoma formation in live mouse models. Cell Res 19(3):370–379. doi:10.1038/cr.2008.329
123. Zhou R, Thomas DH, Qiao H, Bal HS, Choi SR, Alavi A, Ferrari VA, Kung HF, Acton PD (2005) In vivo detection of stem cells grafted in infarcted rat myocardium. J Nucl Med 46(5):816–822
124. Zhou Y, Sun M, Li H, Yan M, He Z, Wang W, Lu S (2013) Recovery of behavioral symptoms in hemi-parkinsonian rhesus monkeys through combined gene and stem cell therapy. Cytotherapy 15(4):467–480. doi:10.1016/j.jcyt.2013.01.007

125. Mitkari B, Kerkela E, Nystedt J, Korhonen M, Mikkonen V, Huhtala T, Jolkkonen J (2013) Intra-arterial infusion of human bone marrow-derived mesenchymal stem cells results in transient localization in the brain after cerebral ischemia in rats. Exp Neurol 239:158–162. doi:10.1016/j.expneurol.2012.09.018
126. Bindslev L, Haack-Sorensen M, Bisgaard K, Kragh L, Mortensen S, Hesse B, Kjaer A, Kastrup J (2006) Labelling of human mesenchymal stem cells with indium-111 for SPECT imaging: effect on cell proliferation and differentiation. Eur J Nucl Med Mol Imaging 33(10):1171–1177. doi:10.1007/s00259-006-0093-7
127. Phulpin B, Dolivet G, Marie PY, Poussier S, Huger S, Bravetti P, Graff P, Merlin JL, Tran N (2011) Feasibility of treating irradiated bone with intramedullary delivered autologous mesenchymal stem cells. J Biomed Biotechnol 2011:560257. doi:10.1155/2011/560257
128. Vrtovec B, Poglajen G, Lezaic L, Sever M, Domanovic D, Cernelc P, Socan A, Schrepfer S, Torre-Amione G, Haddad F, Wu JC (2013) Effects of intracoronary CD34 + stem cell transplantation in nonischemic dilated cardiomyopathy patients: 5-year follow-up. Circ Res 112(1):165–173. doi:CIRCRESAHA.112.276519
129. Mader EK, Butler G, Dowdy SC, Mariani A, Knutson KL, Federspiel MJ, Russell SJ, Galanis E, Dietz AB, Peng KW (2013) Optimizing patient derived mesenchymal stem cells as virus carriers for a phase I clinical trial in ovarian cancer. J Transl Med 11:20. doi:10.1186/1479-5876-11-20
130. Chen SL, Fang WW, Qian J, Ye F, Liu YH, Shan SJ, Zhang JJ, Lin S, Liao LM, Zhao RC (2004) Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. Chin Med J (Engl) 117(10):1443–1448
131. Musialek P, Tekieli L, Kostkiewicz M, Majka M, Szot W, Walter Z, Zebzda A, Pieniazek P, Kadzielski A, Banys RP, Olszowska M, Pasowicz M, Zmudka K, Tracz W (2011) Randomized transcoronary delivery of CD34(+) cells with perfusion versus stop-flow method in patients with recent myocardial infarction: Early cardiac retention of (9)(9)(m)Tc-labeled cells activity. J Nucl Cardiol 18(1):104–116. doi:10.1007/s12350-010-9326-z
132. Perin EC, Silva GV, Zheng Y, Gahremanpour A, Canales J, Patel D, Fernandes MR, Keller LH, Quan X, Coulter SA, Moore WH, Herlihy JP, Willerson JT (2012) Randomized, double-blind pilot study of transendocardial injection of autologous aldehyde dehydrogenase-bright stem cells in patients with ischemic heart failure. Am Heart J 163(3):415–421, 421 e411. doi:10.1016/j.ahj.2011.11.020
133. Tzukerman M, Rosenberg T, Ravel Y, Reiter I, Coleman R, Skorecki K (2003) An experimental platform for studying growth and invasiveness of tumor cells within teratomas derived from human embryonic stem cells. Proc Natl Acad Sci USA 100(23):13507–13512. doi:10.1073/pnas.2235551100
134. Tao W, Evans BG, Yao J, Cooper S, Cornetta K, Ballas CB, Hangoc G, Broxmeyer HE (2007) Enhanced green fluorescent protein is a nearly ideal long-term expression tracer for hematopoietic stem cells, whereas DsRed-express fluorescent protein is not. Stem Cells 25(3):670–678. doi:10.1634/stemcells.2006-0553
