Chapter 1
Introduction to Stem Cell Therapy and Its Application in Vascular Diseases

Lara Lellis Navarro Minchillo Lopes, Tulio Pinho Navarro, and Alan Dardik

1.1 Introduction

1.1.1 Brief History of Stem Cells

Stem cells are undifferentiated cells capable of both self-renewal and differentiation into various specialized cells [1]. Stem cell therapy is the therapeutic administration of stem cells to repair or replace tissue function [2]. The term "stem cell" was proposed by Alexander Maksimov in 1908 when developing "the unitarian theory of hematopoiesis," which proposed a common stem cell progenitor for all blood elements (Fig. 1.1) [3]. However, it was only in 1961 that the existence of murine cells capable of self-renewal was proven by Till et al. while assessing radiation sensitivity of bone marrow tissue [4].

In 1962, John Gurdon performed a classic experiment in frogs, in which he replaced the immature cell nucleus in an egg cell with the nucleus from a mature intestinal cell, resulting in a normal tadpole; this experiment showed that the DNA of the mature cell had all the information needed to develop all cells in the organism, challenging the dogma that the specialized cell is irreversibly committed to its fate [5].

In 1968, Friedenstein et al. reported the discovery of a human bone marrow cell population with high proliferative potential and osteogenic activity in vivo [6]. In the same year, Thomas et al. performed the first hematopoietic stem cell

L. L. N. M. Lopes
Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

T. P. Navarro (✉)
Department of Surgery, Vascular Surgery Section, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

A. Dardik
Vascular Biology and Therapeutics Program and the Departments of Surgery and Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA

T. P. Navarro et al. (eds.), *Stem Cell Therapy for Vascular Diseases*,
https://doi.org/10.1007/978-3-030-56954-9_1

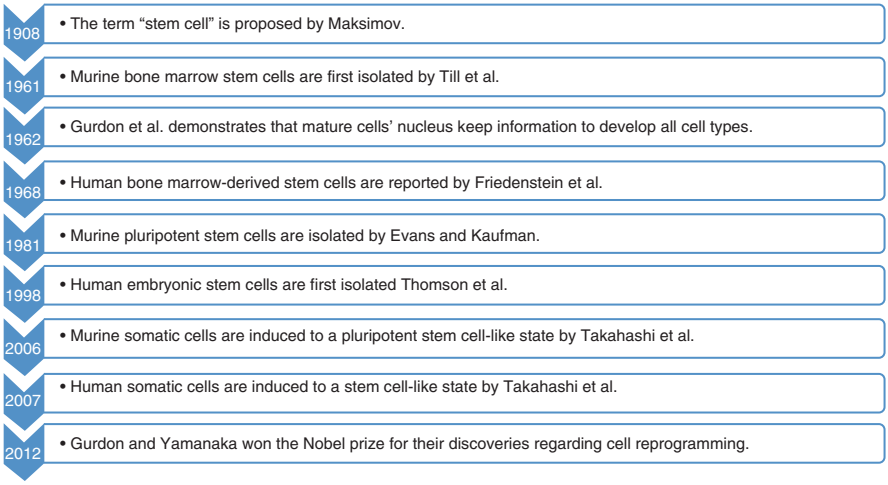

Fig. 1.1 Timeline with the main events related to stem cell therapy history

transplantation for the treatment of leukemia [7]. Since then, theoretical implications of human stem cells and their potential clinical applications have been extensively studied.

In 1981, Evans and Kaufman reported the isolation of murine pluripotent embryonic stem cells, and this was followed by the report of Thomson et al., who first isolated human embryonic stem cells in 1998 [8, 9]. Shinya Yamanaka and Kazutoshi Takahashi identified several genes that kept cells immature while performing research on murine embryonal stem cells in 2006. After that, they were able to reprogram fibroblasts into immature stem cells; these resulting induced pluripotent stem cells (iPSC) could develop into mature cell types such as fibroblasts, nerve cells, and gut cells, demonstrating that intact, mature cells could be reprogrammed into pluripotent stem cells [10]. Later, in 2007, they reported induction of human fibroblasts into a pluripotent state [11]. Takahashi’s experiments reinforced the concept of using iPSC as a novel technological frontier in stem cell therapy perspectives. In 2012, the Nobel Prize was awarded to John B. Gurdon and Shinya Yamanaka in recognition of their findings showing that mature, specialized cells could be reprogrammed, creating new opportunities to study diseases and to develop new methods for diagnosis and treatment [12].

1.1.2 Mesenchymal Stem Cells vs Mesenchymal Stromal Cells and Cell Markers

In 1991, the term “mesenchymal stem cell” (MSC) was proposed by Caplan et al. to designate adult stem cells capable of differentiating into cells of mesodermal origin [13]. However, as these cells showed limited capacity for self-renewal, thus they

failed to meet the criteria to be called stem cells. As such, the term "mesenchymal stromal cell," also abbreviated MSC, was then suggested as a more appropriate designation to these regenerative cells [14].

In 2005, the International Society for Cell and Gene Therapy (ISCT) declared that the terms "stem cell" and "stromal cell" were not equivalent [15]. Furthermore, the term "stem cell" should be limited to a population of cells with demonstrable self-renewal and differentiation capacities, while the term "stromal cell" referred to cells with notable secretory, immunomodulatory, and homing features [16]. In addition, the ISCT provided the following minimal criteria to identify mesenchymal stromal cells: being adherent to plastic; capable of differentiation into adipocyte, chondrocyte, and osteoblast lineages; expression of CD73, CD90, and CD105; and lack of expression of CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA-DR (Fig. 1.2).

Since then, stromal cell surface markers were shown to be plastic and influenced by microenvironmental conditions and stem cell origin among other variables [16–18]. Therefore, there are no specific and unambiguous cell surface markers to distinguish stem cells from stromal cells [16]. Ironically, since the aforementioned ISCT statement, the interchangeable use of the terms "stromal" and "stem" cells by the scientific community has spread. A search in the US National Library of Medicine database (clinicaltrials.gov), performed in November 21, 2019, reported 1009 clinical trials related to the term "mesenchymal stem cell," while only 211 results were related to the term "mesenchymal stromal cell" [19]. Few authors have reported complete mesenchymal stromal cell characterization in both preclinical and clinical publications, and the use of the term "mesenchymal stem cell" remains controversial [16]. Hence, for the purpose of this chapter, the abbreviation "MSC" will be employed to designate populations of regenerative adult mesenchymal cells.

Most of the available data on clinical stem cell therapy relies on adult stem cells and most frequently MSC. Moreover, the use of embryonal stem cells and induced

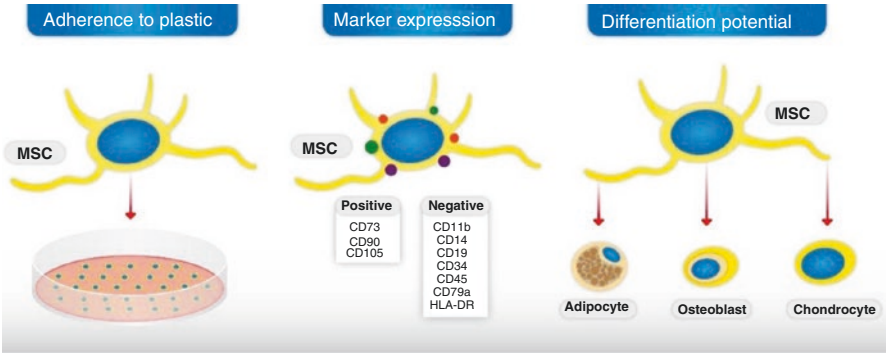

Fig. 1.2 MSC criteria according to the International Society for Cell and Gene Therapy

pluripotent stem cells raises important ethical and safety concerns that impair the use of these two cell types in clinical situations. Therefore, MSC are the main focus of this chapter.

1.2 Differentiation Potential

Stem cells can be classified according to their differentiation potential into totipotent, pluripotent, multipotent, and oligopotent cells. Totipotent stem cells are found in the zygote and can differentiate in both embryonic and extraembryonic cells. Pluripotent cells are also capable of giving rise to any type of embryonic cell, but not to extraembryonic tissue. Multipotent cells give rise to multiple cells of the same lineage whereas oligopotent stem cells have a narrower differentiation spectrum within a same lineage [1].

1.3 Stem Cell Types

Stem cells can be classified into embryonic stem cells (ESC), adult stromal cells, and iPSC according to their origin (Fig. 1.3). The diverse characteristics, advantages, and limitations of these groups (Fig. 1.4) lead to different clinical applications.

1.3.1 Embryonic Stem Cells (ESC)

ESC are stem cells derived from the embryonic inner cell mass and possess unlimited self-renewal capability as well as the ability to differentiate into any type of somatic cell. Even though these cells have potential clinical application, clinical use of these cells is scarce due to ethical conflicts involving the harvest and manipulation of human embryos to obtain these cells. Additionally, embryonic stem cells' unlimited

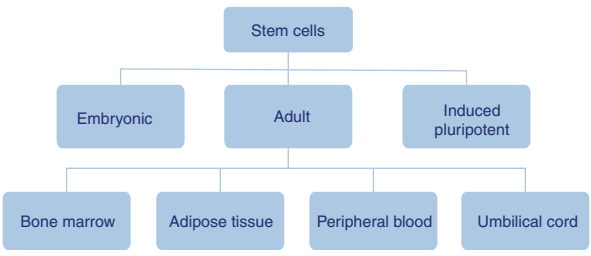

Fig. 1.3 Stem cell types

	Stem Cell Type					
	Adult Stem Cells				Embryonic	IPSC
	BM-MSC	PB-MSC	UC-MSC	ADSC		
Advantages	• Donor-specific therapy • Lower malignancy risk • Cell-lineage committed (targeting differentiation) • No ethical conflict	• Donor-specific therapy • Lower malignancy risk • Cell-lineage committed (targeting differentiation) • No ethical conflict • Relatively disposable tissue • Vein puncture has low surgical risk • Simple cell harvesting protocol	• Future donor-specific therapy • Lower malignancy risk • Cell-lineage committed (targeting differentiation) • Disposable tissue • UC tissue harvesting has low surgical risk • Donor UCB banking storage	• Donor-specific therapy • Lower malignancy risk • Cell-lineage committed (targeting differentiation) • No ethical conflict • Disposable tissue • Liposuction has low surgical risk	• High differentiation potential (pluripotent)	• High differentiation potential (pluripotent) • Somatic-cell memory (targeting differentiation) • Donor-specific therapy • No ethical conflict • Disposable tissue • Low cell harvesting procedure risk
Disadvantages	• Cell lineage committed • Biopsy high surgical risk • Non-disposable tissue • Low stem cell concentration • Cell concentration and performance influenced by comorbities	• Cell lineage committed • Cell concentration and performance influenced by comorbities • G-CSF administration needed	• Cell lineage committed • Immuno incompatibility • Ethical conflict • Low stem cell concentration • Need of UCB banking	• Cell lineage committed • Cell concentration and performance influenced by comorbities	• Increased malignancy risk • Immuno incompatibility • Ethical conflicts	• Increased malignancy risk • Complex induction protocol • Somatic-cell memory (biased differentiation)

Fig. 1.4 Stem cell types, advantages and disadvantages [36]

differentiation potential lead to a higher risk of malignancy and requires immunomodulation as the use of allogeneic cells is mandatory for clinical therapy [20, 21].

