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COMPREHENSIVE REVIEW

Same or Not the Same? Comparison of Adipose Tissue-Derived Versus Bone Marrow-Derived Mesenchymal Stem and Stromal Cells

Marius Strioga,[1] Sowmya Viswanathan,[2] Adas Darinskas,[3] Ondrej Slaby,[4,5] and Jaroslav Michalek[4,6,7]

Mesenchymal stem/stromal cells (MSCs) comprise a heterogeneous population of cells with multilineage differentiation potential, the ability to modulate oxidative stress, and secrete various cytokines and growth factors that can have immunomodulatory, angiogenic, anti-inflammatory and anti-apoptotic effects. Recent data indicate that these paracrine factors may play a key role in MSC-mediated effects in modulating various acute and chronic pathological conditions. MSCs are found in virtually all organs of the body. Bone marrow-derived MSCs (BM-MSCs) were discovered first, and the bone marrow was considered the main source of MSCs for clinical application. Subsequently, MSCs have been isolated from various other sources with the adipose tissue, serving as one of the alternatives to bone marrow. Adipose tissue-derived MSCs (ASCs) can be more easily isolated; this approach is safer, and also, considerably larger amounts of ASCs can be obtained compared with the bone marrow. ASCs and BM-MSCs share many biological characteristics; however, there are some differences in their immunophenotype, differentiation potential, transcriptome, proteome, and immunomodulatory activity. Some of these differences may represent specific features of BM-MSCs and ASCs, while others are suggestive of the inherent heterogeneity of both BM-MSC and ASC populations. Still other differences may simply be related to different isolation and culture protocols. Most importantly, despite the minor differences between these MSC populations, ASCs seem to be as effective as BM-MSCs in clinical application, and, in some cases, may be better suited than BM-MSCs. In this review, we will examine in detail the ontology, biology, preclinical, and clinical application of BM-MSCs versus ASCs.

Introduction

More than 40 years ago, Friedenstein et al. originally described nonphagocytic, nonhematopoietic, fibroblast-like cells, which were isolated ex vivo in small numbers by plastic adherence from rat whole bone marrow cultures [1]. The cells were clonogenic, formed colonies in culture conditions (when established at clonal density), could differentiate in vitro into bone, cartilage, adipose tissue, tendon, muscle, and fibrous tissue, and were defined as colony-forming unit fibroblasts [1–3]. Since then, these cells have been extensively investigated and given various names, until Caplan proposed the definition "mesenchymal stem cells" (MSCs) in 1991 [4], which gained common usage. However, the use of this term has been long debated, as there is a lack of convincing in vivo data that supports the "stemness," that is, the long-term survival with self-renewal capacity and multipotency of an unfractioned plastic-adherent marrow cell population [5]. In fact, Sacchetti et al. have convincingly demonstrated the existence of bona fide self-renewing, CD146-expressing stem cells in a plastic-adherent marrow cell population using an in vivo model of immunocompromised mice [6]. Most likely, the "true" stem cells constitute only a subset of the bone marrow cell population, as selected by rapid plastic adherence, implying that this cell population is heterogeneously composed of bona fide stem cells, their progeny (with multilineage differentiation potential, but without salient characteristics of stem cells), and possibly senescent cells [5,7,8]. Based on such a conception, the Mesenchymal and Tissue Stem Cell Committee of International Society for Cellular Therapy (ISCT) proposed that marrow

[1]Department of Immunology, Center of Oncosurgery, Institute of Oncology, Vilnius University, Vilnius, Lithuania.
[2]Cell Therapy Program, University Health Network, Toronto, Ontario, Canada.
[3]Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania.
[4]Advanced Cell Immunotherapy Unit, Department of Pharmacology, Medical Faculty, Masaryk University, Brno, Czech Republic.
[5]Masaryk Memorial Cancer Institute, Brno, Czech Republic.
[6]Department of Pediatrics, University Hospital Brno, Brno, Czech Republic.
[7]Cellthera, Ltd., Brno, Czech Republic.

plastic-adherent cells generally described as "mesenchymal stem cells" should be retermed "multipotent mesenchymal stromal cells," while the term "mesenchymal stem cells" should be reserved for a subset of these cells that show stem cell activity by clearly stated criteria [7]. Since the acronym MSC may be used to define both cell populations, the combined definition "mesenchymal stem/stromal cells" is probably more relevant, especially when the true "stemness" of the whole MSC population is not proved.

Although MSCs were initially isolated from bone marrow cultures, currently it is thought that they reside within the connective tissue of virtually all organs [9], including adipose tissue [10], and constitute a whole complex system, diffused throughout the body [11]. MSCs have also been isolated from human placenta (MSCs of both fetal and maternal origin) [12], umbilical cord blood [13], umbilical cord/Wharton's jelly [14], amniotic fluid [15], amnion [16], and various fetal tissues, including blood, liver, bone marrow, spleen [17,18], and lung [19].

ISCT has provided 3 minimal criteria to define MSCs, independent of their source [20]: (1) plastic adherence in standard culture conditions; (2) expression of nonspecific markers CD105, CD90, and CD73 along with the lack of expression of CD34, CD45, CD14 or CD11b, CD79α or CD19, and class-II major histocompatibility complex (MHC-II) molecules, mainly HLA-DR; and (3) differentiation into osteoblasts, adipocytes, and chondroblasts under specific stimulus in vitro. There are several additional immunophenotypic markers (such as CD29, CD44, CD146, CD166, CD271, etc.) that can be used to characterize the MSC populations obtained from various sources [11,21,22]. Some of these markers may allow for a more precise isolation of MSCs compared with "traditional" markers; for example, CD271 seems to be more specific to bone marrow-derived MSCs (BM-MSCs) and enables a selective isolation of BM-MSCs with a higher clonogenicity, lower hematopoietic contamination [22], higher paracrine secretion of cytokines, and significantly pronounced lymphohematopoietic engraftment-promoting properties [23]. Another phenotypic characteristic of MSCs is the lack of expression of costimulatory molecules (such as CD80, CD86, CD40, and CD40L), even after interferon-γ (IFN-γ) stimulation. This is in contrast to MHC-II molecules whose expression can be induced by IFN-γ stimulation [24].

It should be emphasized that the immunophenotype of MSCs is dynamic and changes over the course of culturing; some of these changes may represent alterations in the biological features of MSCs [11], for example, the loss of expression of CD90 [thymocyte differentiation antigen-1 (Thy-1)], CD15 [stage-specific embryonic antigen-1 (SSEA-1)], and CD309 [fetal liver kinase-1 (Flk-1)] was shown to be associated with the spontaneous neoplastic transformation of murine, but not human, BM-MSCs after numerous passages [25]. Human MSCs have largely been shown to be free of such transformative events [26–28].

Current View to Origin and Physiological Functions of MSCs

Although the immunophenotype of MSCs is quite well characterized in vitro, much less is known about their in vivo counterpart. The prevailing view is that all MSCs, irrespective of their in vivo source, are of a perivascular origin and may be regarded as a subset of pericytes (subendothelial cells that lie on the abluminal side of blood vessel lining) [29,30]. Indeed, it was found that cultured pericytes derived from various human fetal and adult tissues are adherent, display a phenotype that is similar to MSCs (similar to MSCs, they express CD73, CD90, CD105, and CD44 in situ), can differentiate into osteocytes, chondrocytes, and adipocytes under certain culture conditions or in long-term cultures, and show evident myogenic potential in vitro and in vivo [29,31]; similar to MSCs, they can also secrete multiple growth factors and cytokines [31]. Caplan proposed that once a pericyte is released from a blood vessel (ie, displaced from its natural position) in the case of local injury, it functions as an immunomodulatory and trophic MSC, which actively participates in (1) suppression of immune surveillance of the injured tissue in order to impede autoimmune reactions; (2) wound repair and tissue regeneration; and (3) angiogenesis [32]. In this regard, MSCs act as site-regulated natural "drugstores"; however, if the injury is extensive and/or occurs in elderly individuals, then the natural supply of MSCs may be insufficient and, therefore, needs to be supplemented by their local or systemic delivery [33]. The presence of activated MSCs at sites of inflammation or tissue damage is quite well understood, given that pericytes would be released from their endothelial interactions in vascularized locations; however, the relative number and source of MSCs that are mobilized from either remote or proximate vascularized sites remain to be determined [34]. Sacchetti et al. have clearly demonstrated that CD146-positive clonogenic human BM-MSCs with self-renewal capacity (as determined in vivo) reside in the "pericytic location," that is, in the abluminal side of the bone marrow sinusoidal wall, and play a key role in the formation of a special microenvironment (niche) for hematopoietic stem cells (HSCs) and hematopoiesis [6], lending much credence to the theory on the pericyte origin of MSC.