135. Frangioni JV (2003) In vivo near-infrared fluorescence imaging. Curr Opin Chem Biol 7(5):626–634
136. Lin S, Xie X, Patel MR, Yang YH, Li Z, Cao F, Gheysens O, Zhang Y, Gambhir SS, Rao JH, Wu JC (2007) Quantum dot imaging for embryonic stem cells. BMC Biotechnol 7:67. doi:10.1186/1472-6750-7-67
137. Boddington SE, Henning TD, Jha P, Schlieve CR, Mandrussow L, DeNardo D, Bernstein HS, Ritner C, Golovko D, Lu Y, Zhao S, Daldrup-Link HE (2010) Labeling human embryonic stem cell-derived cardiomyocytes with indocyanine green for noninvasive tracking with optical imaging: an FDA-compatible alternative to firefly luciferase. Cell Transplant 19(1):55–65. doi:10.3727/096368909X478579
138. Tsuji O, Miura K, Okada Y, Fujiyoshi K, Mukaino M, Nagoshi N, Kitamura K, Kumagai G, Nishino M, Tomisato S, Higashi H, Nagai T, Katoh H, Kohda K, Matsuzaki Y, Yuzaki M, Ikeda E, Toyama Y, Nakamura M, Yamanaka S, Okano H (2010) Therapeutic potential of

appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci USA 107(28):12704–12709. doi:10.1073/pnas.0910106107
139. Daadi MM, Davis AS, Arac A, Li Z, Maag AL, Bhatnagar R, Jiang K, Sun G, Wu JC, Steinberg GK (2010) Human neural stem cell grafts modify microglial response and enhance axonal sprouting in neonatal hypoxic-ischemic brain injury. Stroke 41(3):516–523. doi:10.1161/STROKEAHA.109.573691
140. Wang X, Rosol M, Ge S, Peterson D, McNamara G, Pollack H, Kohn DB, Nelson MD, Crooks GM (2003) Dynamic tracking of human hematopoietic stem cell engraftment using in vivo bioluminescence imaging. Blood 102(10):3478–3482. doi:10.1182/blood-2003-05-1432
141. Tang Y, Shah K, Messerli SM, Snyder E, Breakefield X, Weissleder R (2003) In vivo racking of neural progenitor cell migration to glioblastomas. Hum Gene Ther 14(13):1247–1254. doi:10.1089/104303403767740786
142. Wu JC, Chen IY, Sundaresan G, Min JJ, De A, Qiao JH, Fishbein MC, Gambhir SS (2003) Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. Circulation 108(11):1302–1305. doi:10.1161/01.CIR.0000091252.20010.6E
143. Raikwar SP, Zavazava N (2012) PDX1-engineered embryonic stem cell-derived insulin producing cells regulate hyperglycemia in diabetic mice. Transplant Res 1(1):19. doi:10.1186/2047-1440-1-19
144. Cao F, van der Bogt KE, Sadrzadeh A, Xie X, Sheikh AY, Wang H, Connolly AJ, Robbins RC, Wu JC (2007) Spatial and temporal kinetics of teratoma formation from murine embryonic stem cell transplantation. Stem Cells Dev 16(6):883–891. doi:10.1089/scd 2007.0160
145. Priddle H, Grabowska A, Morris T, Clarke PA, McKenzie AJ, Sottile V, Denning C, Young L, Watson S (2009) Bioluminescence imaging of human embryonic stem cells transplanted in vivo in murine and chick models. Cloning Stem Cells 11(2):259–267. doi:10.1089/clo 2008.0056
146. Tian X, Hexum MK, Penchev VR, Taylor RJ, Shultz LD, Kaufman DS (2009) Bioluminescent imaging demonstrates that transplanted human embryonic stem cell-derived CD34(+) cells preferentially develop into endothelial cells. Stem Cells 27(11):2675–2685. doi:10.1002/stem.204
147. Jung-Joon Min UCNL, Han Ho Jae, Lee Hyun-Suk, Moon Sung-Min, Heo Jung-Sun, Lee Yun Jung, Ahn Kyuyoun, Park Kwangsung (2008) Visualization of embryonic stem cell survival in vivo by adenoviral-driven bioluminescence reporter in rat corpus cavernosum. Tissue Eng Regen Med 5(4):743–749
148. Sher F, van Dam G, Boddeke E, Copray S (2009) Bioluminescence imaging of Olig2-neural stem cells reveals improved engraftment in a demyelination mouse model. Stem Cells 27(7):1582–1591. doi:10.1002/stem.76