1.3.2 Adult Stem Cells (ASC)

ASC are partially undifferentiated cells that can be found within nearly any organ or tissue. Peripheral blood, umbilical cord, bone marrow, and adipose tissue are the most common sources of such cells. Endometrium, amniotic fluid and membrane, placenta, dental tissues, thymus, and spleen are unconventional sources of ASC. ASC may be isolated from the same patient in whom the cell therapy will be applied (donor-specific therapy) with no risk of immune rejection. There is no ethical conflict regarding the origin of these adult-derived cells or their isolation; however, isolation protocols for ASC can be laborious, and a significant quantity of tissue is frequently needed to obtain high stem cell counts [20].

ASC are lineage-committed multipotent cells, being able to differentiate only into cells of the same germ layer [20]. This feature accounts for the lower malignancy potential of ASC, and therefore these cells may be desirable to be used in therapies that target specific cell differentiation, such as host tissue replacement.

1.3.3 Induced Pluripotent Stem Cells (iPSC)

iPSC are stem cells generated through somatic cell reprogramming into an embryonic stem cell-like state, first reported in 2006 [10]. In theory, iPSC can be generated from any somatic cell, and thus the use of these cells is emerging as an abundant and accessible source of donor-specific stem cells free of ethical conflicts. These totipotent cells demonstrate a broad differentiation potential, being capable of generating any somatic or trophoblastic cell, but they also have a high malignant potential [22].

Because iPSC show advantages of both ASC, e.g., donor-specific therapy and absence of ethical conflicts, and ESC, e.g., differentiation potential, this novel source of stem cells is considered very promising. Nonetheless, strategies to enhance the yield of cell induction into the stem cell-like state and simultaneously control cell differentiation with absence of malignancy are still hurdles to be overcome.

1.4 Stem Cell Origin

Stem cell therapy can be classified according to the cell origin as either autologous, allogeneic, or xenogeneic transplantation (Fig. 1.5).

	Stem cell origin		
	Autologous	**Allogeneic**	**Xenotransplantation**
Advantages	• Immunoincompatibility • No ethical conflict • No infection transmission risk	• Healthy stem cell source • No cell harvesting risk for the host patient • Donor banking creation	• No ethical conflict • Heathy stem cell source • No cell harvesting risk for the host patient • Donor baking creation
Disadvantages	• Lower stem cell concentration and limited healing potential • Cell harvestmg procedural risk	• Relative immunoincompatibility • Need of disease screening • Ethical conflict	• High immunoincompatibility • Need of disease screening

Fig. 1.5 Stem cell origins, advantages, and disadvantages [36]

1.4.1 Autologous Transplantation

Autologous transplantation involves the administration of the recipient's own cells, typically using either ASC or iPSC. Autologous cells have the advantages of being immunocompatible, having no risk of infectious disease transmission and involving no ethical or legal issues. On the other hand, donor characteristics such as advanced age, diabetes, obesity, and atherosclerosis exert negative impact on stem cell function, potentially decreasing the effectiveness of cell therapy [23–27]. Therefore, past medical history and advanced patient age may be some of the limitations to the use of autologous ASC for cell therapy.

1.4.2 Allogeneic Transplantation

Allogeneic cell transplantation is the exchange of cells between a donor and a recipient of the same species. The use of cells isolated from a healthier or younger donor could enhance the efficacy of stem cell therapy. However, allogeneic transplantation has the drawbacks of lower immunocompatibility, risk of disease transmission, and potential legal issues regarding exchange of biomaterials.

It has been hypothesized that allogeneic MSC are immunoprivileged. Nonetheless, further research has shown that allogeneic MSC are not privileged, but demonstrate diminished immunogenicity compared with other allogeneic cell types [28]. The therapeutic implications of allogeneic MSC immunogenicity are controversial as cell apoptosis is crucial for immunomodulatory effects but reduces therapeutic cell longevity and engraftment [29].

Strict donor screening is needed to avoid disease transmission by allogeneic cell therapy [2]. The use of cadaveric cells may be an alternative to enhance donor availability and to avoid the risks associated to tissue harvesting therapeutic cells [30].

1.4.3 Xenotransplantation

Xenotransplantation involves the administration of either nonhuman live cells or human biological material that has had ex vivo contact with live nonhuman animal cells [31]. The use of animal stem cells has the potential to increase availability of donors and to reduce the financial burden related to stem cell transplantation. However, this source raises concerns regarding immunologic rejection and disease transmission.

1.5 Stem Cell Isolation and Induction Protocols

The tissue source of stem cells is the main determinant of the required isolation protocol and influences MSC phenotypes and function [14]. Here we discuss basic aspects of stem cell isolation and induction protocols and their clinical implications.

1.5.1 Adult Stem Cells

1.5.1.1 Bone Marrow-Derived MSC (BM-MSC)

BM-MSC are isolated from the bone marrow by density gradient centrifugation, washing, and seeding on culture dishes. BM-MSC can be selected by survival in minimum essential media with fetal bovine serum and by their adherence to plastic [14, 32]. However, these isolation protocols usually result in a heterogeneous cell population, contaminated by other cells [33].

Bone marrow tissue is not a disposable tissue and is necessarily obtained by bone marrow aspiration. The aspiration is an invasive procedure, which leads to higher risks to the donor when compared to other MSC isolation protocols.

1.5.1.2 Adipose-Derived Stem Cells (ADSC)

Adipose tissue is a relatively disposable cell source and can be easily accessed by lipoaspiration, which is considered a minimally invasive procedure. Adipose tissue processing leads to a heterogenic population termed the “stromal vascular fraction” (SVF). The SVF is composed of adipose-derived stem cells (ADSC), pericytes, endothelial cells, pre-adipocytes and immune cells as well as other cell types [34].

Isolation of the SVF may be performed either by enzymatic or mechanical protocols that generally comprise washes, agitation, centrifugation, and collagenase digestion (in enzymatic protocols) [35]. Currently, semi-automated SVF isolation devices are commercially available [34]. ADSC and SVF have been used in clinical

trials, but ADSC isolation protocols frequently fail to exclude the other components of the SVF, leading to questions of reproducibility and translatability [14, 34].

1.5.1.3 Peripheral Blood-Derived MSC (PB-MSC)

Peripheral blood is a relatively disposable stem cell source and can be obtained by venipuncture, a minimally invasive procedure. Peripheral blood-derived MSC (PB-MSC) are isolated by centrifugation and dilution protocols. PB-MSC usually circulate in low concentrations; therefore, bone marrow stimulation by granulocyte colony-stimulating factor (G-CSF) is an important adjunct to mobilize PB-MSC into the circulation before collecting blood [36].

G-CSF administration itself is effective in the treatment of diabetic foot ulcers [36]. Thus, the administration of G-CSF may be a confounding variable in study interpretation as well as its associated financial cost.

1.5.1.4 Other Adult Stem Cell Sources

Umbilical cord blood, placenta, Wharton's jelly, amniotic fluid, dental pulp, synovial fluid, and skin are also MSC sources, although less commonly used.

1.5.2 Embryonic Stem Cells (ESC)

The isolation of human embryonic stem cells (hESC) implies the destruction of the embryo. Due to associated ethical and political concerns, research involving the use of these cells has been deferred if not banned. Nevertheless, isolation, culture, and characterization protocols for hESC have been developed [37].

hESC are found in the inner cell mass of both fresh and frozen embryos. Several isolation techniques are described in the literature including mechanical dissection, laser dissection, and immunosurgery [9, 38, 39]. There is no consensus regarding the best method as each of these techniques have specific advantages and disadvantages regarding success rate and financial and time constraints [37].

1.5.3 Induced Pluripotent Stem Cells (iPSC)

iPSC are stem cells induced from somatic cells; as such they are not isolated per se. The use of iPSC has obviated ethical conflicts regarding the use of hESC and has provided insights into early embryo development. The first method to successfully induce human iPSC was the use of four transcription factors Oct3/4, Sox2, Klf4, and c-Myc, under ES cell culture conditions [11]. Currently, several induction

methods have been used to generate iPSC including integrating vectors, non-integrating vectors, non-DNA reprogramming, and small molecules [40]. Pluripotency induction and maintenance are based on the interaction of both extrinsic and intrinsic factors [41]. The extrinsic factors involve cytokines, growth factors, and extracellular matrix; extrinsic elements influence intrinsic pathways that reverse epigenetic programming of differentiated somatic cells, such as octamer-binding transcription factor 4 (Oct4) and sex-determining region Y-box 2 (Sox2) [42].

The low cell reprogramming yield (0.01–0.02% when first described), expression of transgenic viral genes, and tumorigenesis by overexpression of oncogenes (c-Myc, kfl4) are major concerns of iPSC induction protocols [11, 43]. Further advances on cell reprogramming techniques are required to enable iPSC use in clinical scenarios.

1.6 Stem Cell Culture and Priming

Culture conditions such as media composition, oxygen tension, and extracellular structure affect stem cell survival, differentiation, and function [14, 43]. Furthermore, several cell priming techniques have been proposed as strategies to enhance stem cell therapeutic effects. Understanding and electing the best cell culture and priming protocol is important not only to enhance their regenerative potential but also for reproducible cell expansion after low yield isolation protocols and target differentiation.

1.6.1 Culture Media

Medium containing either fetal bovine serum (FBS), human AB serum (HABS), human platelet lysate (HPL), or chemically defined media (CMD) is commonly used in MSC culture [14]. FBS is the most common supplement, although it carries the risk of xenogeneic immune reaction and has a variable composition [44]. HABS and HPL are derived from peripheral blood that have been proposed as human-derived immunocompatible alternatives to FBS. However, their composition is also highly variable, and there is a risk of infection transmission [45]. In addition, HPL has pro-inflammatory factors and may alter MSC cell markers, affecting its functionality [14, 46]. Serum-free, xenobiotic-free CMD can improve cell expansion and differentiation and is considered a desirable alternative for MSC culture for clinical use [47].