Interestingly, Maumus et al. performed immunostaining of intact human adipose tissue and demonstrated that native, CD34-expressing adipose tissue-derived MSCs (ASCs) (clonogenic and multipotential in vitro) are neither perivascularly localized (the majority of them are scattered throughout the stroma of adipose tissue with only a few being perivascularly localized) nor express markers of pericytes (such as CD140b, NG2, and α-smooth muscle actin) in situ, although the expression of these markers appears on ASCs during the in vitro culture process [35]. There are conflicting data emerging from other groups; for example, Zannettino et al. demonstrated that human ASCs are intimately associated with perivascular cells surrounding the blood vessels [36]. They used a different set of antibodies than the previous group focusing on stromal cell precursor antigen (STRO-1), 3G5, and CD146 and found that the ASCs isolated using these cell-surface markers had characteristics of MSCs, were clonogenic, and multipotential. Taken together, these findings probably do not negate the pericytic origin of MSCs in adipose tissue but rather suggest that once pericytes are released from the vascular wall and become MSCs, the latter do not necessarily retain the entire phenotype and localization of their ancestors (ie, pericytes). However, there is no definitive answer for the pericyte origin of ASC, and further assessment is needed. To make the picture more complex,

recent data indicate that MSCs in various human tissues may originate not only from pericytes ($CD146^+CD34^-CD31^-CD45^-$), but also from a recently identified histologically and phenotypically distinct subset of adventitial cells ($CD34^+CD146^-CD31^-CD45^-$), which reside in the outermost layer of larger vessels' wall, natively express surface markers of MSCs (such as CD90), and behave similar to MSCs in a long-term in vitro culture [37,38].

Differentiation Potential of MSCs—How Wide Is It?

Although minimal criteria defined by ISCT state that MSCs should show trilineage differentiation into bone, cartilage, and adipose tissues in vitro, it has been shown that under certain culture conditions, MSCs can differentiate into other mesodermal tissues such as skeletal muscle [39], tendon [40], myocardium [41], smooth muscle [42], and endothelium [43]. Furthermore, recent studies have indicated that under certain culture conditions, in the presence of specific mediators, MSCs may show plasticity, that is, the ability to cross germinal boundaries and differentiate into cells of ectodermic origin, for example, neurons [44], and into cells of endodermic origin, for example, various epithelial cells [45–48]. However, the nonmesodermal differentiation of MSCs is still controversial [5,21] in the absence of convincing in vivo data.

There is some evidence that MSCs fuse with particular cell types (eg, epitheliocytes) in vivo rather than transdifferentiate [49]; however, other groups have shown that this is not the case [47,50]. Recent in vitro data show that MSCs may partially (not permanently) fuse with and transfer mitochondria (or other cytoplasmic components) to fully differentiated cells (such as cardiomyocytes) and induce their reprogramming back to a progenitor-like state [51]. Thus, the exact differentiation potential of MSCs is yet to be elucidated, although there is some evidence that MSCs (or, at least, particular subsets, most likely bona fide stem cells) tend to posses some level of inherent plasticity, both in vitro and in vivo [21,52]. It should be noted that the in vitro differentiation potential of MSCs does not necessary predict or correlate with their in vivo differentiation capacity [53,54]. Additionally, the differentiation efficiency of MSCs may vary with age; for example, Zhu et al. showed that the osteogenic potential of human female ASCs decreases with age, but the adipogenic potential remains unchanged [55].

Several populations with a broader differentiation potential have been characterized. Jiang et al. were the first who demonstrated at a single cell level the existence of rare pluripotent stem cells (cultured from adult rodent bone marrow) with the capacity to differentiate into neuroectodermal, mesodermal, and endodermal cell types, both in vitro and in vivo. These cells were defined as multipotent adult progenitor cells (MAPCs) [56]; their equivalents were also cultured from mouse brain, muscle [57], pancreas, dermis, human skin, and adipose tissue [29]. MAPCs are phenotypically and biologically distinct from MSCs; human MAPCs can be expanded for more than 70 population doublings without cytogenetic abnormalities [58–60]. MAPCs were hypothesized to be embryonic stem cells reserved during embryogenesis and retained in the tissues of the adult body for future use [61]. However, this does not seem to be the case, as currently, there is no evidence that MAPCs exist in vivo; they are isolated from MSC cultures after several population doublings under specific conditions and, therefore, seem to be a product of culture-induced changes, such as reprogramming and dedifferentiation, resulting from genetic or epigenetic alterations [58,59]. Therefore, MAPCs may be regarded as a mere culture artifact [62], although there is some evidence that cells closely resembling murine MAPCs may originate from a rare subset within mouse bone marrow under specific MAPC culture conditions [58,63]. Various other populations similar to MAPCs have been identified and include, but are not confined to, marrow-isolated adult multilineage inducible cells [64], very small embryonic-like cells [65,66], and others. The origin of and relationship between these populations remain to be elucidated.

The Pleiotropic Role of MSCs in Tissue Repair—Which Mechanism Predominates?

Since MSCs are able to differentiate into cells of various tissues, it was thought that they were responsible for the normal turnover and maintainance of adult tissues (at least of mesenchymal origin), just as HSCs are responsible for the turnover and maintainance of blood cells [32]. Since MSCs can be easily isolated from bone marrow and other sources, it was originally thought that after the delivery of culture-expanded MSCs to the injured host, they would migrate to the site of injury and directly differentiate into the cells of an appropriate phenotype and function, thus contributing to the repair of the injured tissue [67]. These expectations were reinforced by evidence that injected MSCs preferentially home to injured areas, in particular to the foci of hypoxia, apoptosis, or inflammation [68].

MSCs were shown to demonstrate therapeutic efficacy in animal models of meniscus injury [69], neurological disorders [70], myocardial infarction (MI) [71], lung injury [72], ischemic acute renal failure [73], and others. Clinical studies have also shown that MSCs can be used safely and in some cases effectively for the treatment of various conditions, including osteogenesis imperfecta [74].

However, it eventually became evident that MSCs could mediate robust tissue repair, but exhibited low or/and transient engraftment into the injured tissue [75,76]. Furthermore, several animal studies clearly demonstrated that injected MSCs reconstituted the structure and function of the injured organ without differentiation into (or fusion with) specialized cells of that organ [73,77]. Many studies have also demonstrated that MSC-conditioned media alone could have therapeutic effects, for example, the stimulation of endothelial cell proliferation and migration in vitro and in a hindlimb ischemia model in vivo [78]. Collectively, these data suggest that typically MSCs do not differentiate into specialized resident cells of the injured tissue; rather, they exert their reparative functions through paracrine effects [8,21,32,79]. Indeed, an analysis of human [80] and murine [81] MSC transcriptome revealed that they express transcripts encoding proteins involved in immunomodulatory and trophic activities. Furthermore, there are data that MSCs may exert their therapeutic functions through systemic (endocrine) activity, and this provides an explanation, at least in part, for how intravenously injected MSCs can act on distant injured or diseased tissues [82], as data from animal studies have

shown that often, most of the systemically administered MSCs are entrapped within the lungs [83,84]. MSCs may also exert their therapeutic activity through direct cell-to-cell contact with the cells of the immune system [85,86] and/or the tissue to be repaired [51].

The MSC-mediated trophic activities include (1) inhibition of apoptosis and fibrosis (this limits the field of damage or injury and ensures optimal wound healing with minimal scarring); (2) stimulation of angiogenesis and recovery of blood supply; (3) stimulation of recruitment, retention, proliferation, and differentiation of tissue-specific and tissue-intrinsic stem/progenitor cells [32,87]; and (4) attenuation of oxidative stress [79].

MSC-mediated immunomodulatory activities include suppression of naïve and memory $CD4^+$ and $CD8^+$ T-cell proliferation and differentiation, promotion of regulatory T-cell expansion and enhancement of their immunosuppressive activity, impairment of dendritic cell (DC) phenotype and functions, and secretion of immunosuppressive substances [such as nitric oxide (NO), prostaglandin E_2, hepatocyte growth factor, indoleamine 2,3-dioxygenase, etc.], and other mechanisms as reviewed in detail in [88–90].