149. Ling X, Marini F, Konopleva M, Schober W, Shi Y, Burks J, Clise-Dwyer K, Wang RY, Zhang W, Yuan X, Lu H, Caldwell L, Andreeff M (2010) Mesenchymal stem cells overexpressing IFN-beta inhibit breast cancer growth and metastases through Stat3 signaling in a syngeneic tumor model. Cancer Microenviron 3(1):83–95. doi:10.1007/s12307-010-0041-8
150. Deuse T, Peter C, Fedak PW, Doyle T, Reichenspurner H, Zimmermann WH, Eschenhagen T, Stein W, Wu JC, Robbins RC, Schrepfer S (2009) Hepatocyte growth factor or vascular endothelial growth factor gene transfer maximizes mesenchymal stem cell-based myocardial salvage after acute myocardial infarction. Circulation 120(11 Suppl):S247–S254. doi:10.1161/CIRCULATIONAHA.108.843680
151. Togel F, Yang Y, Zhang P, Hu Z, Westenfelder C (2008) Bioluminescence imaging to monitor the in vivo distribution of administered mesenchymal stem cells in acute kidney injury. Am J Physiol Renal Physiol 295(1):F315–F321. doi:10.1152/ajprenal.0 0098.2008

152. Zachos T, Diggs A, Weisbrode S, Bartlett J, Bertone A (2007) Mesenchymal stem cell-mediated gene delivery of bone morphogenetic protein-2 in an articular fracture model. Mol Ther 15(8):1543–1550. doi:10.1038/sj.mt.6300192
153. Gao J, Li Y, Lu S, Wang M, Yang Z, Yan X, Zheng Y (2009) Enhanced in vivo motility of human umbilical cord blood hematopoietic stem/progenitor cells introduced via intra-bone marrow injection into xenotransplanted NOD/SCID mouse. Exp Hematol 37(8):990–997. doi:10.1016/j.exphem.2009.05.006
154. Yong RL, Shinojima N, Fueyo J, Gumin J, Vecil GG, Marini FC, Bogler O, Andreeff M, Lang FF (2009) Human bone marrow-derived mesenchymal stem cells for intravascular delivery of oncolytic adenovirus Delta24-RGD to human gliomas. Cancer Res 69(23):8932–8940. doi:10.1158/0008-5472.CAN-08-3873
155. Wang H, Cao F, De A, Cao Y, Contag C, Gambhir SS, Wu JC, Chen X (2009) Trafficking mesenchymal stem cell engraftment and differentiation in tumor-bearing mice by bioluminescence imaging. Stem Cells 27(7):1548–1558. doi:10.1002/stem.81
156. Pearl JI, Lee AS, Leveson-Gower DB, Sun N, Ghosh Z, Lan F, Ransohoff J, Negrin RS, Davis MM, Wu JC (2011) Short-term immunosuppression promotes engraftment of embryonic and induced pluripotent stem cells. Cell Stem Cell 8(3):309–317. doi:10.1016/j.stem.2011.01.012
157. Boheler KR, Joodi RN, Qiao H, Juhasz O, Urick AL, Chuppa SL, Gundry RL, Wersto RP, Zhou R (2011) Embryonic stem cell-derived cardiomyocyte heterogeneity and the isolation of immature and committed cells for cardiac remodeling and regeneration. Stem Cells Int 2011:214203. doi:10.4061/2011/214203
158. Lee AS, Xu D, Plews JR, Nguyen PK, Nag D, Lyons JK, Han L, Hu S, Lan F, Liu J, Huang M, Narsinh KH, Long CT, de Almeida PE, Levi B, Kooreman N, Bangs C, Pacharinsak C, Ikeno F, Yeung AC, Gambhir SS, Robbins RC, Longaker MT, Wu JC (2011) Preclinical derivation and imaging of autologously transplanted canine induced pluripotent stem cells. J Biol Chem 286(37):32697–32704. doi:10.1074/jbc.M111.235739
159. Williams AR, Hatzistergos KE, Addicott B, McCall F, Carvalho D, Suncion V, Morales AR, Da Silva J, Sussman MA, Heldman AW, Hare JM (2013) Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation 127(2):213–223. doi:10.1161/CIRCULATIONAHA.112.131110
160. Liu J, Cheng EC, Long RC, Yang SH, Wang L, Cheng PH, Yang J, Wu D, Mao H, Chan AW (2009) Noninvasive monitoring of embryonic stem cells in vivo with MRI transgene reporter. Tissue Eng Part C Methods 15(4):739–747. doi:10.1089/ten.TEC2008.0678
161. Burgess A, Ayala-Grosso CA, Ganguly M, Jordao JF, Aubert I, Hynynen K (2011) Targeted delivery of neural stem cells to the brain using MRI-guided focused ultrasound to disrupt the blood-brain barrier. PLoS ONE 6(11):e27877. doi:10.1371/journal.pone.0027877