1.6.2 Hypoxia

Hypoxic culture conditions (1–10% O_2 partial pressure) mimic hypoxic areas within the bone marrow where BM-MSC can be found physiologically (4–7% O_2 tension) [14]. However, hypoxia affects stem cells from other sources by influencing cell

metabolism, proliferation, differentiation, and the secretome [48]. Since hypoxia intensity is variable among stem cell studies that use hypoxic conditioning, conclusions regarding the effects of hypoxia need to be cautiously interpreted.

Low oxygen availability lowers metabolism and production of reactive oxygen species (ROS), a mechanism that is thought to be responsible for reduced stem cell injury [48]. In addition, the decrease in ROS added to hypoxia-mediated upregulation of c-jun leads to delayed stem cell senescence and increases immunosuppression [49].

Upregulation of hypoxia-inducible factors 1α and 2α (HIF-1α and HIF-2α), vascular endothelial growth factor (VEGF), and angiopoietin-1 (Ang-1) lead to increase angiogenic potential [50, 51]. This feature is especially desirable when treating ischemic and inflammatory pathologies. Moreover, HIF-2α inhibits p53, increasing the regenerative potential of stem cells [51].

Hypoxic-cultured MSC (2% O_2 tension) were reported to be more proliferative when compared to MSC cultured in normoxic conditions (20%) [52]. The increased cell proliferation compensates for the initial hypoxia-mediated MSC apoptosis in subsequent cell passages. Furthermore, hypoxia favors maintenance of stem cells' undifferentiated state and increases cell motility [50, 53].

1.6.3 Culture Matrices and Devices

Stem cells are often cultured in plastic containers such as T-flasks and well plates. However, these two-dimensional devices are associated with alteration of cell markers as well as reduced capacity for differentiation and proliferation [54, 55]. As a result, a variety of three-dimensional culture systems and matrices composed of collagen, hyaluronic acid, glycosaminoglycan, polyethylene glycol, and alginate have been investigated as alternative matrices for stem cell culture [14, 56].

In an attempt to reproduce the three-dimensional native environment of MSC, spinner flasks, wavy-walled cultures, and bioreactors were proposed and developed as three-dimensional culture systems. Bioreactors demonstrate important advantages besides cost: faster cell expansion, reduced risk of contamination, higher rate of cytokine production, rigorous monitoring, and control of culture parameters [57–59].

Besides three-dimensional structure, the inherent material properties of the cell culture system, such as rigidity and composition, directly influence expression of MSC markers, the cell secretome, and cell viability and are considered as part of the strategy to target stem cell differentiation into specific cell populations [60]. MSC marker expression shifts from neurogenic toward myogenic or osteogenic markers as rigidity increases from low to intermediate and high stiffness, respectively [61]. In addition, the use of extracellular matrix as a surface for cell growth enhances VEGF and glial-derived neurotropic factor production while decreasing synthesis of interleukin-6 when compared to the use of tissue-culture plastic [62].

Besides the aforementioned inherent properties of the culture systems, the mechanism of cell release from the matrix and the route of cell administration need to be considered when choosing an optimal culture matrix, as they affect cell yield and viability. Stem cells cultured in biocompatible microcarriers, such as poly-ε-caprolactone, can be directly administrated without the need of enzymatic release [63]. The administration of cells associated with these types of carriers preserves yield and functionality while providing a microarchitecture design for tissue regeneration [63, 64].

Another trypsin-free alternative is the use of thermoresponsive polymers, such as poly-N-isopropylacrilamide, which are able to reversibly expand and adhere according to the temperature. Hydrogels composed of these polymers are able to transit in aqueous solutions upon temperature increase [65]. Thermoresponsive polymers have been investigated as an alternative to increase stem cell yield, homing, and engraftment.

1.6.4 Stem Cell Priming

Cell priming refers to techniques used to trigger stem cell "memory," inducing cell activity toward a specific therapeutic purpose. Stem cells may be primed for different therapeutic purposes such as to promote immunomodulation, cell homing, target differentiation, and decrease apoptosis [14]. Priming techniques include the use of various stimuli such as pharmaceutical (valproic acid, progesterone), interleukins (IL-1, IFN-γ), genetic (dsRNA), and environmental (three-dimensional structure, hypoxia), among others [14].

Priming is an important strategy to enhance the overall effectiveness of stem cell therapy. Nonetheless, it is especially important when guiding and limiting ESC and iPSC proliferation and differentiation as these pluripotent stem cells demonstrate increased oncogenicity.

1.7 Routes of Stem Cell Administration

Cell delivery is a crucial step in stem cell therapy since administration routes can enhance cell survival and functionality, increasing stem cell therapy effectiveness in hostile microenvironmental conditions [66]. Intravascular administration is considered systemic therapy, whereas injections into a particular site and topical administration are local delivery methods (Fig. 1.6). Both systemic (intravascular) and local delivery methods have demonstrated effectiveness in the treatment of vascular pathologies [67]. Various diseases and clinical scenarios require different cell mechanisms of action, and there are a lack of studies comparing cell delivery methods [68]. Accordingly, no consensus regarding the optimal administration route has been achieved.

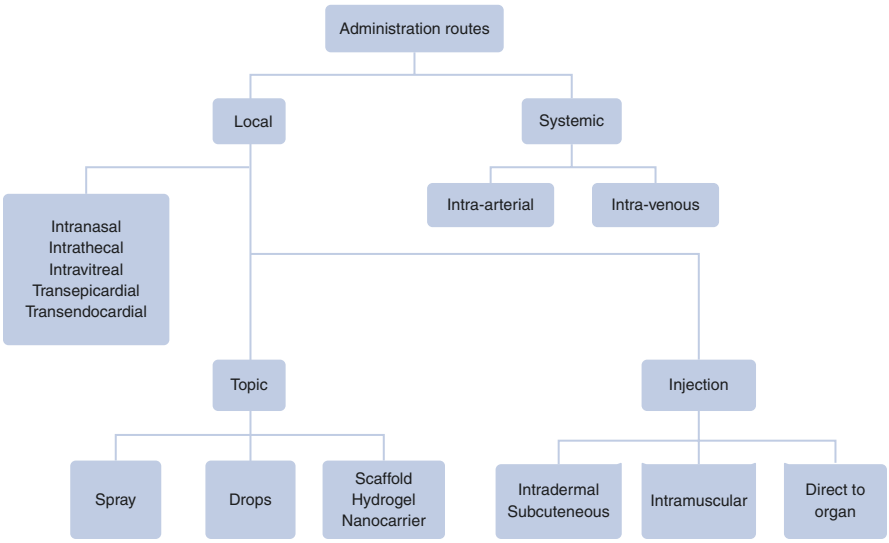

Fig. 1.6 Routes of stem cell administration

Since treating different diseases requires several cell-induced regenerative pathways and proper cell homing, understanding the advantages and disadvantages of different cell delivery methods is critical to better address the disease and tissue of interest (Fig. 1.7).

1.7.1 Local Administration Routes

Injections into the diseased site and topical administration are the main local stem cell delivery techniques. Local delivery methods enhance cell homing, a key feature in the treatment of patients with peripheral vascular diseases, which by definition prevents robust intravascular cell delivery [69].

Topical delivery typically consists of administration of a cell suspension directly to the target lesion using a spray or drops. This is a local, simple, and painless delivery method that has been used as tissue replacement strategy for chronic wound and burn treatment [68]. Topical cell delivery is a lower-risk alternative to local injections and systemic routes and enhances engraftment at the site of interest [68]. However, preliminary procedures to optimize cell homing, such as debridement, may be unavoidable [66]. Moreover, inaccurate cell density and spacing and the lack of extracellular protective environment lead to premature cell differentiation and increased cell mortality [66].

Intramuscular, intradermal, and translesional injections are safe and simple local administration routes [36]. Intramuscular administration is associated with a higher cell dwell time and provides a highly vascular support for the therapeutic

	Stem cell administration route				
	Systemic		Local		
			Topic		
	Intraarterial	Intravenous	Spray or drops	Hydrogel, scaffold or nanocarrier	Injection
Advantages	• Can be performed during angioplasty angioplasty • Possible immunomodulation and glucose homeostasis optimizing effect	• Can be performed during angioplasty angioplasty • Possible immunomodulation and glucose homeostasis optimizing effect	• Painless • Simple • Low risk • Inexpensive	• Low risk • Cell density and spacing control • Better retention and engraftment • Mechanical anchorage • Stem cell behavior modulation	• Simple • Low risk • Inexpensive • Better vascular support • Crosses anatomical barriers
Disadvantages	• High surgical risk • Low addressing and poor engraftment • Thrombosis/embolia • Expensive	• High surgical risk • Low addressing and poor engraftment • Thrombosis/embolia • Expensive • Lung entrapping	• High cell death • Low addressing and poor engraftment • No cell density and spacing control • Premature differentiation	• High protocol complexity • Expensive	• High cell death • Low addressing and poor engraftment • No cell density and spacing control • Infection risk • Ectopic stem cell transformation

Fig. 1.7 Stem cell administration routes, advantages, and disadvantages [36]

cells, enhancing local and systemic flow of cytokines [70]. Direct injections into the target tissue have the potential to overcome delivery obstacles such as the blood-brain barrier. Nonetheless, ectopic stem cell transformation and increased surgical risk can be associated with direct injections. Additionally, intraparenchymal cell injection leads to decreased systemic response and can result in tissue trauma [68].

Bioscaffolds, fibrin, nanofibers, and other exogenous support systems have been extensively investigated as local stem cell therapy adjuvants [71, 72]. The use of these components is a strategy to modulate stem cell behavior and provide mechanical anchorage, ultimately enhancing the efficiency of local administration [69, 73].

1.7.2 Systemic Administration Routes

Intra-arterial and intravenous infusion are the main routes of systemic stem cell administration. Systemic cell delivery leads to enhanced interaction with the host immune system and regeneration signaling pathways and is appropriate to treat systemic conditions or large areas of pathology not amenable to local treatment [68].

Intra-arterial infusion enables cell delivery to the site of interest with lower cell loss when compared to intravenous delivery [74]. Thrombi and emboli formation raise concerns regarding the intra-arterial route, especially in intra-coronary and intra-carotid infusion [68]. These complications can be avoided by controlling infusion speed, cell dosage, and size [75].

Intravenous infusion may be a more accessible and safer route compared with intra-arterial infusion, as microthrombi can be captured by the lungs with fewer significant clinical consequences [68]. However, intravenous infused cells are retained in lung vasculature, at up to 90% in animal models, and therefore may be removed by the host immune system [76]. After being entrapped in the lung, stem cells are phagocytosed by monocytes, which switch toward immunoregulatory phenotype and are redistributed systemically [77]. As a result, a reduced number of therapeutic cells may reach the organ of interest by this administration route, even though there is the potential to have systemic immunomodulation.