The immunomodulatory properties of MSCs likely play an important role in protecting the injured organ from a potential autoimmune attack (since many self-antigens may be abundantly exposed during tissue damage) [32], as well as they may have obvious therapeutic benefits in controlling graft-versus-host disease (GvHD), solid organ allogeneic rejection [91], and autoimmune diseases [88], as will be discussed later in this review. On the other hand, it has been demonstrated that under certain conditions, MSCs can act as proinflammatory cells and even as efficient antigen-presenting cells that are able to present intracellular antigens and cross-present extracellular antigens with MHC-I class molecules and induce specific $CD8^+$ T-cell responses [92,93]. MSCs can also up-regulate the expression of MHC-II molecules (on stimulation by IFN-γ) and present exogenous antigens to $CD4^+$ T cells [92,94]. Thus, current data suggest that immunosuppression is not an inherent constitutive property of MSCs, that is, the acquisition of either immunosuppressive or immunostimulating properties by MSCs depends on a complex and delicate balance between the multiple stimuli of different origins within the context of the microenvironment [95]. The concentration and duration of the stimulus, as well as the nature of signal involved (eg, infection vs. ischemia), seem to have an important role in MSC functional polarization (reviewed in [34,95,96]). Besides, these microenvironmental factors, the concentration of MSCs at the site of injury (MSC-to-lymphocyte ratio), may also play an important role in their immunostimulatory versus immunosuppressive polarization [97]. Furthermore, a tissue microenvironment may "shape" not only the immunomodulatory properties of MSCs, but also their overall secretory profile, depending on the kind of damage in a given tissue [8].

Although the prevailing view is that MSCs help tissue repair without directly contributing to it (reviewed in [32,33]), the results showing that these cells (both human and nonhuman) have the ability to differentiate into distinctive specialized cells in vivo [47,50,98–103] cannot be completely ignored. Moreover, there are data stating that a combined action of the paracrine effects of MSCs as well as their differentiation into specialized cells may contribute to the regeneration of the damaged tissue, as shown in a rat dilated cardiomyopathy model [104]. Furthermore, there is some evidence that MSCs may need to be cocultured with cells of a particular tissue (to mimic the tissue microenvironment) for the effective induction of differentiation into cells of that tissue [45,105], or specific biomaterials may need to be added to provide a scaffold that induces specific differentiation [106]. However, it is crucial to confirm that the differentiated progenies of MSCs adopt not only the phenotype of particular specialized cells, but also gain their function and persist for sufficient time in the restored tissue, because some studies have shown that while MSCs may acquire a differentiated phenotype, they lack the functional activity of specialized cells [107,108]. It should be also kept in mind that the differentiated progenies should be derived from autologous or HLA-matched MSCs in order to survive in an immunocompetent organism. Indeed, Niemeyer et al. showed that in the great majority of cases, differentiated osteoblasts from human MSCs (both BM-MSCs and ASCs) were eliminated by the immune system of immunocompetent mice, while undifferentiated MSCs survived [109].

Biology of Human MSCs from Different Sources

MSCs from different sources share many biological features, although there are some differences in (1) immunophenotype, for example, a proportion of ASCs is positive for CD34, at least in early in vitro culture stages, whereas MSCs from bone marrow or other sources do not express this marker [11,110,111]; (2) proliferative capacity, for example, Kern et al. compared MSCs isolated from bone marrow, adipose tissue, and umbilical cord and found that BM-MSCs had the lowest proliferative capacity (in terms of both proliferation rates and number of cell doublings), while umbilical cord-derived MSCs showed the highest proliferation potency [112]; (3) differentiation potential, for example, it has been shown (using in vivo assays) that MSCs isolated from dental pulp tend to preferentially differentiate into dentin rather than bone, although grown and transplanted under the same conditions as BM-MSCs [113]; (4) gene expression profile, for example, gene expression analysis of BM-MSCs and umbilical cord vein MSCs indicated that the former showed a higher expression of genes associated with osteogenic differentiation, while the latter exhibited a higher expression of genes involved in angiogenesis [114]; and (5) utility for specific medical applications, for example, it was demonstrated that ASCs show a significantly greater angiogenic potential compared with BM-MSCs [115], and may, therefore, be more effective in cardiovascular pathologies associated with ischemia.

BM-MSCs and ASCs—the Most Prevalent MSCs in Clinical Practice

Despite many unanswered biological questions around MSC origin, their exact role, and mechanism of action in various pathological conditions, they have already gained acceptance in a number of clinical investigations over a broad spectrum of indications. From the clinical perspective, one of the most attractive properties of MSCs (besides their ability to preferentially home to the sites of damage, tissue-regenerative, and immunomodulating capacities, as already

discussed) is their low immunogenicity (hypoimmunogenicity), which allows their safe therapeutic application in allogeneic, donor-mismatched [116–121], and even xenogeneic [122,123] settings. There is some evidence, however, that both murine [124] and human [125] nonself MSCs (at least BM-MSCs) may be immunogenic in animal models; however, to our knowledge, clinical trials have not demonstrated any adverse events associated with the use of allogeneic and HLA-mismatched MSCs to date.

Since BM-MSCs were the first MSCs identified, they have been extensively studied and are, therefore, best characterized. However, it was eventually found that MSCs can be easily and safely isolated not only from bone marrow but also from other sources, including adipose tissue [10], placenta [12], umbilical cord blood [13], umbilical cord [14], and dental pulp [113]. These alternative sources are very attractive, because bone marrow harvesting is rather invasive, painful, and associated with potential donor-site morbidity and potentially low yields. Thus, bone marrow aspirates should be mostly reserved for hematopoietic stem cell transplantation (HSCT) [85].

In 1976, adipogenic precursors were isolated from human adipose tissue by plastic adherence [126]; however, in 2001, ASCs were identified and characterized in human lipoaspirates by Zuk et al. [10], and this led to a recognition of adipose tissue as an alternative to bone marrow for MSCs. Large amounts of ASCs can be readily isolated from subcutaneous adipose tissue, which can be easily and repeatedly obtained by lipectomy or even more often by liposuction, a minimally invasive, safe, and well-established surgical procedure that is widely performed in clinical practice; ~400.000 liposuction surgeries are performed in the United States each year [127]. The removed excess adipose tissue is often discarded, as medical waste, and can serve as a valuable source of ASCs suitable for therapeutic applications [127].

Adipose tissue is composed of adipocytes and a heterogeneous cell population that surrounds and supports adipocytes and on isolation is termed the stromal vascular fraction (SVF) [128]. Mature adipocytes constitute less than 50% (generally about 20%–30%) of all cells in intact adipose tissue; however; they are very large cells and occupy more than 90% of adipose tissue volume [129,130].

The SVF is separated from mature lipid-laden adipocytes by centrifugation (the supernatant contains low-density floating adipocytes, whereas the SVF forms the denser cellular pellet). ASCs are subfractionated from the SVF population by plastic adherence in cultures (isolation and culture protocols are briefly reviewed in [11]). Crude SVF contains ASCs (about 30%–40%), vascular endothelial cells and their progenitors, vascular mural cells (smooth muscle cells and pericytes), and various numbers of circulating blood-derived cells such as leucocytes and erythrocytes [129–131].

Differences Between Human BM-MSCs and ASCs

Although BM-MSCs and ASCs share many biological features, there are also some differences between these distinct MSC populations. For instance, human ASCs are more genetically and morphologically stable in a long-term culture [132], display a lower senescence ratio, show a higher proliferative capacity [112,132], and retain differentiation potential for a longer period in culture [132] compared with human BM-MSCs. A very recent study clearly demonstrated that human ASCs support hematopoiesis both in vitro and in vivo and unexpectedly seem to exert this activity more efficiently than human BM-MSCs [133].