162. Qiu B, Yang X (2008) Molecular MRI of hematopoietic stem-progenitor cells: in vivo monitoring of gene therapy and atherosclerosis. Nat Clin Pract Cardiovas Med 5(7):396–404. doi:10.1038/ncpcardio1217
163. Chung J, Yamada M, Yang PC (2009) Magnetic resonance imaging of human embryonic stem cells. Curr Protoc Stem Cell Biol. Chapter 5 (Unit 5A):3. doi:10.1002/9780470151808.sc05a03s10
164. Himes N, Min JY, Lee R, Brown C, Shea J, Huang X, Xiao YF, Morgan JP, Burstein D, Oettgen P (2004) In vivo MRI of embryonic stem cells in a mouse model of myocardial infarction. Magn Res Med 52(5):1214–1219. doi:10.1002/mrm.20220
165. Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK (2009) Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. Mol Ther 17(7):1282–1291. doi:10.1038/mt.2009.104
166. Shen J, Zhong XM, Duan XH, Cheng LN, Hong GB, Bi XB, Liu Y (2009) Magnetic resonance imaging of mesenchymal stem cells labeled with dual (MR and fluorescence)

agents in rat spinal cord injury. Acad Radiol 16(9):1142–1154. doi:10.1016/j.acra.2009.03.016
167. Sykova E, Jendelova P (2006) Magnetic resonance tracking of transplanted stem cells in rat brain and spinal cord. Neurodegener Dis 3(1–2):62–67. doi:10.1159/000092095
168. Campan M, Lionetti V, Aquaro GD, Forini F, Matteucci M, Vannucci L, Chiuppesi F, Di Cristofano C, Faggioni M, Maioli M, Barile L, Messina E, Lombardi M, Pucci A, Pistello M, Recchia FA (2011) Ferritin as a reporter gene for in vivo tracking of stem cells by 1.5-T cardiac MRI in a rat model of myocardial infarction. Am J Physiol Heart Circ Physiol 300(6):H2238–H2250. doi:10.1152/ajpheart.0 0935.2010
169. Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, Baumgartner WW, Martin BJ, Heldman AW, Hare JM (2005) Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA 102(32):11474–11479. doi:10.1073/pnas.0504388102
170. Wood JA, Chung DJ, Park SA, Zwingenberger AL, Reilly CM, Ly I, Walker NJ, Vernau W, Hayashi K, Wisner ER, Cannon MS, Kass PH, Cherry SR, Borjesson DL, Russell P, Murphy CJ (2012) Periocular and intra-articular injection of canine adipose-derived mesenchymal stem cells: an in vivo imaging and migration study. J Ocul Pharmacol Ther 28(3):307–317. doi:10.1089/jop2011.0166
171. Lu SS, Liu S, Zu QQ, Xu XQ, Yu J, Wang JW, Zhang Y, Shi HB (2013) In vivo MR imaging of intraarterially delivered magnetically labeled mesenchymal stem cells in a canine stroke model. PLoS ONE 8(2):e54963. doi:10.1371/journal.pone.0054963
172. Meng Y, Zhang F, Blair T, Gu H, Feng H, Wang J, Yuan C, Zhang Z, Qiu B, Yang X (2012) MRI of auto-transplantation of bone marrow-derived stem-progenitor cells for potential repair of injured arteries. PLoS ONE 7(2):e31137. doi:10.1371/journal.pone.0031137
173. Watts AE, Yeager AE, Kopyov OV, Nixon AJ (2011) Fetal derived embryonic-like stem cells improve healing in a large animal flexor tendonitis model. Stem Cell Res Ther 2(1):4. doi:10.1186/scrt45
174. Leng X, Wang J, Fu H, Fisher A, Chen X, Villanueva FS (2009) In-vivo stem cell tracking using ultrasound imaging. Circulation 120(Suppl 18):S326
175. Arpornmaeklong P, Wang Z, Pressler MJ, Brown SE, Krebsbach PH (2010) Expansion and characterization of human embryonic stem cell-derived osteoblast-like cells. Cell Reprogram 12(4):377–389. doi:10.1089/cell.2009.0079
176. Nienhuis AW, Persons DA (2012) Development of gene therapy for thalassemia. Cold Spring Harb Perspect Med 2(11). doi:2/11/a011833
177. Siqueira RC (2011) Stem-cell therapy for retinal diseases In: Kallos PMS (ed) Embryonic stem cells—differentiation and pluripotent alternatives. InTech, p 506