1.7.3 Other Administration Routes

Intranasal and intrathecal stem cell administration have been investigated in the treatment of neurologic pathologies such as subarachnoid hemorrhage and neuropathic pain [78, 79]. Intravitreal stem cell infusion was described as therapeutic alternative to diabetic retinopathy and retinal vein occlusion [80]. Transepicardial and transendocardial routes are important local administration routes used in stem cell therapy for ischemic cardiomyopathy [81, 82].

1.8 Stem Cell-Mediated Mechanisms of Regeneration

The therapeutic potential of stem cells was originally accredited to the cells' broad differentiation capability and potential for host tissue replacement by cell engraftment. However, substantial evidence regarding direct cell interaction and trophic paracrine effects has challenged the importance of stem cell differentiation in tissue regeneration [83, 84]. This evidence was further supported by studies demonstrating low MSC engraftment in both clinical settings and in vivo models [85–87]. As such, the focus of stem cell therapeutic potential has shifted from direct differentiation toward secondary signaling effector cells. Cytokines, growth factors, and chemokines released by stem cells induce angiogenesis, immunomodulation, neuroregeneration, and extracellular matrix production while reducing cell apoptosis and fibrosis [83, 88].

In recent years, investigations demonstrated that inactivated, apoptotic, and fragmented mesenchymal stem cells retain some immunomodulatory capacity and are potentially regenerative [89–91]. Nonetheless, direct stem cell differentiation continues to play an important role in specific therapeutic scenarios such as tissue engineering and tissue replacing therapies [92, 93].

1.8.1 Immunomodulation

Stromal cells are associated with a spectrum of different immunomodulatory mechanisms, attained via both soluble factors and direct cell-cell interaction [77]. Host microenvironmental characteristics, stromal cell source, culture, and administration conditions influence the expression of surface markers and the profile of secreted cytokines [94–96]. Although a clear picture of stem cell-induced immunomodulation is not well understood, some pathways are consistent.

1.8.1.1 Monocytes and Macrophages Interaction

Mesenchymal stem cells inhibit monocyte and macrophage differentiation into the type 1 phenotype and dendritic cells, inducing type 2 anti-inflammatory and immunoregulatory differentiation by secreting interleukin-1 receptor antagonist (IL-1 RA) [97]. These induced anti-inflammatory monocytes play an important role in MSC-mediated beneficial effects in the treatment of sepsis and induction of tolerance against alloimmunity and autoimmunity by secreting high levels of IL-10 and suppressing T-cell activity, respectively [98, 99].

Recent investigations in asthma and peritonitis animal models show that MSC phagocytosis by host monocytes induces anti-inflammatory type 2 differentiation in a cytokine-independent pathway [100, 101]. Furthermore, stromal cells increase monocyte count and phagocytic activity besides inhibiting dendritic cell maturation and migration [100, 102, 103].

1.8.1.2 T-Cell, B-Cell, and Natural Killer Cell Interaction

Mesenchymal stromal cells suppress T-cell proliferation and induce a shift from Th1 pro-inflammatory to Th2 anti-inflammatory subtypes [104]. MSC induce conventional T-cell differentiation into regulatory T-cells (T-reg), important mediators of graft immune tolerance and prevention of autoimmunity [105, 106]. Stromal cells inhibit proliferation of alloreactive CD4+ and CD8+ T-cells [107].

MSC suppress plasmablast production and induce regulatory B-cell (B-reg) formation, which promotes immunological tolerance [108, 109]. These effects are thought to be promoted by direct cell-cell contact, whereas inhibition of B-cell proliferation and differentiation by MSC is ascribed to soluble factors such as IFN-γ and IL-1 RA [94, 110]. Natural killer cell activity and proliferation are also inhibited by MSC via secretion of prostaglandin E2, TGF-β, nitric oxide, and other factors [111–113].

1.8.1.3 Complement and Coagulation Systems

MSC exert procoagulant activity by triggering both host coagulation and the innate immune system, increasing C3 activation, D-dimer, and thrombin-antithrombin complex formation while decreasing platelet counts [68, 114, 115]. Christy et al. demonstrated increased tissue factor expression in MSC that varies according to the cell source [116]. BM-MSC express less tissue factor compared to ADSC, which are highly procoagulant [116]. Thus, theoretically, BM-MSC may be preferable in the treatment of ischemic conditions and in systemic MSC infusion, whereas ADSC may be preferable in the treatment of hemorrhagic conditions.

Procoagulant MSC activity increases with increased cell passages and can be reduced by cell dilution, heparin, or tissue factor blockers [117, 118]. Adverse thrombotic events reported by clinical studies will be discussed below.

1.8.2 Angiogenesis

Angiogenesis is the growth of blood vessels from pre-existing vessels. The angiogenic potential of MSC has been the focus not only in investigations involving diseases caused by limited angiogenesis, such as peripheral arterial disease, myocardial ischemia, and stroke, but also in pathologic angiogenesis associated with tumors [87]. Since MSC engraftment is typically low, the pro-angiogenic effects of MSC are generally attributed to paracrine activity of secreted factors. The MSC secretome is composed of various soluble factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), angiopoietin-1 (ang-1), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), among others. Investigations using the chicken chorioallantoic membrane and mouse matrigel plug assay showed that BM-MSC induce angiogenesis in vitro [119, 120].

VEGF and FGF-2 are critical factors for wound healing and are capable of inducing endothelial cell (EC) proliferation, migration, and remodeling of the extracellular matrix [121]. Hypoxic conditions, conditioned medium from tumor necrosis factor α (TNF-α), and lipopolysaccharide (LPS) enhance human MSC production of VEGF, FGF-2, and insulin-like growth factor (IGF-1) and, therefore, are potential strategies to enhance MSC-induced angiogenesis [122].

However, evidence regarding MSC differentiation in endothelial cells as a mechanism of angiogenesis is scarce [87]. However, the use of MSC conditioned by EC-differentiation medium significantly increased differentiation into a more angiogenic cell type, leading to better therapeutic outcomes in wire injury model and in vivo angiogenesis assay [123, 124]. MSC differentiation toward EC phenotype is especially promising for vascular graft tissue engineering and intima replacing therapies. The potential of MSC angiogenesis to form tumors remains controversial and will be discussed below.

1.8.3 Apoptotic, Inactivated, and Fragmented Stem Cells

In 2005, Thum et al. proposed "the dying stem cell hypothesis," suggesting that apoptosis of therapeutic stem cells was responsible for modulation of host immune reactivity [125]. Recent studies showed that immunomodulation of mesenchymal stem cells not only occur by soluble mediators but also rely on cell-cell interaction; since these interactions do not require intact cell metabolism, cell viability is not a prerequisite for the therapeutic effects of stem cells [126].

Sun et al. demonstrated that cytotoxic activity against mesenchymal stem cells was a vital step in inducing immunomodulation [29]. In addition, the need for the host cytotoxic response could be bypassed by using apoptotic MSC. Moreover, apoptotic adipose-derived MSC were more effective in reducing oxidative stress, inflammation, and apoptosis compared with living MSC in a sepsis animal model [127].

Heat inactivated stem cells exhibited similar effects on monocyte function as living MSC in an ischemic kidney model despite lack of proliferative and metabolic activities [91]. MSC membrane particles retained immunomodulatory capacity by inducing selective apoptosis of pro-inflammatory monocytes [90].

1.8.4 Mesenchymal Stem Cell-Derived Extracellular Vesicles (MSC-EV)

MSC-derived microvesicles and exosomes are extracellular vesicles secreted by mesenchymal stromal cells containing proteins, miRNA, and mRNA. These structures are involved in cell-cell communication acting on regenerative pathways such

as coagulation, inflammation, angiogenesis, and immune responses [83]. The therapeutic potential of MSC extracellular vesicles has been demonstrated in several disease models such as acute kidney injury, liver fibrosis, myocardial ischemia-reperfusion, and stroke [128–131]. MSC-EV have been proposed as a promising non-cellular therapy since their biological activity is similar to that of MSC [132].

1.8.5 Stem Cells as a Delivery System

Stem cells are considered an innovative drug and gene delivery system due to their affinity to travel to injured tissue and tumors [133]. MSC can take up drugs such as paclitaxel, doxorubicin, and gemcitabine and release these drugs at a specific site of interest [134]. Studies performed using in vitro pancreatic adenocarcinoma showed the ability of MSC to deliver these three drugs [134, 135]. Moreover, MSC loaded with paclitaxel impaired tumor growth and angiogenesis in vivo, using a murine leukemia model [136]. In addition, MSC loaded with organic and inorganic nanoparticles have been proposed as photothermal cancer drugs and as diagnostic agents for laser-induced thermal ablation and magnetic resonance imaging [137–139].

Stem cells have also emerged as a gene therapy alternative. MSC transduced by vectors or three-dimensional/reverse transfection systems express genes for cytokines, drugs, and cell receptors and other proteins [133]. However, transient gene expression and carcinogenesis are important issues in stem cell-mediated gene therapy [133]. All in all, current studies suggest that stem cell therapy may be a promising gene and target drug delivery system, capable of increasing the effectiveness of therapy and reducing side effects.

1.9 Stem Cell Therapy Safety and Adverse Events

Stem cell therapy potential adverse events are various and depend on multiple factors such as disease to be treated, donor medical history, stem cell characteristics, the cell manufacturing process, administration route, and host response to the therapy. Here, we summarize main safety concerns regarding stem cell therapy safety (Fig. 1.8).

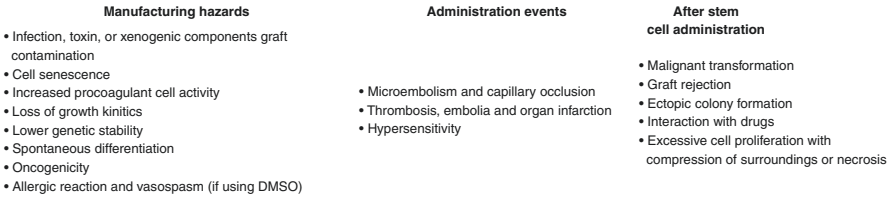

Fig. 1.8 Stem cell therapy risks

1.9.1 Stem Cell Manufacturing Hazards

Stem cell isolation and induction and cell culture and priming can all potentially lead to adverse clinical events. Allogeneic transplantation with inadequate donor screening and suboptimal culture conditions may lead to transplantation of cells contaminated by xenogenic components, toxins, and infectious microorganisms [14, 140]. Dimethylsulfoxide (DMSO), a cell protectant used in cryopreservation protocols, is associated with allergic reactions and vasospasm [141, 142].