Differences in localization and yield between human BM-MSCs and ASCs

BM-MSCs reside in the bone marrow stroma in relatively small quantities. It has been calculated that they constitute about 0.001%–0.01% of the total marrow nucleated cells [134], whereas the amount of ASCs is approximately 500-fold greater when isolated from an equivalent amount of adipose tissue [135,136]. This difference may be particularly relevant for making ASCs more suited for clinical applications due to their ease of accessibility. Additionally, sufficient numbers of BM-MSCs may be more difficult to obtain from a conventional marrow harvest, particularly from the elderly [85], as the number of MSCs decreases with age [137]. Furthermore, ASCs can be clinically used without expansion, if harvested from a sufficiently large volume (typically hundreds of mililiters of adipose tissue) of lipoaspirate [138]. It may be presumed that the use of freshly isolated cells would be more safe and efficacious compared with the cells expanded by culture, as ex vivo manipulations may lead to the accumulation of genetic and epigenetic alterations that may affect the functional and biological properties of the cells [134], even though human MSCs generally do not seem to undergo malignant transformation in long-term in vitro cultures [26–28] as well as in vivo [26,27]. Indeed, most clinical applications of ASCs are focusing on minimizing or even eliminating cell expansion in vitro before the implantation of the cells. Some clinical studies even apply freshly isolated autologous crude SVF rather than purified or cultured ASCs to eliminate additional processing steps [116,139,140]. There is evidence that various components of SVF (such as vascular endothelial cells, pericytes, and macrophages) may act synergistically with ASCs and, therefore, may be superior to ASCs alone [138]. The application of autologous crude SVF may be advantageous in acute clinical situations when it is not possible to use autologous expanded ASCs, which require additional time for culturing, unless off-the-shelf cryopreserved, allogeneic ASCs are available. The efficacy of crude SVF versus expanded ASCs is debated by Bai et al., showing equal efficacy of human SVF or cultured ASCs in a mouse model of acute MI [50], while Garcia-Olmo et al. demonstrated the superiority of ASCs over crude SVF in the treatment of enterocutaneous fistula in Crohn's disease [141]. Further investigation is needed to clarify which cell fraction may be most efficacious.

Differences in the immunophenotype between human BM-MSCs and ASCs

It has been shown that the immunophenotypes of BM-MSCs and ASCs are greater than 90% identical [127,142]; however, some minor differences seem to exist. A proportion of ASCs express CD34 when freshly isolated, although the expression of CD34 gradually declines with successive passages [35,110,111,143,144]; it may not be entirely lost [144]. CD34 is generally not expressed by adult BM-MSCs or by

MSCs from other sources [11,35]. ASCs moderately or strongly express CD49d (integrin α4), but not CD106 [vascular cell adhesion molecule-1 (VCAM-1)], while BM-MSCs highly express CD106, but not CD49d [145,146]. ASCs express high levels of CD54 [intercellular adhesion molecule-1 (ICAM-1)], while BM-MSCs show a minimal expression of this marker [147,148]. BM-MSCs moderately or strongly express CD49f (integrin α6) and podocalyxin-like protein 1 (PODXL), while the expression of these surface markers is low in ASCs [149].

In fact, there is still much debate about the immunophenotypic differences between BM-MSCs and ASCs. For example, some authors claim that CD106 is neither expressed in ASCs [142,145] nor is its expression found in negligible numbers of cells [149,150], while others have shown CD106 expression in ASCs [110]. Similarly, several groups have demonstrated the expression of STRO-1 [142,151] and CD34 [110,111] in ASCs, while others have reported the absence or low levels of STRO-1 [110] and CD34 expression [142,147,148] in ASCs. Such apparent discrepancies can, at least in part, be explained by differences in antibody sources (eg, different epitopes or isoforms recognized by different antibodies) [127,136], as well as by variables in culture medium and duration, cell density, number of culture doublings, proliferative stage of cells in culture, donor age, and various other factors [128,150,152,153]. There are data that distinct subsets with different immunophenotypes, proliferation capacity, and differentiation potential exist in the general MSC population from the same source, and the predominance of a particular subset (with a slightly distinct phenotype) may be influenced by various factors, such as isolation and culture protocols (reviewed in [152] and [153]). It can also be presumed that some immunophenotypic differences between BM-MSCs and ASCs (including those still unidentified) may contribute to differential responses of ASCs versus BM-MSCs to growth factors and biomaterial scaffolds.

Differences in the differentiation potential between human BM-MSCs and ASCs

The differentiation capacity of ASCs versus BM-MSCs is debated with data on each side supporting the superiority of one cell type over the other. Some studies have found that ASCs display pronounced adipogenic differentiation compared with BM-MSCs in vitro [149,154]; however; others have not reproduced those differences in the adipogenic differentiation capacity [112,145,146,155]. Similarly, while some studies demonstrate that BM-MSCs are more prone to osteogenic differentiation than ASCs in vitro [145,154,155], others show that the osteogenic response of BM-MSCs is not significantly greater than that of ASCs [112,146,149]. Gender differences appear to influence the osteogenic capacity of ASCs, with male ASCs differentiating more rapidly and more effectively than female ASCs in vitro; moreover; the osteogenic potential of female ASCs decreases with age, while the adipogenic potential remains unchanged [55,128]. It was found by several groups that ASCs show decreased chondrogenic differentiation capacity compared with BM-MSCs in vitro [156–158]; however, other groups report no differences in chondrogenic potential [112] or show that the use of a greater dose combination of particular growth factors, such as transforming growth factor-β2 and insulin-like growth factor-1, enhances the chondrogenic potential of ASCs (which was initially lower) to a level that is comparable to that of BM-MSCs [159]. It was also shown that the addition of bone morphogenetic protein 6 (BMP-6) enhances the chondrogenesis of ASCs [160].

Collectively, these often conflicting data imply that MSCs from different sources can respond differently to different stimuli, such that conditions optimal for BM-MSC differentiation might not be well suited for ASCs [136]. Culture conditions [eg, media supplemented with either human serum or fetal calf serum (FCS), or serum-free] may also affect the differentiation potential of even MSCs of the same origin [128]. In all likelihood, the differences in the differentiation efficiencies are more reflective of the heterogeneity of MSC populations (ie, the presence of distinct subpopulations) [151,152,161,162]. Different isolation and culture protocols used by various groups may account for the predominance of a particular MSC subpopulation with a distinct differentiation potential [151–153].

Differences in transcriptome and proteome between human BM-MSCs and ASCs

Several studies clearly demonstrated that there are some differences in the global transcriptomic and proteomic profiles of BM-MSCs and ASCs [132,145,163,164]. For example, Noël et al. investigated the expression of 384 genes in BM-MSCs and ASCs and found that 3.4% of the analyzed genes were specifically expressed by only one MSC population [145]. In addition, 9.7% of the analyzed genes were differentially expressed between BM-MSCs and ASCs. Taken together, these results indicated that the differential expression of 13.2% of genes was able to discriminate between the 2 populations, but without identifying markers specific to each MSC population. Genes expressed only in BM-MSCs were involved in WNT signaling and differentiation pathways (*WNT11*, *WNT7B*, and *SOX6*), while genes expressed uniquely in ASCs were involved in cellular communication (*CCL3*, *FGF9*, *IL1R2*, and *KDR*) and transcription control (*PAX3*, *SPI1*, and *ZNF45*). Proteomic analysis (2D electrophoresis of the whole cell extract) of BM-MSCs and ASCs performed by the same group showed that 23% of proteins were expressed specifically in one or the other MSC population; 18% of the total proteins were found to be differentially expressed between BM-MSCs and ASCs [145].

Differences in the immunomodulatory activity between human BM-MSCs and ASCs

Although it was initially shown that BM-MSCs and ASCs exhibit very similar immunosuppressive properties in vitro [85,148], more recent studies indicate that there are additional differences [155,165]. Bochev et al. incubated peripheral blood mononuclear cells (PBMC) alone or with isolated allogeneic BM-MSCs or ASCs at a 10:1 ratio in the presence or absence of pokeweed mitogen (PWM) and after 7 days of culture, assessed the immunoglobulin (Ig) amount in supernatants by ELISA. They found that Ig production in the presence of MSCs was significantly inhibited only in PWM-stimulated PBMC cultures and, most importantly, ASCs suppressed Ig production to a much greater extent than BM-MSCs [155]. In another study, the same group evaluated the

effect of BM-MSCs and ASCs on the maturation and differentiation of human monocytes into DCs and the secretory profile of these DCs [165]. They found that ASCs were better than BM-MSCs, significantly inhibiting both the differentiation of blood monocytes into DCs (defined by CD83 expression) and the expression of functionally important costimulatory molecules (CD80, CD86) on the surface of mature monocyte-derived DCs. Moreover, it was found that ASCs were better than BM-MSCs at stimulating the secretion of immunosuppressive cytokine IL-10 by DCs [165]. Collectively, these in vitro results suggest that ASCs can be more effective suppressors of immune response compared with BM-MSCs. Although the underlying mechanisms of this phenomenon are not known, the authors postulated that ASCs may express a different set of molecules responsible for the immunosuppressive activity than BM-MSCs [165]. This viewpoint is consistent with the results, showing differences in the gene expression profile between BM-MSCs and ASCs [145], as discussed in the previous section.