178. Dunnett SB, Bjorklund A (1987) Mechanisms of function of neural grafts in the adult mammalian brain. J Exp Biol 132:265–289
179. Drucker-Colin R, Verdugo-Diaz L (2004) Cell transplantation for Parkinson's disease: present status. Cell Mol Neurobiol 24(3):301–316
180. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. New Engl J Med 344(10):710–719. doi:10.1056/NEJM200103083441002
181. Venkataramana NK, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, Dixit A, Rao DK, Das M, Jan M, Gupta PK, Totey SM (2010) Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. Transl Res 155(2):62–70. doi:S1931-5244(09)00220-5
182. Davies T, Amy Greenwood P, Opitz S, Girard L (2008) Stem cell science: overviews of selected disease areas stem cells and neurodegenerative disease
183. Bachoud-Levi AC, Gaura V, Brugieres P, Lefaucheur JP, Boisse MF, Maison P, Baudic S, Ribeiro MJ, Bourdet C, Remy P, Cesaro P, Hantraye P, Peschanski M (2006) Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term

follow-up study. Lancet Neurol. 5 (4):303–309. doi:S1474-4422(06)70381-7[pii] 10.1016/S1474-4422(06)70381-7
184. Gaura V, Bachoud-Levi AC, Ribeiro MJ, Nguyen JP, Frouin V, Baudic S, Brugieres P, Mangin JF, Boisse MF, Palfi S, Cesaro P, Samson Y, Hantraye P, Peschanski M, Remy P (2004) Striatal neural grafting improves cortical metabolism in Huntington's disease patients. Brain. 127(Pt 1):65–72. doi:10.1093/brain/awh003
185. Mazzini L, Fagioli F, Boccaletti R (2004) Stem-cell therapy in amyotrophic lateral sclerosis. Lancet 364(9449):1936–1937. doi:S0140673604174709
186. Mazzini L, Fagioli F, Boccaletti R, Mareschi K, Oliveri G, Olivieri C, Pastore I, Marasso R, Madon E (2003) Stem cell therapy in amyotrophic lateral sclerosis: a methodological approach in humans. Amyotroph Lateral Scler Other Motor Neuron Disord 4(3):158–161. doi:WEYT8N4CMFY5MVM6
187. Mazzini L, Ferrero I, Luparello V, Rustichelli D, Gunetti M, Mareschi K, Testa L, Stecco A, Tarletti R, Miglioretti M, Fava E, Nasuelli N, Cisari C, Massara M, Vercelli R, Oggioni GD, Carriero A, Cantello R, Monaco F, Fagioli F (2010) Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: a phase I clinical trial. Exp Neurol 223(1):229–237. doi:S0014-4886(09)00317-3
188. Trounson A, Thakar RG, Lomax G, Gibbons D (2011) Clinical trials for stem cell therapies. BMC Med. 9:52. doi:1741-7015-9-52
189. Acharya VN (2012) Stem cell therapy for kidney disease—present and future. J Assoc Physicians India 60:32–33
190. Trivedi HL, Vanikar AV, Vakil JM, Shah VR, Modi PR, Trivedi VB (2004) A strategy to achieve donor-specific hyporesponsiveness in cadaver renal allograft recipients by donor haematopoietic stem cell transplantation into the thymus and periphery. Nephrol Dial Transplant 19(9):2374–2377. doi:10.1093/ndt/gfh27419/9/2374
191. Thrasher AJ, Hacein-Bey-Abina S, Gaspar HB, Blanche S, Davies EG, Parsley K, Gilmour K, King D, Howe S, Sinclair J, Hue C, Carlier F, von Kalle C, de Saint Basile G, le Deist F, Fischer A, Cavazzana-Calvo M (2005) Failure of SCID-X1 gene therapy in older patients. Blood 105(11):4255–4257. doi:2004-12-4837
192. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, Al Ghonaium A, Bayford J, Brown L, Davies EG, Kinnon C, Thrasher AJ (2011) Long-term persistence of a polyclonal T cell repertoire after gene therapy for X-linked severe combined immunodeficiency. Sci Transl Med 3(97):97ra79. doi:3/97/97ra79
193. Hacein-Bey-Abina S, Le Deist F, Carlier F, Bouneaud C, Hue C, De Villartay JP, Thrasher AJ, Wulffraat N, Sorensen R, Dupuis-Girod S, Fischer A, Davies EG, Kuis W, Leiva L, Cavazzana-Calvo M (2002) Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. New Engl J Med 346(16):1185–1193. doi:10.1056/NEJMoa012616346/16/1185
194. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, Down J, Denaro M, Brady T, Westerman K, Cavallesco R, Gillet-Legrand B, Caccavelli L, Sgarra R, Maouche-Chretien L, Bernaudin F, Girot R, Dorazio R, Mulder GJ, Polack A, Bank A, Soulier J, Larghero J, Kabbara N, Dalle B, Gourmel B, Socie G, Chretien S, Cartier N, Aubourg P, Fischer A, Cornetta K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P (2010) Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 467(7313):318–322. doi:nature09328
195. Riviere I, Dunbar CE, Sadelain M (2012) Hematopoietic stem cell engineering at a crossroads. Blood 119(5):1107–1116. doi:blood-2011-09-349993
196. Lehmann R, Pavlicek V, Spinas GA, Weber M (2005) Islet transplantation in type I diabetes mellitus. Ther Umsch 62(7):481–486
197. Gu W, Hu J, Wang W, Li L, Tang W, Sun S, Cui W, Ye L, Zhang Y, Hong J, Zhu D, Ning G (2012) Diabetic ketoacidosis at diagnosis influences complete remission after treatment with hematopoietic stem cell transplantation in adolescents with type 1 diabetes. Diabetes Care 35(7):1413–1419. doi:dc11-2161

198. Haller MJ, Wasserfall CH, McGrail KM, Cintron M, Brusko TM, Wingard JR, Kelly SS, Shuster JJ, Atkinson MA, Schatz DA (2009) Autologous umbilical cord blood transfusion in very young children with type 1 diabetes. Diabetes Care 32(11):2041–2046. doi:dc09-0967
199. Miki T (2011) Amnion-derived stem cells: in quest of clinical applications. Stem Cell Res Ther 2(3):25. doi:scrt66
200. Hou Y, Huang Q, Liu T, Guo L (2008) Human amnion epithelial cells can be induced to differentiate into functional insulin-producing cells. Acta Biochim Biophys Sin (Shanghai) 40(9):830–839
201. Rancea M, Skoetz N, Monsef I, Hubel K, Engert A, Bauer K (2012) Fourteenth biannual report of the Cochrane haematological malignancies group–focus on autologous stem cell transplantation in hematological malignancies. J Natl Cancer Inst 104(14):NP. doi:djs278
202. Michallet M, Dreger P, Sutton L, Brand R, Richards S, van Os M, Sobh M, Choquet S, Corront B, Dearden C, Gratwohl A, Herr W, Catovsky D, Hallek M, de Witte T, Niederwieser D, Leporrier M, Milligan D (2011) Autologous hematopoietic stem cell transplantation in chronic lymphocytic leukemia: results of European intergroup randomized trial comparing autografting versus observation. Blood 117(5):1516–1521. doi:blood-2010-09-308775
203. Sutton L, Chevret S, Tournilhac O, Divine M, Leblond V, Corront B, Lepretre S, Eghbali H, Van Den Neste E, Michallet M, Maloisel F, Bouabdallah K, Decaudin D, Berthou C, Brice P, Gonzalez H, Chapiro E, Radford-Weiss I, Leporrier N, Maloum K, Nguyen-Khac F, Davi F, Lejeune J, Merle-Beral H, Leporrier M (2011) Autologous stem cell transplantation as a first-line treatment strategy for chronic lymphocytic leukemia: a multicenter, randomized, controlled trial from the SFGM-TC and GFLLC. Blood 117(23):6109–6119. doi:blood-2010-11-317073
204. Socie G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, Volin L, Ruutu T, Heim DA, Schwerdtfeger R, Kolbe K, Mayer J, Maertens JA, Linkesch W, Holler E, Koza V, Bornhauser M, Einsele H, Kolb HJ, Bertz H, Egger M, Grishina O, Finke J (2011) Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. Blood 117(23):6375–6382. doi:blood-2011-01-329821
205. George RE, Li S, Medeiros-Nancarrow C, Neuberg D, Marcus K, Shamberger RC, Pulsipher M, Grupp SA, Diller L (2006) High-risk neuroblastoma treated with tandem autologous peripheral-blood stem cell-supported transplantation: long-term survival update. J Clin Oncol 24(18):2891–2896. doi:24/18/2891