Stem cell quality diminishes with age, a process termed cell senescence that is characterized by several genetic, phenotypic, and functional modifications that result in loss of the state of "stemness" [143]. Advanced donor age and in vitro stem cell expansion are associated with cell senescence [144]. Furthermore, prolonged stem cell culture is associated with increased procoagulant activity as well as loss of growth kinetics and immunomodulatory properties [145, 146]. After the fifth passage, MSC show significant drop of differentiation potential [145]. Moreover, late stem cell passages show lower genetic stability, leading to spontaneous cell differentiation and oncogenicity [14].

Rigorous donor screening, control of isolation, induction, and culture conditions and the use of automated systems are effective alternatives to reduce the risk of infections. DMSO use should be avoided in cryopreservation for therapeutic purposes. Limited cell passage, the cytokinesis-block micronucleus assay, and monitoring of miRNA and proteins are recommended in order to avoid adverse effects of stem cell aging in clinical therapeutic scenarios [14, 147].

1.9.2 Stem Cell Administration Adverse Events

Stem cell administration is specially challenging since, unlike therapeutic drugs, these cellular therapies tend to aggregate in specific sites, depending on the route of administration [148]. As previously discussed, MSC exert procoagulant activity, which is especially worrisome in systemic stem cell administration. Large cell size, high infusion speed, and ADSC cell type are variables related to increased procoagulant properties [116].

Intravenous and intra-arterial stem cell infusion may cause microembolism, although generally without clinical sequelae [140]. Nonetheless, several studies have reported thrombotic events such as pulmonary embolism, pulmonary infarction, and venous thrombosis of brachial and portal veins as major complications of intravenous MSC infusion [149–151]. Intra-arterial stem cell delivery can cause not only capillary obstruction but also occlusion at the precapillary level, raising concerns especially when treating high oxygen consumption organs such as heart and brain [152]. Microvascular obstruction after intra-coronary BM-MSC administration has also been reported [153].

Acosta el al. reported peripheral microthrombosis following intra-arterial administration of autologous ADSC in the treatment of critical limb ischemia [154]. Importantly, this report showed that MSC from diabetic patients have decreased fibrinolytic activity, raising concerns regarding intravascular infusion of autologous stromal cells in diabetic patients [154].

The risk of thrombotic complications following intra-arterial infusion can be reduced by controlling infusion speed, cell dosage, and size [75]. Nonetheless, lower MSC concentration may be ineffective in avoiding pulmonary microthrombosis following intravenous stem cell administration [140]. Heparin, bivalirudin, and other anticoagulant drugs can prevent cell priming and limit progression of MSC-mediated thrombogenic events [155].

There are several reports of hypersensitivity reactions following systemic administration of allogeneic placenta-derived MSC and ADSC [115, 156–158]. It remains unclear, however, if the reported events were related to anti-donor immune responses [159].

1.9.3 Adverse Events After Stem Cell Administration

Uncontrolled stem cell differentiation is a key obstacle that needs to be solved in order to ensure the safety of stem cell therapy. Excessive cell proliferation may result in compression of the surrounding structures and cell necrosis among other detrimental effects [140]. Depending on the route of administration, transplanted cells may migrate and form undesired ectopic colonies that can differentiate in ectopic tissue or tumors [160]. Pluripotent cells such as ESC and iPSC are particularly concerning due to their unlimited proliferation capacity that can cause neoplastic formation in animal models [161, 162]. Interestingly, neoplasia may arise from malignant transformation of host cells surrounding the graft after administration of senescent stem cells [163].

Graft rejection and graft versus host disease are potential complications of allogeneic and xenogeneic cell transplantation. Major histocompatibility complex (MHC) matching and the use of less immunogenic stem cells have been proposed as alternatives to avoid these reactions [164]. This recommendation is, however, highly controversial since recent studies suggest the importance of host immune reaction in the effectiveness of MSC therapy.

Stem cell interaction with host drugs is an under-explored theme. Murine in vivo studies have reported that MSC infusion may abolish the effects of G-CSF [165]. Corticosteroid administration was reported to inhibit MSC-mediated immunomodulation in a cirrhosis model and, therefore, should be avoided while performing stem cell therapy [166]. Further investigation regarding medication interactions with transplanted stem cells are needed.

Tumor formation and adverse immune reactions can be avoided by using autologous stem cells and following safety recommendations mentioned above.

1.10 Stem Cell Therapy for Vascular Diseases

Vascular disorders are frequently chronic conditions characterized by impaired blood flow; the general goal of stem cell therapy for these diseases is to restore blood flow, improving the perfusion and function of the ischemic end organ. Stem cell therapy was shown to be safe and effective to treat some vascular disorders such as diabetic foot ulcers [36] and is considered a promising alternative for other diseases such as vasculitis and lymphedema.

1.10.1 The Vascular Patient

The potential for stem cell therapy to induce regeneration relies on the interaction between stem cells and the host. The high prevalence of risk factors that impact stem cell therapy, including age, diabetes, hypertension, smoking, dyslipidemia, obesity, and coagulation and immunity disorders, remains a key challenge in the treatment of vascular patients.

Lower cell yield, hostile administration site microenviroment, and decreased and dysfunctional cell activity are important limitations and barriers of autologous stem cell therapy in vascular patients. Stem cells from diabetic patients show decreased fibrinolytic activity, leading to higher risk of adverse events. It is not clear whether other metabolic disorders are associated with higher risk of complications.

The management of chronic diseases frequent requires the use multiple medications whose interactions with stem cell therapy are yet to be discovered. The inhibition of MSC-mediated immunomodulation by corticosteroids is a special concern for patients diagnosed with immunological disorders. The use of allogeneic cells and strict clinical management of comorbidities is essential to optimize stem cell therapy in vascular patients. Further investigation regarding the interactions of stem cell therapy with medications is needed (Fig. 1.9).

1.10.2 Stem Cell Manufacturing and Administration for Vascular Diseases

Several strategies have been developed to enhance stem cell therapy-induced angiogenesis in the treatment of vascular disease. Regarding cell manufacturing, hypoxic preconditioning and pretreatment with curcumin and angiotensin II increased VEGF secretion and angiogenesis to treat myocardial ischemia [87, 167–169]. Another strategy is the use of EC-conditioned differentiation medium to increase MSC differentiation into a more angiogenic cell type [123, 124].

Blood flow impairment and organ ischemia caused by vascular disorders hinders stem cell delivery, engraftment, and function. Recently, Liu et al. showed the use of

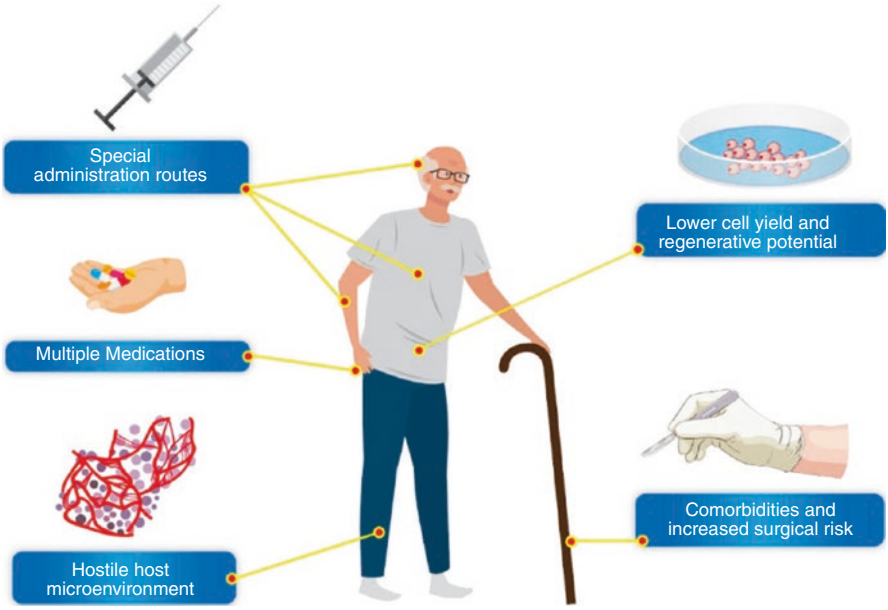

Fig. 1.9 Particularities of stem cell therapy for vascular diseases

BM-MSC coated with dendrimer nanocarriers modified with adhesion molecules to increase stem cell anchoring, transendothelial migration, extravasation, and homing to the targeted tissues [170].

Both local and systemic routes of administration have several advantages and disadvantages. Specific administration routes have been studied in the treatment of stroke (intranasal and intrathecal) [78, 79], ophthalmic (intravitreal), and cardiovascular [81, 82] diseases (transepicardial and transendocardial). Individualized decisions based on the disease and/or organ to be treated are essential to choose the appropriate administration route and adjuvants and to avoid suboptimal therapy.

1.11 Conclusion

Stem cell therapy has emerged as a promising therapeutic option for several clinical conditions. In addition to embryos, stem cells can be isolated from several adult tissues, particularly bone marrow, peripheral blood, adipose tissue, and umbilical cord derivatives. Along with adult cells, induced pluripotent stem cells are ethical alternatives for stem cell therapy and enhance the availability of therapeutic cells. Autologous tissue is a safe therapeutic cell origin, whereas allogeneic and xenogeneic stem cells raise concerns regarding disease transmission and immunogenicity.

The mechanisms of regeneration induced by stem cells are yet to be fully understood. As various protocols for isolation, culture, priming, and administration result

in different therapeutic effects, understanding the effect of these processes remains particularly challenging. Stem cell therapy is generally safe, demonstrating limited risks which rely particularly on the stem cell type and administration route. Treatment of vascular diseases is especially challenging due to the presence of patient-associated comorbidities that frequently impair stem cell-induced regeneration.

Stem cell therapy comprises a spectrum of cell-based regenerative strategies characterized by three sets of variables: (1) stem cell intrinsic properties (origin, type, isolation/induction, culture, and priming features); (2) therapy protocol (dose, concentration, administration route, adjuvants); and (3) patient characteristics (medical history, immune reaction, disease of interest). Understanding and controlling the effects and interactions between these variables is the key for progress in clinical stem cell therapy.