Animal Models and Preclinical Studies with BM-MSCs and ASCs

Various animal models have been used to characterize the biological properties and functions of BM-MSCs and ASCs in healthily and diseased models to evaluate their therapeutic potential and clinical safety (see Tables 1 and 2). The majority of preclinical studies have been conducted in rodents (due to their size, cost, access of inbred and genetically modified strains, etc.); however, a small but growing number of studies have been performed in large animal models, although there are often limitations in terms of cell tracking due to the relative inavailability of species-specific antibodies [166].

Important results were obtained using BM-MSCs and ASCs in animal models of GvHD. Sudres et al. showed that there is no prevention of GvHD when a single dose (5×10^5, 3×10^6 or 4×10^6) of allogeneic BM-MSCs is co-administered with hematopoietic stem cell transplantation (HSCT) in a mouse model of GvHD [167]. In another study, Polchert et al. [168] demonstrated that a similar co-injection of allogeneic BM-MSCs with the BM graft could not prevent the development of GvHD; however, if BM-MSCs were administered at day 2 or at day 20, then they significantly increased animal survival. The administration of BM-MSCs at day 30, on the other hand, had no significant beneficial effect on GvHD mortality, suggesting an optimum window of opportunity for MSC-mediated effects. This effect was likely mediated by IFN-γ secreted by activated and robustly proliferating donor alloreactive T cells. It was found that no serum IFN-γ could be detected at day 0, but was present at days 2 and 20 to activate BM-MSCs' immunosuppressive activity. At day 30, BM-MSCs could not manage GvHD, possibly due to their inability to overwhelm an already too robust donor T-cell response and/or an insufficient concentration of IFN-γ for optimal BM-MSC activation (it was found that by day 30, the serum levels of IFN-γ were considerably decreased, despite the increased number of T cells that had reduced IFN-γ secretion).

Similar results have been shown using ASCs in a mouse haploidentical HSCT model. ASCs could prevent GvHD when they were repeatedly administered at the same doses (5×10^4 ASCs) on days 0, 7 and 14, but not at later days 14, 21, and 28, and the infusion of a single high dose (5×10^5) on day 0 did not show a therapeutic effect [148].

Collectively, these results clearly indicate that the timing of MSC administration plays a critical role in preventing and/or treating GvHD. However, it should be noted that the timing of MSC administration may be more important in an allogeneic setting, because it has been shown that the coinfusion of autologous BM-MSCs with donor bone marrow reverses GvHD in a rat model [169].

Preclinical results show that MSCs may also have a potential role in the treatment of diseases and disorders that are not associated with high morbidity or mortality. Lin et al. [170] in a rat model of stress urinary incontinence demonstrated a significant therapeutic effect (improvement of internal urethral sphincter function, assessed by cystometric analysis) mediated by autologous ASCs isolated from periovary fat pads. Once in the urethra, the tail vein-injected ASCs were able to perform functions similar to ASCs transplanted directly in the urethra. It was found that a small fraction of the directly transplanted ASCs might have differentiated into smooth muscle cells (as determined by α-smooth muscle actin staining). However, since the great majority of the transplanted ASCs appeared to remain undifferentiated, their therapeutic effects were likely mediated by trophic factors that promote host tissue regeneration. Urethral histological analysis indicated that normal-voiding rats from the ASC-treated group had significantly higher smooth muscle and elastin content after treatment than either the control group or ASC-treated abnormal-voiding rats. There was no statistical difference in the smooth muscle and elastin content between normal-voiding and abnormal-voiding rats within the control group. The therapeutic effect of allogeneic BM-MSCs was also investigated in a rat model of stress urinary incontinence, induced by bilateral pudental nerve dissection [171]. BM-MSCs injected bilaterally into the proximal periurethral tissues of female rats had a significant effect after 4 weeks on leak point pressure (LPP) and closing pressure (CP). Moreover, in the MSC-treated group, LPP and CP values were restored to levels of the sham group. Immunohistochemical analysis revealed that the injected BM-MSCs persisted in periurethral tissues, and showed expression of smooth muscle markers (α-smooth muscle actin, desmin, and vimentin), suggestive of the possible differentiation of BM-MSCs into smooth muscle cells.

In a model of rat erectile dysfunction (induced by bilateral cavernous nerve crush injury), the direct delivery of autologous ASCs into the rat penis was associated with a significantly improved erectile function compared with the control groups [172]. The erectile function was evaluated by measuring the intracavernosal pressure (ICP) 5 weeks after an injection of ASCs or phosphate-buffered saline (PBS). Furthermore, there was evidence that some ASCs labeled with 5-bromo-2′-deoxyuridine (BrdU) appeared to directly differentiate into endothelial cells and smooth muscle cells within the erectile tissue. The authors postulated that besides the possible direct differentiation of ASCs into the components of erectile tissue, paracrine secretion likely contributed to the observed effects.

Bivalacqua et al. investigated the therapeutic effect of syngeneic wild-type BM-MSCs (wt-BM-MSCs) and genetically modified [adenovirally transduced with endothelial NO synthase (*eNOS* gene)] BM-MSCs (eBM-MSCs)

Table 1. BM-MSCs in Experimental Disease Models

Disease model	*Animal*	*Preclinical outcome*	*References*
Cisplatin-induced acute renal failure	Mouse	Protection of renal function	[245]
Ischemia-reperfusion-induced acute renal failure	Rat	Protection of renal function	[73]
Mesangioproliferative glomerulonephritis	Rat	Acceleration of glomerular healing	[246]
Stress urinary incontinence	Rat	Improvement of voiding function	[171]
Erectile dysfunction	Rat	Improvement of erectile function	[173]
MI	Rabbit	Improvement of heart function	[247]
	Pig		[248]
Chronic myocardial ischemia	Dog	Improvement of heart function	[249]
Dilated cardiomyopathy	Rat	Improvement of heart function	[104]
Experimental colitis	Rat	Promotion of gut mucosa healing	[250]
Acute hepatic failure	Rat	Hepatoprotection mediated by BM-MSC-derived molecules	[251]
Bleomycin-induced or endotoxin-induced lung injury	Mouse	Attenuation of lung injury	[72,75]
Ovalbumin–induced asthma	Mouse	Attenuation of acute asthma-associated inflammation	[252]
Traumatic brain injury	Rat	Improvement of neurological function	[122]
Spinal cord injury	Rat	Promotion on neuronal function recovery	[107]
Cerebral ischemic stroke	Rat	Improvement of neurological function	[229]
Neurodegenerative diseases (HD, AMLS, PD)	Rat Mouse	Protection of neuronal loss and improvement of neurological outcomes	[253–255]
EAE (model of MS)	Mouse	Improvement of neurological function	[256]
EAU	Rat	Attenuation of EAU (reduction of severity and delay of onset)	[257]
Experimental type 1 diabetes	Mouse	Amelioration of diabetes	[258]
	Pig		[259]
SLE	Mouse	Improvement of multiple organ function	[223]
Colagen-induced autoimmune arthritis	Mouse	No clinical effect or worsening of clinical parameters [260], prevention from developing arthritis [261]	[260] [261]
Osteoarthritis	Goat	Regeneration of meniscus	[69]
Critical size bone defect	Dog	Enhancement of defect repair	[121]
Cardiotoxin-induced muscle damage	Rat	Regeneration of myofibers	[39]
Muscular dystrophy (model of DMD)	Mouse (*mdx*-nude)	Regeneration of myofibers	[39]
Solid organ (skin, heart, liver, and kidney) transplantation	Baboon	Attenuation of acute rejection and prolongation of graft survival	[262]
	Mouse		[263]
	Rat		[264–266]
Sepsis	Mouse	Amelioration of the disease	[267]
GvHD	Mouse	No effect when coadministered with HSCT once	[167]
GvHD	Mouse	Prevention or treatment of GvHD when injected once at day 2 or 20 respectively; no effect when injected at days 0 or 30	[168]
Persistent pain (neuropathy)	Rat	Long-term reversion of pain hypersensitivity	[268]
Deep burn wound	Pig	Enhancement of wound healing	[269]