206. Berthold F, Boos J, Burdach S, Erttmann R, Henze G, Hermann J, Klingebiel T, Kremens B, Schilling FH, Schrappe M, Simon T, Hero B (2005) Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. Lancet Oncol 6(9):649–658. doi:S1470-2045(05)70291-6
207. Wollert KC, Drexler H (2010) Cell therapy for the treatment of coronary heart disease: a critical appraisal. Nat Rev Cardiol. 7(4):204–215. doi:nrcardio.2010.1
208. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H (2004) Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 364(9429):141–148. doi:10.1016/S0140-6736(04)16626-9S0140673604166269
209. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, Kalantzi M, Herbots L, Sinnaeve P, Dens J, Maertens J, Rademakers F, Dymarkowski S, Gheysens O, Van Cleemput J, Bormans G, Nuyts J, Belmans A, Mortelmans L, Boogaerts M, Van de Werf F (2006) Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 367(9505):113–121. doi:S0140-6736(05)67861-0
210. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM

(2006) Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. New Engl J Med 355(12):1210–1221. doi:355/12/1210
211. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjornerheim R, Brekke M, Muller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K (2006) Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. New Engl J Med 355(12):1199–1209. doi:355/12/1199
212. Huikuri HV, Kervinen K, Niemela M, Ylitalo K, Saily M, Koistinen P, Savolainen ER, Ukkonen H, Pietila M, Airaksinen JK, Knuuti J, Makikallio TH (2008) Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 29(22):2723–2732. doi:ehn436
213. Tendera M, Wojakowski W, Ruzyllo W, Chojnowska L, Kepka C, Tracz W, Musialek P, Piwowarska W, Nessler J, Buszman P, Grajek S, Breborowicz P, Majka M, Ratajczak MZ (2009) Intracoronary infusion of bone marrow-derived selected CD34+ CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre myocardial regeneration by intracoronary infusion of selected population of stem cells in acute myocardial infarction (REGENT) trial. Eur Heart J 30(11):1313–1321. doi:ehp073
214. van der Laan A, Hirsch A, Nijveldt R, van der Vleuten PA, van der Giessen WJ, Doevendans PA, Waltenberger J, Ten Berg JM, Aengevaeren WR, Zwaginga JJ, Biemond BJ, van Rossum AC, Tijssen JG, Zijlstra F, Piek JJ (2008) Bone marrow cell therapy after acute myocardial infarction: the HEBE trial in perspective, first results. Neth Heart J 16(12):436–439
215. Losordo DW, Schatz RA, White CJ, Udelson JE, Veereshwarayya V, Durgin M, Poh KK, Weinstein R, Kearney M, Chaudhry M, Burg A, Eaton L, Heyd L, Thorne T, Shturman L, Hoffmeister P, Story K, Zak V, Dowling D, Traverse JH, Olson RE, Flanagan J, Sodano D, Murayama T, Kawamoto A, Kusano KF, Wollins J, Welt F, Shah P, Soukas P, Asahara T, Henry TD (2007) Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. Circulation 115(25):3165–3172. doi:CIRCULATIONAHA.106.687376
216. Jonas JB, Witzens-Harig M, Arseniev L, Ho AD (2010) Intravitreal autologous bone-marrow-derived mononuclear cell transplantation. Acta Ophthalmol 88(4):e131–e132. doi:AOS1564
217. Jonas JB, Witzens-Harig M, Arseniev L, Ho AD (2008) Intravitreal autologous bone marrow-derived mononuclear cell transplantation: a feasibility report. Acta Ophthalmol 86(2):225–226. doi:AOS987
218. Yong Zhao M, PhD phase 1/phase 2 study of stem cell educator therapy in alopecia areata
219. Lataillade JJ, Doucet C, Bey E, Carsin H, Huet C, Clairand I, Bottollier-Depois JF, Chapel A, Ernou I, Gourven M, Boutin L, Hayden A, Carcamo C, Buglova E, Joussemet M, de Revel T, Gourmelon P (2007) New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy. Regen Med 2(5):785–794. doi:10.2217/17460751.2.5.785