References

1. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019;10(1):68.
2. Kirby GTS, Mills SJ, Cowin AJ, Smith LE. Stem cells for cutaneous wound healing. Biomed Res Int. 2015;2015:285869.
3. Konstantinov IE. In search of Alexander a. Maximow: the man behind the unitarian theory of hematopoiesis. Perspect Biol Med. 2000;43(2):269–76.
4. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res. 1961;14(2):213–22.
5. Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J Embryol Exp Morphol. 1962;10:622–40.
6. Friedenstein AJ, Piatetzky S II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol. 1966;16(3):381–90.
7. de la Morena MT, Gatti RA. A history of bone marrow transplantation. Hematol Oncol Clin North Am. 2011;25(1):1–15.
8. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292(5819):154–6.
9. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145–7.
10. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–76.
11. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–72.
12. NobelPrize.org. The Nobel prize in physiology or medicine 2012. [cited 2020 04 April]; Available from: https://www.nobelprize.org/prizes/medicine/2012/press-release/.
13. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991;9(5):641–50.
14. Yin JQ, Zhu J, Ankrum JA. Manufacturing of primed mesenchymal stromal cells for therapy. Nat Biomed Eng. 2019;3(2):90–104.
15. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al. Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement. Cytotherapy. 2005;7(5):393–5.
16. Viswanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, et al. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT(R)) mes-

enchymal stromal cell committee position statement on nomenclature. Cytotherapy. 2019;21(10):1019–24.
17. Lin CS, Ning H, Lin G, Lue TF. Is CD34 truly a negative marker for mesenchymal stromal cells? Cytotherapy. 2012;14(10):1159–63.
18. Bellagamba BC, Grudzinski PB, Ely PB, Nader PJH, Nardi NB, da Silva Meirelles L. Induction of expression of CD271 and CD34 in mesenchymal stromal cells cultured as spheroids. Stem Cells Int. 2018;2018:7357213.
19. Available from: https://clinicaltrials.gov/ct2/results?cond=&term=mesenchymal+stem+cell&cntry=&state=&city=&dist=.
20. Leeper NJ, Hunter AL, Cooke JP. Stem cell therapy for vascular regeneration: adult, embryonic, and induced pluripotent stem cells. Circulation. 2010;122(5):517–26.
21. Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res. 2008;100:133–58.
22. Mali P, Ye Z, Hommond HH, Yu X, Lin J, Chen G, et al. Improved efficiency and pace of generating induced pluripotent stem cells from human adult and fetal fibroblasts. Stem Cells (Dayton, Ohio). 2008;26(8):1998–2005.
23. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. J Transl Med. 2014;12:8.
24. Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. PLoS One. 2014;9(12):e115963.
25. Duscher D, Rennert RC, Januszyk M, Anghel E, Maan ZN, Whittam AJ, et al. Aging disrupts cell subpopulation dynamics and diminishes the function of mesenchymal stem cells. Sci Rep. 2014;4:7144.
26. Hequet O. Hematopoietic stem and progenitor cell harvesting: technical advances and clinical utility. J Blood Med. 2015;6:55–67.
27. Serena C, Keiran N, Ceperuelo-Mallafre V, Ejarque M, Fradera R, Roche K, et al. Obesity and type 2 diabetes alters the immune properties of human adipose derived stem cells. Stem Cells. 2016;34(10):2559–73.
28. Berglund AK, Fortier LA, Antczak DF, Schnabel LV. Immunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. Stem Cell Res Ther. 2017;8(1):288.
29. Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med. 2017;9(416):3–4.
30. Hodgetts SI, Stagg K, Sturm M, Edel M, Blancafort P. Long live the stem cell: the use of stem cells isolated from post mortem tissues for translational strategies. Int J Biochem Cell Biol. 2014;56:74–81.
31. Administration FUSFD. Xenotransplantation. 2017 [updated 04/28/2017; cited 2017 12/05/2017]; Available from: https://www.fda.gov/BiologicsBloodVaccines/Xenotransplantation/.
32. Hass R, Kasper C, Bohm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal. 2011;9:12.
33. Murray IR, West CC, Hardy WR, James AW, Park TS, Nguyen A, et al. Natural history of mesenchymal stem cells, from vessel walls to culture vessels. Cell Mol Life Sci. 2014;71(8):1353–74.
34. Andia I, Maffulli N, Burgos-Alonso N. Stromal vascular fraction technologies and clinical applications. Expert Opin Biol Ther. 2019;19(12):1289–305.
35. Gentile P, Calabrese C, De Angelis B, Pizzicannella J, Kothari A, Garcovich S. Impact of the different preparation methods to obtain human adipose-derived stromal vascular fraction cells (AD-SVFs) and human adipose-derived mesenchymal stem cells (AD-MSCs): enzymatic digestion versus mechanical centrifugation. Int J Mol Sci. 2019;20(21):4–7.

36. Lopes L, Setia O, Aurshina A, Liu S, Hu H, Isaji T, et al. Stem cell therapy for diabetic foot ulcers: a review of preclinical and clinical research. Stem Cell Res Ther. 2018;9(1):188.
37. Khan FA, Almohazey D, Alomari M, Almofty SA. Isolation, culture, and functional characterization of human embryonic stem cells: current trends and challenges. Stem Cells Int. 2018;2018:1429351.
38. Tanaka N, Takeuchi T, Neri QV, Sills ES, Palermo GD. Laser-assisted blastocyst dissection and subsequent cultivation of embryonic stem cells in a serum/cell free culture system: applications and preliminary results in a murine model. J Transl Med. 2006;4:20.
39. Strom S, Inzunza J, Grinnemo KH, Holmberg K, Matilainen E, Stromberg AM, et al. Mechanical isolation of the inner cell mass is effective in derivation of new human embryonic stem cell lines. Hum Reprod. 2007;22(12):3051–8.
40. Hayes M, Zavazava N. Strategies to generate induced pluripotent stem cells. Methods Mol Biol. 2013;1029:77–92.
41. Yeo JC, Ng HH. The transcriptional regulation of pluripotency. Cell Res. 2013;23(1):20–32.
42. Zhang S, Cui W. Sox2, a key factor in the regulation of pluripotency and neural differentiation. World J Stem Cells. 2014;6(3):305–11.
43. Dakhore S, Nayer B, Hasegawa K. Human pluripotent stem cell culture: current status, challenges, and advancement. Stem Cells Int. 2018;2018:7396905.
44. Gstraunthaler G, Lindl T, van der Valk J. A plea to reduce or replace fetal bovine serum in cell culture media. Cytotechnology. 2013;65(5):791–3.
45. Burnouf T, Strunk D, Koh MB, Schallmoser K. Human platelet lysate: replacing fetal bovine serum as a gold standard for human cell propagation? Biomaterials. 2016;76:371–87.
46. Abdelrazik H, Spaggiari GM, Chiossone L, Moretta L. Mesenchymal stem cells expanded in human platelet lysate display a decreased inhibitory capacity on T- and NK-cell proliferation and function. Eur J Immunol. 2011;41(11):3281–90.
47. Oikonomopoulos A, van Deen WK, Manansala AR, Lacey PN, Tomakili TA, Ziman A, et al. Optimization of human mesenchymal stem cell manufacturing: the effects of animal/xeno-free media. Sci Rep. 2015;5:16570.
48. Ejtehadifar M, Shamsasenjan K, Movassaghpour A, Akbarzadehlaleh P, Dehdilani N, Abbasi P, et al. The effect of hypoxia on mesenchymal stem cell biology. Adv Pharmaceut Bullet. 2015;5(2):141–9.
49. Nold P, Hackstein H, Riedlinger T, Kasper C, Neumann A, Mernberger M, et al. Immunosuppressive capabilities of mesenchymal stromal cells are maintained under hypoxic growth conditions and after gamma irradiation. Cytotherapy. 2015;17(2):152–62.
50. Rosova I, Dao M, Capoccia B, Link D, Nolta JA. Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells. 2008;26(8):2173–82.
51. Das B, Bayat-Mokhtari R, Tsui M, Lotfi S, Tsuchida R, Felsher DW, et al. HIF-2alpha suppresses p53 to enhance the stemness and regenerative potential of human embryonic stem cells. Stem Cells. 2012;30(8):1685–95.
52. Grayson WL, Zhao F, Izadpanah R, Bunnell B, Ma T. Effects of hypoxia on human mesenchymal stem cell expansion and plasticity in 3D constructs. J Cell Physiol. 2006;207(2):331–9.
53. Basciano L, Nemos C, Foliguet B, de Isla N, de Carvalho M, Tran N, et al. Long term culture of mesenchymal stem cells in hypoxia promotes a genetic program maintaining their undifferentiated and multipotent status. BMC Cell Biol. 2011;12:12.
54. Roobrouck VD, Vanuytsel K, Verfaillie CM. Concise review: culture mediated changes in fate and/or potency of stem cells. Stem Cells. 2011;29(4):583–9.
55. Shekaran A, Sim E, Tan KY, Chan JKY, Choolani M, Reuveny S, et al. Enhanced in vitro osteogenic differentiation of human fetal MSCs attached to 3D microcarriers versus harvested from 2D monolayers. BMC Biotechnol. 2015;15(1):102.
56. Haugh MG, Heilshorn SC. Integrating concepts of material mechanics, ligand chemistry, dimensionality and degradation to control differentiation of mesenchymal stem cells. Curr Opin Solid State Mater Sci. 2016;20(4):171–9.