MSCs, mesenchymal stem/stromal cells; BM-MSCs, bone marrow-derived MSCs; EAE, experimental autoimmune encephalomyelitis; EAU, experimental autoimmune uveoretinitis; AMLS, amyotrophic lateral sclerosis; DMD, Duchenne muscular dystrophy; GvHD, graft-versus-host disease; HD, Huntington's disease; HSCT, hematopoietic stem cell transplantation; MS, multiple sclerosis; PD, Parkinson's disease; SLE, systemic lupus erythematosus.

administered intracavernosally in age-associated erectile dysfunction in elderly male rats [173]. Erectile function was assessed by measuring changes in ICP in response to cavernous nerve stimulation at days 7 and 21. Histological analysis showed that the injected BM-MSCs were present in corporal tissue for at least 21 days. Both eBM-MSCs and wt-BM-MSCs significantly increased erectile function compared with the PBS injection; however, the effect of eBM-MSCs emerged at earlier time points relative to wt-BM-MSCs (7 days vs. 21 days). The improvement of erectile function was associated with increased eNOS protein expression, calcium-dependent (constitutive) NOS activity, and cyclic guanosine monophosphate (cGMP) levels in erectile tissue, suggesting that both eBM-MSCs and wtBM-MSCs increased endothelial-

Table 2. ASCs and SVF in Experimental Disease Models

Disease model	*Animal/cell type*	*Preclinical outcome*	*References*
Ischemia-reperfusion-induced kidney injury	Rat/ASCs	Amelioration of kidney damage	[270]
Erectile dysfunction	Rat/ASCs	Improvement of erectile function	[172]
Stress urinary incontinence	Rat/ASCs	Improvement of voiding function	[170]
MI	Mouse/ASCs/SVF	Improvement of heart function	[50]
	Rat/SVF		[271]
	Rabbit/ASCs		[272]
	Pig/ASCs		[273]
Hindlimb ischemia	Mouse/ASCs	Induction of angiogenesis and recovery of limb muscle injury	[274]
	Mouse/SVF		[275]
Experimental colitis (model of Crohn's disease)	Mouse/ASCs	Amelioration of clinical and histopathologic severity of experimental colitis	[276]
Liver injury	Mouse/ASCs (predifferentiated into hepatocytes)	Liver regeneration	[277]
Spinal cord injury	Rat/ASCs	Improvement of motor function	[278]
	Dog/ASCs	Improvement of neurological function	[279]
Acute ischemic stroke	Rat/ASCs	Limitation of brain infarction area, enhancement of neurological function recovery	[230]
Hemorrhagic stroke	Rat/ASCs	Amelioration of neurological function	[123]
EAE (model of MS)	Mouse/ASCs	Improvement of neurological function	[280]
Huntington's disease	Rat/ASCs	Retardation of striatal degeneration and behavioral deterioration	[281]
Peripheral nerve injury	Rat/ASCs	Increase in axonal regeneration	[282]
Experimental type 1 diabetes	Mouse/ASCs (predifferentiated into pancreatic islet-like cells)	Amelioration of diabetes	[283]
	Rat/ASCs (predifferentiated into insulin-producing cells by *Pdx1* gene transduction)		[284]
Osteoarthritis	Dog/ASCs	Improvement of joint function	[285]
Collagen-induced autoimmune arthritis	Mouse/ASCs	Amelioration of arthritis	[286]
Collagenase-induced tendinitis	Horse/SVF	Improvement of tendon architectural organization	[287]
Tendon injury	Rabbit/ASCs	Acceleration of tendon repair	[288]
Critical size bone defects	Mouse/ASCs	Healing of the defect	[102]
	Rabbit/ASCs		[106]
	Rat/ASCs		[289]
Full-thickness cartilage defect	Rabbit/ASCs	Complete healing of the defect	[290]
Bilateral decortication of the L4/L5 transverse processes	Rat/ASCs	Acceleration of posterior lumbar spinal fusion	[291]
Muscular dystrophy	Mouse/SVF	Formation of dystrophin-expressing muscle fibers in dystrophin-deficient *mdx* mice	[275]
Solid organ - liver -transplantation	Rat/ASCs	Alleviation of acute rejection and prolongation of graft survival	[292]
Full-thickness dermal wound	Mouse/ASCs	Promotion of dermal wound healing	[293]
Sepsis	Mouse/ASCs	Amelioration of sepsis	[294]
Alergic rhinitis	Mouse/ASCs	Alleviation of symptoms of alergic rhinitis, inhibition of eosinophilic inflammation	[295]
PAH	Rat/ASCs	Amelioration of PAH	[296]
SLE	Mouse/ASC	Amelioration of SLE and restoration of immune homeostasis	[226]
GvHD	Mouse/ASCs	Prevention of GvHD	[148]

Pdx-1, pancreatic duodenal homeobox 1 (gene); ASCs, adipose tissue-derived MSCs; PAH, pulmonary arterial hypertension; SVF, stromal vascular fraction.

derived NO biosynthesis with a subsequent increase in cGMP signaling in the aged penile vasculature. The increase in peak ICP and biochemical parameters (NOS activity and cGMP levels) were significantly greater in eBM-MSCs-treated rats compared with wtBM-MSCs-treated and PBS-treated rats. There was evidence that the transplanted wtBM-MSCs may have differentiated into endothelial cells [determined immunohistochemically (IHC) by de novo expression of eNOS and platelet endothelial cell adhesion molecule-1 (PECAM-1)] and smooth muscle cells (determined IHC by de novo expression of smooth muscle myosin heavy chain SM-MHC) within the aged erectile tissue.

Clinical Application of BM-MSCs and ASCs

Promising preclinical studies using BM-MSCs and ASCs has prompted the initiation of a number of clinical trials. Some of the representative trials in the field of plastic surgery, orthopedics, cardiovascular diseases, GvHD, autoimmune, central nervous system, and liver diseases are briefly discussed in this section. Other clinical applications of MSCs are also being investigated in the treatment of various diseases, some of which are summarized in Table 3.

Plastic surgery

Autologous fat transplantation (lipoinjection) is a promising cosmetic tool for soft tissue augmentation (eg, breast, buttock augmentation), correction of various types of depressed facial deformities (eg, pectus excavatum, hemifacial microsomia, and lipoatrophy), and for age-related facial rejuvenation [140,174].

Autologous fat transplantation has many advantages in plastic surgery, such as the lack of incisional scarring or complications associated with foreign materials. However, there are some disadvantages such as unpredictability (the transplanted fat often loses volume) and low rates of graft survival due to partial necrosis and atrophy during the first 6 months, post-transplantation [140,175]. Yoshimura et al. originally showed that aspirated adipose tissue contains fewer vessels and approximately half the number of ASCs found in excised intact fat tissue, because during the liposuction procedure, a major portion of ASCs is either left unremoved at the donor site (ASCs are located mainly around vessels) [176], or released into the fluid portion of the lipoaspirate [131]. They postulated that the relatively low numbers of ASCs in aspirated fat tissue may be associated with low survival rate and progressive long-term atrophy of the autotransplant [140,175,176]. To address this, in 2003, Yoshimura et al. developed a novel strategy known as cell-assisted lipotransfer (CAL), in which half the volume of the aspirated fat is processed for isolation of the SVF containing up to 40% of ASCs, and the remaining half of the aspirated fat is harvested as graft material. The freshly isolated SVF is added to the harvested graft, with the latter acting as a living bioscaffold for transplantation. In this way, relatively ASC-poor fat can be converted into ASC-rich fat, which is subsequently used for lipoinjection [140,175]. It is believed that the turnover of adipose grafts happens during the first 2–3 months after transplantation (generally, adipose tissue turnover is usually slow, taking 2–10 years), because the grafted nonvascularized fat tissue experiences temporary ischemia followed by reperfusion [138]. ASCs that are more resistant to hypoxia may be responsible for the turnover of adipose tissue; thus, their relative deficiency in grafts may lead to the postoperative atrophy of the graft, which is circumvented by the CAL strategy [138,140,175].