220. Heile A, Brinker T (2011) Clinical translation of stem cell therapy in traumatic brain injury: the potential of encapsulated mesenchymal cell biodelivery of glucagon-like peptide-1. Dialogues Clin Neurosci 13(3):279–286
221. Marcacci M, Kon E, Moukhachev V, Lavroukov A, Kutepov S, Quarto R, Mastrogiacomo M, Cancedda R (2007) Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng 13(5):947–955. doi:10.1089/ten 2006.0271
222. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK (1999) Transplantability and therapeutic

effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5(3):309–313. doi:10.1038/6529
223. Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, McCarville ME, Orchard PJ, Pyeritz RE, Brenner MK (2001) Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. Blood 97(5):1227–1231
224. Gordon MY, Levicar N, Pai M, Bachellier P, Dimarakis I, Al-Allaf F, M'Hamdi H, Thalji T, Welsh JP, Marley SB, Davies J, Dazzi F, Marelli-Berg F, Tait P, Playford R, Jiao L, Jensen S, Nicholls JP, Ayav A, Nohandani M, Farzaneh F, Gaken J, Dodge R, Alison M, Apperley JF, Lechler R, Habib NA (2006) Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells 24(7):1822–1830. doi:2005-0629
225. Levicar N, Pai M, Habib NA, Tait P, Jiao LR, Marley SB, Davis J, Dazzi F, Smadja C, Jensen SL, Nicholls JP, Apperley JF, Gordon MY (2008) Long-term clinical results of autologous infusion of mobilized adult bone marrow derived CD34+ cells in patients with chronic liver disease. Cell Prolif 41(Suppl 1):115–125. doi:CPR491
226. Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, Yokoyama Y, Uchida K, Yamasaki T, Fujii Y, Okita K, Sakaida I (2006) Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. Stem Cells 24(10):2292–2298. doi:2005-0542
227. Lyra AC, Soares MB, da Silva LF, Fortes MF, Silva AG, Mota AC, Oliveira SA, Braga EL, de Carvalho WA, Genser B, dos Santos RR, Lyra LG (2007) Feasibility and safety of autologous bone marrow mononuclear cell transplantation in patients with advanced chronic liver disease. World J Gastroenterol 13(7):1067–1073
228. Gasbarrini A, Rapaccini GL, Rutella S, Zocco MA, Tittoto P, Leone G, Pola P, Gasbarrini G, Di Campli C (2007) Rescue therapy by portal infusion of autologous stem cells in a case of drug-induced hepatitis. Dig Liver Dis 39(9):878–882. doi:S1590-8658(06)00312-4
229. Pai M, Spalding D, Xi F, Habib N (2012) Autologous bone marrow stem cells in the treatment of chronic liver disease. Int J Hepatol 2012:307165. doi:10.1155/2012/307165
230. Zhang Z, Lin H, Shi M, Xu R, Fu J, Lv J, Chen L, Lv S, Li Y, Yu S, Geng H, Jin L, Lau GK, Wang FS (2012) Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. J Gastroenterol Hepatol 27(Suppl 2):112–120. doi:10.1111/j.1440-1746.2011.07024.x
231. Kharaziha P, Hellstrom PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, Telkabadi M, Atashi A, Honardoost M, Zali MR, Soleimani M (2009) Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. Eur J Gastroenterol Hepatol 21(10):1199–1205. doi:10.1097/MEG.0b013e32832a1f6c
232. Persidis A (1999) Tissue engineering. Nat Biotechnol 17(5):508–510. doi:10.1038/8700
233. McKernan R, McNeish J, Smith D (2010) Pharma's developing interest in stem cells. Cell Stem Cell 6(6):517–520. doi:S1934-5909(10)00222-5
234. Kim HJ, Jin CY (2012) Stem cells in drug screening for neurodegenerative disease. Korean J Physiol Pharmacol 16(1):1–9. doi:10.4196/kjpp.2012.16.1.1

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