57. Mo M, Zhou Y, Li S, Wu Y. Three-dimensional culture reduces cell size by increasing vesicle excretion. Stem Cells. 2018;36(2):286–92.
58. Petrenko Y, Sykova E, Kubinova S. The therapeutic potential of three-dimensional multipotent mesenchymal stromal cell spheroids. Stem Cell Res Ther. 2017;8(1):94.
59. Rashedi I, Talele N, Wang XH, Hinz B, Radisic M, Keating A. Collagen scaffold enhances the regenerative properties of mesenchymal stromal cells. PLoS One. 2017;12(10):e0187348.
60. Murphy WL, McDevitt TC, Engler AJ. Materials as stem cell regulators. Nat Mater. 2014;13(6):547–57.
61. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126(4):677–89.
62. Shakouri-Motlagh A, O'Connor AJ, Brennecke SP, Kalionis B, Heath DE. Native and solubilized decellularized extracellular matrix: a critical assessment of their potential for improving the expansion of mesenchymal stem cells. Acta Biomater. 2017;55:1–12.
63. Carrier-Ruiz A, Evaristo-Mendonca F, Mendez-Otero R, Ribeiro-Resende VT. Biological behavior of mesenchymal stem cells on poly-epsilon-caprolactone filaments and a strategy for tissue engineering of segments of the peripheral nerves. Stem Cell Res Ther. 2015;6:128.
64. Lam A, Li J, Toh J, Sim E, Chen A, Chan J, et al. Biodegradable poly-ε-caprolactone microcarriers for efficient production of human mesenchymal stromal cells and secreted cytokines in batch and fed-batch bioreactors. Cytotherapy. 2016;19:8–12.
65. Jun I, Lee YB, Choi YS, Engler AJ, Park H, Shin H. Transfer stamping of human mesenchymal stem cell patches using thermally expandable hydrogels with tunable cell-adhesive properties. Biomaterials. 2015;54:44–54.
66. Duscher D, Barrera J, Wong VW, Maan ZN, Whittam AJ, Januszyk M, et al. Stem cells in wound healing: the future of regenerative medicine? A mini-review. Gerontology. 2016;62(2):216–25.
67. Isakson M, de Blacam C, Whelan D, McArdle A, Clover AJP. Mesenchymal stem cells and cutaneous wound healing: current evidence and future potential. Stem Cells Int. 2015;2015:831095.
68. Caplan H, Olson SD, Kumar A, George M, Prabhakara KS, Wenzel P, et al. Mesenchymal stromal cell therapeutic delivery: translational challenges to clinical application. Front Immunol. 2019;10:1645.
69. O'Loughlin A, O'Brien T. Topical stem and progenitor cell therapy for diabetic foot ulcers. In: Gholamrezanezhad DA, editor. Stem cells clinic and research. Published online: InTech; 2011. p. 578–604.
70. Hamidian Jahromi S, Davies JE. Concise review: skeletal muscle as a delivery route for mesenchymal stromal cells. Stem Cells Transl Med. 2019;8(5):456–65.
71. Qazi TH, Mooney DJ, Duda GN, Geissler S. Biomaterials that promote cell-cell interactions enhance the paracrine function of MSCs. Biomaterials. 2017;140:103–14.
72. Jenkins TL, Little D. Synthetic scaffolds for musculoskeletal tissue engineering: cellular responses to fiber parameters. NPJ Regen Med. 2019;4:15.
73. Falanga V, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, et al. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng. 2007;13(6):1299–312.
74. Watanabe M, Yavagal DR. Intra-arterial delivery of mesenchymal stem cells. Brain Circ. 2016;2(3):114–7.
75. Janowski M, Lyczek A, Engels C, Xu J, Lukomska B, Bulte JW, et al. Cell size and velocity of injection are major determinants of the safety of intracarotid stem cell transplantation. J Cereb Blood Flow Metab. 2013;33(6):921–7.
76. Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev. 2009;18(5):683–92.

77. de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, et al. Immunomodulation by therapeutic Mesenchymal Stromal Cells (MSC) is triggered through phagocytosis of MSC by Monocytic cells. Stem Cells. 2018;36(4):602–15.
78. Nijboer CH, Kooijman E, van Velthoven CT, van Tilborg E, Tiebosch IA, Eijkelkamp N, et al. Intranasal stem cell treatment as a novel therapy for subarachnoid hemorrhage. Stem Cells Dev. 2018;27(5):313–25.
79. Vaquero J, Zurita M, Rico MA, Aguayo C, Bonilla C, Marin E, et al. Intrathecal administration of autologous mesenchymal stromal cells for spinal cord injury: safety and efficacy of the 100/3 guideline. Cytotherapy. 2018;20(6):806–19.
80. Park SS. Cell therapy applications for retinal vascular diseases: diabetic retinopathy and retinal vein occlusion. Invest Ophthalmol Vis Sci. 2016;57(5):ORSFj1–ORSFj10.
81. van der Spoel TI, Vrijsen KR, Koudstaal S, Sluijter JP, Nijsen JF, de Jong HW, et al. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischaemic cardiomyopathy: a study on delivery efficiency. J Cell Mol Med. 2012;16(11):2768–76.
82. Kanelidis AJ, Premer C, Lopez J, Balkan W, Hare JM. Route of delivery modulates the efficacy of mesenchymal stem cell therapy for myocardial infarction: a meta-analysis of preclinical studies and clinical trials. Circ Res. 2017;120(7):1139–50.
83. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. Stem Cell Res Ther. 2018;9(1):63.
84. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006;98(5):1076–84.
85. Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell. 2011;9(1):11–5.
86. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007;25(10):2648–59.
87. Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, et al. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. Pharmacol Ther. 2014;143(2):181–96.
88. Kean TJ, Lin P, Caplan AI, Dennis JE. MSCs: delivery routes and engraftment, cell-targeting strategies, and immune modulation. Stem Cells Int. 2013;2013:732742.
89. Chang CL, Leu S, Sung HC, Zhen YY, Cho CL, Chen A, et al. Impact of apoptotic adipose-derived mesenchymal stem cells on attenuating organ damage and reducing mortality in rat sepsis syndrome induced by cecal puncture and ligation. J Transl Med. 2012;10:244.
90. Goncalves FDC, Luk F, Korevaar SS, Bouzid R, Paz AH, Lopez-Iglesias C, et al. Membrane particles generated from mesenchymal stromal cells modulate immune responses by selective targeting of pro-inflammatory monocytes. Sci Rep. 2017;7(1):12100.
91. Luk F, de Witte SF, Korevaar SS, Roemeling-van Rhijn M, Franquesa M, Strini T, et al. Inactivated mesenchymal stem cells maintain immunomodulatory capacity. Stem Cells Dev. 2016;25(18):1342–54.
92. Howard D, Buttery LD, Shakesheff KM, Roberts SJ. Tissue engineering: strategies, stem cells and scaffolds. J Anat. 2008;213(1):66–72.
93. Petrella F, Spaggiari L, Acocella F, Barberis M, Bellomi M, Brizzola S, et al. Airway fistula closure after stem-cell infusion. N Engl J Med. 2015;372(1):96–7.
94. Luk F, Carreras-Planella L, Korevaar SS, de Witte SFH, Borras FE, Betjes MGH, et al. Inflammatory conditions dictate the effect of mesenchymal stem or stromal cells on B cell function. Front Immunol. 2017;8:1042.
95. Wu Y, Hoogduijn MJ, Baan CC, Korevaar SS, de Kuiper R, Yan L, et al. Adipose tissue-derived mesenchymal stem cells have a heterogenic cytokine secretion profile. Stem Cells Int. 2017;2017:4960831.
96. De Witte SFH, Peters FS, Merino A, Korevaar SS, Van Meurs JBJ, O'Flynn L, et al. Epigenetic changes in umbilical cord mesenchymal stromal cells upon stimulation and culture expansion. Cytotherapy. 2018;20(7):919–29.

97. Melief SM, Geutskens SB, Fibbe WE, Roelofs H. Multipotent stromal cells skew monocytes towards an anti-inflammatory interleukin-10-producing phenotype by production of interleukin-6. Haematologica. 2013;98(6):888–95.
98. Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15(1):42–9.
99. Ko JH, Lee HJ, Jeong HJ, Kim MK, Wee WR, Yoon SO, et al. Mesenchymal stem/stromal cells precondition lung monocytes/macrophages to produce tolerance against Allo- and autoimmunity in the eye. Proc Natl Acad Sci U S A. 2016;113(1):158–63.
100. Krasnodembskaya A, Samarani G, Song Y, Zhuo H, Su X, Lee JW, et al. Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. Am J Physiol Lung Cell Mol Physiol. 2012;302(10):L1003–13.
101. Braza F, Dirou S, Forest V, Sauzeau V, Hassoun D, Chesne J, et al. Mesenchymal stem cells induce suppressive macrophages through phagocytosis in a mouse model of asthma. Stem Cells. 2016;34(7):1836–45.
102. Ge W, Jiang J, Arp J, Liu W, Garcia B, Wang H. Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. Transplantation. 2010;90(12):1312–20.
103. Miteva K, Pappritz K, El-Shafeey M, Dong F, Ringe J, Tschope C, et al. Mesenchymal stromal cells modulate monocytes trafficking in Coxsackievirus B3-induced myocarditis. Stem Cells Transl Med. 2017;6(4):1249–61.
104. Duffy MM, Ritter T, Ceredig R, Griffin MD. Mesenchymal stem cell effects on T-cell effector pathways. Stem Cell Res Ther. 2011;2(4):34.
105. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.
106. Khosravi M, Karimi MH, Hossein Aghdaie M, Kalani M, Naserian S, Bidmeshkipour A. Mesenchymal stem cells can induce regulatory T cells via modulating miR-126a but not miR-10a. Gene. 2017;627:327–36.
107. Gieseke F, Bohringer J, Bussolari R, Dominici M, Handgretinger R, Muller I. Human multipotent mesenchymal stromal cells use galectin-1 to inhibit immune effector cells. Blood. 2010;116(19):3770–9.
108. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity. 2015;42(4):607–12.
109. Franquesa M, Mensah FK, Huizinga R, Strini T, Boon L, Lombardo E, et al. Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. Stem Cells. 2015;33(3):880–91.
110. Luz-Crawford P, Djouad F, Toupet K, Bony C, Franquesa M, Hoogduijn MJ, et al. Mesenchymal stem cell-derived interleukin 1 receptor antagonist promotes macrophage polarization and inhibits B cell differentiation. Stem Cells. 2016;34(2):483–92.
111. Deng Y, Zhang Y, Ye L, Zhang T, Cheng J, Chen G, et al. Umbilical cord-derived mesenchymal stem cells instruct monocytes towards an IL10-producing phenotype by secreting IL6 and HGF. Sci Rep. 2016;6:37566.
112. Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, et al. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood. 2007;109(1):228–34.
113. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood. 2008;111(3):1327–33.