Yoshimura et al. have performed several hundred successful CAL procedures for breast augmentation (including cosmetic breast augmentation, rescue for breast implant replacement, and postmastectomy reconstruction) without significant side effects [140,175,177]. There were 8 cases of cyst formation (5–15 mm in diameter), 2 cases of microcalcification (possibly due to inappropriate tissue preparation and injection technique; the calcifications were easily distinguished radiologically from those associated with breast cancer), and 2 cases of ectopic fibrogenesis (in both cases, SVF suspension was injected separately from fat grafts) [177]. Successful CAL procedures have also been performed on the face, hip, and hand, sometimes at multiple sites, implying that CAL can potentially be applied for the reconstruction or augmentation of any soft tissue defect [138,174,175,178].

Cytori completed a phase IV postmarketing clinical trial RESTORE-2, which evaluated the transplantation of autologous fat augmented with SVF (generated using Celution® system, a procedure very similar to CAL) in the treatment of recurrence-free breast cancer patients ($n = 71$) with functional and cosmetic breast deformities after segmental mastectomy or quadrantectomy (lumpectomy) with or without radiation therapy. In March 2011, Cytori released final results of the study [179], which show that at 12 months, 85% of physicians and 75% of patients were satisfied with the outcome of the procedure, which is consistent with the reported 6-month results. The satisfaction criteria included functional and cosmetic outcomes, namely breast deformity, breast symmetry, appearance of scarring, and skin pigmentation. There was no defined control for this trial, as there is no generally accepted standard of care. The comprehensive, peer-reviewed data are expected to be published in the near future.

It has been postulated that the immunomodulatory properties of ASCs may create a microenvironment that is favorable for the growth of yet undetectable tumors or rare tumor cells that were not originally removed during breast cancer surgical treatment [180]. To date, no such case has been reported to be directly associated with the CAL procedure or normal lipoinjection, which has been performed worldwide in thousands of women annually [178]. However, longer-term follow-up studies comparing the incidence of breast cancer in ASC/SVF-implanted versus untreated controls are needed to thoroughly evaluate this possibility [166].

The use of BM-MSCs in plastic surgery is less common and is mainly confined to wound therapy [181,182]; for example, a dose-dependent response was observed in patients with acute wounds from skin cancer surgery and chronic, long-standing nonhealing lower extremity wounds receiving cultured BM-derived MSCs along with fibrin polymer spray [181]. However, ASCs/SVF also emerge as a very promising tool for wound treatment, skin rejuvenation, and skin engineering (reviewed in [183] and [184]). For example, it has been demonstrated that purified autologous lipoaspirate transplant dramatically increased the healing of oncologic radiotherapy-induced chronic, degenerative dermal tissue damage such as fibrosis, atrophy, ulcers, and retraction [185].

Table 3. Representative Ongoing Clinical Trials with BM-MSCs and ASCs/SVF

Disease	*Trial phase*	*No of patients*	*Type of MSCs and study design*	*Application*	*Clinical trials.gov reference*
Articular femoral condyle cartilage defect	I/II	30	auto-ASC ($1\times10^6/cm^2$) vs. auto-chondrocytes ($1\times10^6/cm^2$)	Local intraarticular	NCT01399749
Degenerative arthritis	I/II	18	auto-ASC 3 arms: (1) $1\times10^7/3$ mL (2) $5\times10^7/3$ mL (3) $1\times10^8/3$mL	Local intraarticular	NCT01300598
Full-thickness articular knee cartilage defect	I	50	auto-BM-MSC vs. auto-chondrocytes	Local intra-articular	NCT00885729
Osteoarthritis	II	50	auto-BM-MSC in HA vs. HA alone	Local intra-articular	NCT01459640
Atrophic nonunion fracture	I/II	40	auto-BM-MSC 2 arms: (1) ($20\times10^6/3$ mL)+second injection, if no callus after 6 months (2) PBS (3 mL)	Local percutaneous	NCT01429012
Articular cartilage defects	II/III	25	auto-BM-MSC	Local intra-articular	NCT00891501
Osteogenesis imperfecta (type II, III)	I	12	allo-BM-MSC 6 infusions over 20 months 2 arms: (1) previously treated with BMT (2) without history of BMT	Intravenous	NCT01061099
Treatment-refractory Crohn's disease	III	270	allo-BM-MSC (Prochymal™) 3 arms: (1) low dose (600×10^6 total) (2) high dose (1200×10^6 total) (3) placebo	Intravenous	NCT00482092 www.osiris.com
Treatment-refractory Crohn's disease with perianal fistula	I/II	21	allo-BM-MSC 4 arms: (1) sham (2) 10×10^6 (3) 30×10^6 (4) 90×10^6	Local intrafistular	NCT01144962
Fistulizing Crohn's disease	I/II	15	auto-ASC	Local intrafistular	NCT01157650
Fistulizing Crohn's disease	I	6	allo-ASC (1) 10×10^6/mL for 3 patients, and if safe (2) 30×10^6/mL for other 3 patients	Local intrafistular	NCT01440699

(*continued*)

Table 3. (Continued)

Disease	*Trial phase*	*No of patients*	*Type of MSCs and study design*	*Application*	*Clinical trials.gov reference*
Complex perianal fistula	II	40	auto-ASC 2 arms: (1) low dose (10×10^6/mL) (2) high dose (20×10^6/mL)	Local intrafistular	NCT01314092
Acute MI	II	220	allo-BM-MSC (Prochymal) vs. Placebo	Intravenous	NCT00877903 www.osiris.com
Acute MI	III	50	auto-BM-MSC vs. placebo	Local intraendocardial	NCT01394432
Acute MI	I	48	auto-SVF vs. placebo	Local intracoronary	NCT00442806 APOLLO trial www.cytori.com
Acute MI	II/III	360	auto-SVF 3 arms: (1) dose A (2) dose B (3) placebo	Local intracoronary	NCT01216995 ADVANCE trial www.cytori.com
Post-MI chronic myocardial ischemia	I	36	auto-SVF vs. placebo	Local intramyocardial intraventricular	NCT00426868 PRECISE trial www.cytori.com
Post-MI chronic myocardial ischemia	I/II	30	auto-BM-MSC (1) 20×10^6 total (2) 100×10^6 total (3) 200×10^6 total allo-BM-MSC (1) 20×10^6 total (2) 100×10^6 total (3) 200×10^6 total	Local transendocardial	NCT01087996 POSEIDON trial
Diabetes mellitus-associated critical limb ischemia	I/II	36	auto-ASC 4 arms: (1) low dose (0.5×10^6/kg) (2) medium dose (1×10^6/kg) (3) high dose (2×10^6/kg) (4) conventional treatment without intervention	Local intraarterial (*Arteria femoralis*)	NCT01257776
Critical limb ischemia	I/II	20	allo-BM-MSC vs. Plasmalyte A	Local intramuscular	NCT00883870

(*continued*)

Table 3. (Continued)

Disease	Trial phase	No of patients	Type of MSCs and study design	Application	Clinical trials.gov reference
Chronic GvHD	I/II	30	allo-ASC 3 arms: (1) standart treatment + low dose ASC (1×10^6/kg) (2) standart treatment + high dose ASC (3×10^6/kg) (3) standart treatment	Intravenous	NCT01222039
Acute or chronic GvHD	I	10	Clonal allo-BM-MSC (1×10^6/kg)	Intravenous	NCT01318330
Steroid-resistant acute GvHD or poor graft function	II	120	allo-BM-MSC 3 arms: (1) 4×10^6/kg for refractory aGvHD (2) 2×10^6/kg for poor graft function (3) 2×10^6/kg + DLI for poor donor T-cell chimerism	Intravenous	NCT00603330
Liver fibrosis	I	3	auto-BM-MSC (twice with 6 mo interval) + pioglitazone (30 mg daily for 24 months)	Intravenous (via portal vein)	NCT01454336
Wilson cirrhosis	II	10	allo-BM-MSC (from healthy donor with opposite gender)	Intravenous (peripheral or portal vein) Intraarterial (*a. hepatis dex*)	NCT01378182
Refractory primary biliary cirrhosis	I	20	allo-BM-MSC (single dose $5–50\times10^6$/kg) vs. ursodeoxycholic acid (13–15 mg/kg/day *per os*, to the end of the study)	Intravenous	NCT01440309
Kidney or liver transplantation	I/II	40	allo-BM-MSC (third party) 4 arms: (1) kidney transplant standart treatment + $1.5–3\times10^6$/kg (2) kidney transplant standard treatment (3) liver transplant standart treatment + $1.5–3\times10^6$/kg (4) liver transplant standard treatment	Intravenous	NCT01429038
Severe pulmonary emphysema	I	10	auto-BM-MSC	Intravenous	NCT01306513
Bronchiolitis obliterans after lung transplantation	I	10	allo-BM-MSC 2×10^6/kg twice weekly for 2 weeks	Intravenous	NCT01175655
Type I diabetes mellitus	I/II	30	auto-BM-MSC cotransplanted with allogeneic islet cells	Not specified	NCT00646724
Type I diabetes mellitus	II/III	80	auto-BM-MSC ($\sim2\times10^6$/kg)	Intravenous	NCT01157403