114. Moll G, Rasmusson-Duprez I, von Bahr L, Connolly-Andersen AM, Elgue G, Funke L, et al. Are therapeutic human mesenchymal stromal cells compatible with human blood? Stem Cells. 2012;30(7):1565–74.
115. Moll G, Ignatowicz L, Catar R, Luecht C, Sadeghi B, Hamad O, et al. Different Procoagulant activity of therapeutic mesenchymal stromal cells derived from bone marrow and placental decidua. Stem Cells Dev. 2015;24(19):2269–79.
116. Christy BA, Herzig MC, Montgomery RK, Delavan C, Bynum JA, Reddoch KM, et al. Procoagulant activity of human mesenchymal stem cells. J Trauma Acute Care Surg. 2017;83(1 Suppl 1):S164–S9.
117. Liao L, Shi B, Chang H, Su X, Zhang L, Bi C, et al. Heparin improves BMSC cell therapy: anticoagulant treatment by heparin improves the safety and therapeutic effect of bone marrow-derived mesenchymal stem cell cytotherapy. Theranostics. 2017;7(1):106–16.
118. Moll G, Ankrum JA, Kamhieh-Milz J, Bieback K, Ringden O, Volk HD, et al. Intravascular mesenchymal stromal/stem cell therapy product diversification: time for new clinical guidelines. Trends Mol Med. 2019;25(2):149–63.
119. Roobrouck VD, Clavel C, Jacobs SA, Ulloa-Montoya F, Crippa S, Sohni A, et al. Differentiation potential of human postnatal mesenchymal stem cells, mesoangioblasts, and multipotent adult progenitor cells reflected in their transcriptome and partially influenced by the culture conditions. Stem Cells. 2011;29(5):871–82.
120. Gruber R, Kandler B, Holzmann P, Vogele-Kadletz M, Losert U, Fischer MB, et al. Bone marrow stromal cells can provide a local environment that favors migration and formation of tubular structures of endothelial cells. Tissue Eng. 2005;11(5–6):896–903.
121. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004;94(5):678–85.
122. Crisostomo PR, Wang Y, Markel TA, Wang M, Lahm T, Meldrum DR. Human mesenchymal stem cells stimulated by TNF-alpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. Am J Physiol Cell Physiol. 2008;294(3):C675–82.
123. Whyte JL, Ball SG, Shuttleworth CA, Brennan K, Kielty CM. Density of human bone marrow stromal cells regulates commitment to vascular lineages. Stem Cell Res. 2011;6(3):238–50.
124. Takahashi M, Suzuki E, Oba S, Nishimatsu H, Kimura K, Nagano T, et al. Adipose tissue-derived stem cells inhibit neointimal formation in a paracrine fashion in rat femoral artery. Am J Physiol Heart Circ Physiol. 2010;298(2):H415–23.
125. Thum T, Bauersachs J, Poole-Wilson PA, Volk HD, Anker SD. The dying stem cell hypothesis: immune modulation as a novel mechanism for progenitor cell therapy in cardiac muscle. J Am Coll Cardiol. 2005;46(10):1799–802.
126. Weiss ARR, Dahlke MH. Immunomodulation by Mesenchymal Stem Cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. Front Immunol. 2019;10:1191.
127. Sung PH, Chang CL, Tsai TH, Chang LT, Leu S, Chen YL, et al. Apoptotic adipose-derived mesenchymal stem cell therapy protects against lung and kidney injury in sepsis syndrome caused by cecal ligation puncture in rats. Stem Cell Res Ther. 2013;4(6):155.
128. Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol. 2009;20(5):1053–67.
129. Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells Dev. 2013;22(6):845–54.
130. Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res. 2013;10(3):301–12.
131. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cereb Blood Flow Metab. 2013;33(11):1711–5.

132. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. Mol Ther. 2015;23(5):812–23.
133. Wu HH, Zhou Y, Tabata Y, Gao JQ. Mesenchymal stem cell-based drug delivery strategy: from cells to biomimetic. J Control Release. 2019;294:102–13.
134. Coccè V, Farronato D, Brini AT, Masia C, Giannì AB, Piovani G, et al. Drug loaded gingival mesenchymal stromal cells (GinPa-MSCs) inhibit in vitro proliferation of Oral squamous cell carcinoma. Sci Rep. 2017;7(1):9376.
135. Bonomi A, Silini A, Vertua E, Signoroni PB, Coccè V, Cavicchini L, et al. Human amniotic mesenchymal stromal cells (hAMSCs) as potential vehicles for drug delivery in cancer therapy: an in vitro study. Stem Cell Res Ther. 2015;6(1):155.
136. Pessina A, Coccè V, Pascucci L, Bonomi A, Cavicchini L, Sisto F, et al. Mesenchymal stromal cells primed with paclitaxel attract and kill leukaemia cells, inhibit angiogenesis and improve survival of leukaemia-bearing mice. Br J Haematol. 2013;160(6):766–78.
137. Cao B, Yang M, Zhu Y, Qu X, Mao C. Stem cells loaded with nanoparticles as a drug carrier for in vivo breast cancer therapy. Adv Mater. 2014;26(27):4627–31.
138. Huang X, Neretina S, El-Sayed MA. Gold Nanorods: from synthesis and properties to biological and biomedical applications. Adv Mater. 2009;21(48):4880–910.
139. Cheng L, Wang C, Ma X, Wang Q, Cheng Y, Wang H, et al. Multifunctional upconversion nanoparticles for dual-modal imaging-guided stem cell therapy under remote magnetic control. Adv Funct Mater. 2013;23(3):272–80.
140. Boltze J, Arnold A, Walczak P, Jolkkonen J, Cui L, Wagner DC. The dark side of the force – constraints and complications of cell therapies for stroke. Front Neurol. 2015;6:155.
141. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut. 2010;59(12):1662–9.
142. Savolainen H. Encephalopathy, stroke, and myocardial infarction with DMSO use in stem cell transplantation. Neurology. 2007;69(5):494; author reply -5
143. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev. 2008;129(3):163–73.
144. Turinetto V, Vitale E, Giachino C. Senescence in human mesenchymal stem cells: functional changes and implications in stem cell-based therapy. Int J Mol Sci. 2016;17(7):6–8.
145. Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B. Aging of mesenchymal stem cell in vitro. BMC Cell Biol. 2006;7:14.
146. von Bahr L, Sundberg B, Lonnies L, Sander B, Karbach H, Hagglund H, et al. Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. Biol Blood Marrow Transplant. 2012;18(4):557–64.
147. Cornelio DA, Tavares JC, Pimentel TV, Cavalcanti GB Jr, Batistuzzo de Medeiros SR. Cytokinesis-block micronucleus assay adapted for analyzing genomic instability of human mesenchymal stem cells. Stem Cells Dev. 2014;23(8):823–38.
148. Wagner J, Kean T, Young R, Dennis JE, Caplan AI. Optimizing mesenchymal stem cell-based therapeutics. Curr Opin Biotechnol. 2009;20(5):531–6.
149. Jung JW, Kwon M, Choi JC, Shin JW, Park IW, Choi BW, et al. Familial occurrence of pulmonary embolism after intravenous, adipose tissue-derived stem cell therapy. Yonsei Med J. 2013;54(5):1293–6.
150. Wu Z, Zhang S, Zhou L, Cai J, Tan J, Gao X, et al. Thromboembolism induced by umbilical cord mesenchymal stem cell infusion: a report of two cases and literature review. Transplant Proc. 2017;49(7):1656–8.
151. Sokal EM, Stéphenne X, Ottolenghi C, Jazouli N, Clapuyt P, Lacaille F, et al. Liver engraftment and repopulation by in vitro expanded adult derived human liver stem cells in a child with ornithine carbamoyltransferase deficiency. JIMD Rep. 2014;13:65–72.
152. Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics. Circ Res. 2009;104(3):398–402.

153. Gleeson BM, Martin K, Ali MT, Kumar AHS, Pillai MG-K, Kumar SPG, et al. Bone marrow-derived mesenchymal stem cells have innate Procoagulant activity and cause microvascular obstruction following intracoronary delivery: amelioration by Antithrombin therapy. Stem Cells. 2015;33(9):2726–37.
154. Acosta L, Hmadcha A, Escacena N, Perez-Camacho I, de la Cuesta A, Ruiz-Salmeron R, et al. Adipose mesenchymal stromal cells isolated from type 2 diabetic patients display reduced fibrinolytic activity. Diabetes. 2013;62(12):4266–9.
155. Coppin L, Sokal E, Stéphenne X. Thrombogenic risk induced by intravascular mesenchymal stem cell therapy: current status and future perspectives. Cell. 2019;8(10):1160.
156. Melmed GY, Pandak WM, Casey K, Abraham B, Valentine J, Schwartz D, et al. Human placenta-derived cells (PDA-001) for the treatment of moderate-to-severe Crohn's disease: a phase 1b/2a study. Inflamm Bowel Dis. 2015;21(8):1809–16.
157. Baygan A, Aronsson-Kurttila W, Moretti G, Tibert B, Dahllöf G, Klingspor L, et al. Safety and side effects of using placenta-derived Decidual stromal cells for graft-versus-host disease and hemorrhagic cystitis. Front Immunol. 2017;8:795.
158. Kaipe H, Carlson LM, Erkers T, Nava S, Mollden P, Gustafsson B, et al. Immunogenicity of decidual stromal cells in an epidermolysis bullosa patient and in allogeneic hematopoietic stem cell transplantation patients. Stem Cells Dev. 2015;24(12):1471–82.
159. Lohan P, Treacy O, Griffin MD, Ritter T, Ryan AE. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells and their extracellular vesicles: are we still learning? Front Immunol. 2017;8:1626.
160. Steward O, Sharp KG, Yee KM, Hatch MN, Bonner JF. Characterization of ectopic colonies that form in widespread areas of the nervous system with neural stem cell transplants into the site of a severe spinal cord injury. J Neurosci. 2014;34(42):14013–21.
161. Kawai H, Yamashita T, Ohta Y, Deguchi K, Nagotani S, Zhang X, et al. Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain. J Cereb Blood Flow Metab. 2010;30(8):1487–93.
162. Erdo F, Buhrle C, Blunk J, Hoehn M, Xia Y, Fleischmann B, et al. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J Cereb Blood Flow Metab. 2003;23(7):780–5.
163. Minieri V, Saviozzi S, Gambarotta G, Lo Iacono M, Accomasso L, Cibrario Rocchietti E, et al. Persistent DNA damage-induced premature senescence alters the functional features of human bone marrow mesenchymal stem cells. J Cell Mol Med. 2015;19(4):734–43.
164. Schu S, Nosov M, O'Flynn L, Shaw G, Treacy O, Barry F, et al. Immunogenicity of allogeneic mesenchymal stem cells. J Cell Mol Med. 2012;16(9):2094–103.
165. Posel C, Scheibe J, Kranz A, Bothe V, Quente E, Frohlich W, et al. Bone marrow cell transplantation time-dependently abolishes efficacy of granulocyte colony-stimulating factor after stroke in hypertensive rats. Stroke. 2014;45(8):2431–7.
166. Chen X, Gan Y, Li W, Su J, Zhang Y, Huang Y, et al. The interaction between mesenchymal stem cells and steroids during inflammation. Cell Death Dis. 2014;5:e1009.
167. Liu C, Fan Y, Zhou L, Zhu HY, Song YC, Hu L, et al. Pretreatment of mesenchymal stem cells with angiotensin II enhances paracrine effects, angiogenesis, gap junction formation and therapeutic efficacy for myocardial infarction. Int J Cardiol. 2015;188:22–32.
168. Liu J, Zhu P, Song P, Xiong W, Chen H, Peng W, et al. Pretreatment of adipose derived stem cells with curcumin facilitates myocardial recovery via Antiapoptosis and angiogenesis. Stem Cells Int. 2015;2015:638153.
169. Wei ZZ, Zhu YB, Zhang JY, McCrary MR, Wang S, Zhang YB, et al. Priming of the cells: hypoxic preconditioning for stem cell therapy. Chin Med J. 2017;130(19):2361–74.
170. Liu ZJ, Daftarian P, Kovalski L, Wang B, Tian R, Castilla DM, et al. Directing and potentiating stem cell-mediated angiogenesis and tissue repair by cell surface E-selectin coating. PLoS One. 2016;11(4):e0154053.