(*continued*)

Table 3. (Continued)

Disease	*Trial phase*	*No of patients*	*Type of MSCs and study design*	*Application*	*Clinical trials. gov reference*
Type I diabetes mellitus	II	60	allo-BM-MSC (Prochymal) vs. placebo	Intravenous	NCT00690066 www.osiris.com
Type II diabetes mellitus	I/II	10	auto-SVF 2 arms: (1) IA + IV injection (2) IV injection alone	Intravenous (IV) Intraarterial (IA, *a. pancreatica*)	NCT01453751
Refractory SLE	I/II	20	allo-BM-MSC 1×10^6/kg	Intravenous	NCT00698191
MS	I	24	auto-BM-MSC (1×10^6/kg)	Intravenous	NCT00813969
MS	I/II	30	auto-ASC 3 arms: (1) low dose 1×10^6/kg (2) high dose 4×10^6/kg (3) no intervention	Intravenous	NCT01056471
MS	I/II	20	allo-BM-MSC (1×10^6/kg)	Intravenous	NCT00962923
Parkinson's disease	I/II	10	auto-ASC	Intraarterial (*a. vertebralis*) Intravenous	NCT01453803
Ischemic cerebral stroke	II	50	auto-BM-MSC plus standart care vs. standart care alone	Intravenous	NCT01461720
Ischemic cerebral stroke	I/II	35	allo-BM-MSC $0.5–1.5\times10^6$/kg	Intravenous	NCT01297413
Spinal cord injury	I	20	auto-BM-MSC	Local intralesional	NCT01325103
Spinal cord injury	I/II	20	auto-BM-MSC 1×10^6/kg (IV) + 1×10^6/kg (IT)	Intravenous (IV) and Intrathecal (IT)	NCT01446640
Amyotrophic lateral sclerosis	I/II	24	auto-BM-MSC (secreting neurotrophic factors) 2 arms: (1) early ALS $1\times10^6\times24$ IM (2) progressive ALS 60×10^6 IT	Local intramuscular (IM) Intrathecal (IT)	NCT01051882
Cisplatin-induced ARF in cancer patients with solid tumors	I	9	allo-BM-MSC (1) 1×10^6/kg for 3 patients If any efficacy and safe, then (2) 2×10^6/kg for other 3 patients If any efficacy and safe, then (3) 5×10^6/kg for other 3 patient	Intravenous	NCT01275612
Treatment-refractory neuroblastoma	0	15	allo-BM-MSCs + hiHSCT + ^{131}I-MIBG + DLI + ricChT + rituximab	Intravenous	NCT00790413

ARF, acute renal failure; auto, autologous; allo, allogeneic; BMT, bone marrow transplantation; DLI, donor lymphocyte infusion; HA, hyaluronic acid; hiHSCT, haploidentical hematopoietic stem cell transplantation; ^{131}I-MIBG, iodine-131 metaiodobenzylguanidine; MI, myocardial infarction; ricChT, reduced intensity conditioning chemotherapy; PBS, phosphate-buffered saline.

It would seem reasonable that ASCs/SVF are preferred in plastic surgery, as it would be quite arguable to use BM-MSCs when the therapeutic efficacy of ASCs is demonstrated, especially for clinical situations or conditions that are not life threatening, because marrow harvesting is a more invasive and potentially donor-site morbid procedure.

Orthopedics

The capacity of articular cartilage to heal is limited (due to low mitotic potential of chondrocytes in vivo); thus, defects in the joint cartilage progress to osteoarthritis [186]. Traditionally, articular cartilage defects are treated by abrasion, microfracturing, mosaic plasty, or cell-engineering strategies such as autologous culture-expanded chondrocyte implantation (ACI). ACI is associated with difficulties, such as obtaining a sufficient number of autologous chondrocytes, donor-site morbidity, and poor histological repair [186]. To address these limitations, the use of autologous BM-MSCs was investigated. Wakitani et al. tested the role of autologous BM-MSCs in the repair of large articular cartilage defects in the osteoarthritic knees of patients ($n=24$) who underwent high tibial osteotomy [187]. There were no significant differences in clinical improvement between patients treated with BM-MSCs along with periosteal flap ($n=12$) and the controls treated with periosteal flap alone, although the arthroscopic and histological grading score was higher in the BM-MSC-transplanted group. The same group also reported using autologous BM-MSCs in the repair of full-thickness articular cartilage defects of patella in 2 patients [188], and articular cartilage defects in the patello-femoral joint in the knees of 3 patients [189]. Autologous BM-MSCs also promoted the repair of large, full-thickness articular cartilage defect in the medial femoral condyle of a 31-year-old athlete [186]. The role of ASCs in the treatment of cartilage defects is investigated in clinical trials (NCT01399749, NCT01300598 at www.clinicaltrials.gov; see Table 3).

Both BM-MSCs and ASCs have been used for the successful repair of various bone defects, including critical size defects in the long bone [190], hard palate reconstruction [191], and craniomaxillofacial defects [128,166].

BM-MSC have been shown to have superior chondrogenitc potential to ASCs in vitro differentiation assays [156–158], although the modulation of in vitro factors allows for the chondrogenic differentiation of ASCs that are comparable to BM-MSCs [159–160]. Similarly, some authors claim that BM-MSCs are more prone to osteogenic differentiation than ASCs in vitro [145,154,155], whereas others demonstrate that the osteogenic potential of BM-MSCs is not significantly greater than that of ASCs [112,146,149]. However, the osteogenic capacity of ASCs seems to decline with age, and this phenomenon is more prominent in women [128]. The in vitro data suggest that BM-MSCs may, thus, be better suited to repair cartilage defects, although it is not clear whether BM-MSCs or ASCs actually affect cartilage or bone repair by differentiation into chondrogenic or osteogenic cells. If paracrine factors are implicated in the therapeutic effects of these MSCs, there would be little difference between the 2 cell types.

Therapeutic potential and the clinical experience of MSC application for orthopedic diseases are reviewed in greater detail in [192], and summarized in Table 3.

Myocardial infarction

Chen et al. investigated the use of autologous BM-MSCs for treating acute MI in 69 patients who underwent primary percutaneous coronary intervention (PCI) [193]. Patients were randomized into 2 groups, and 34 patients received an intracoronary BM-MSC injection, with controls receiving saline injections. Three months of follow-up showed that both wall movement velocity over the infarcted region and left ventricular ejection fraction (LVEF) were significantly increased, while regional functional defects (percentage of hypokinetic, akinetic, and dyskinetic segments), perfusion defects (evaluated by positron emission tomography), and left ventricular end-systolic and end-dyastolic volumes significantly decreased in the BM-MSC-treated group when compared with the control group.

Mohyeddin-Bonab et al. investigated the therapeutic efficacy of autologous BM-MSCs in 16 patients with old MI undergoing coronary artery bypass grafting or PCI [194]. They showed that while patients receiving local injections of BM-MSCs ($n=8$) had an increased LVEF compared with the control group, this difference was not statistically significant. However, there were significant differences in terms of clinical improvement (as assessed by the New York Heart Association classification) and reduced infarction size in the BM-MSC treated group relative to controls.

A randomized phase I clinical study assessing the systemic delivery of allogeneic BM-MSCs (Prochymal™) without immunosuppression after acute MI similarly showed significant improvements in symptomatic global assessments, cardiac arrhythmias, and pulmonary function in the MSC-treated groups relative to controls (receiving saline) without concomitant differences in the ejection fraction between the 2 groups [117]. Subset analysis revealed that LVEF was improved as assessed by magnetic resonance imaging in MSC-treated MI patients compared with placebo controls 1 year after treatment, although the difference was again not statistically significant.

There are emerging data on the use of ASCs and the more heterogeneous SVF in cardiac applications. Results from the APOLLO trial show that the intracoronary delivery of autologous ASCs is safe [195]. Similar to the early phase I/II autologous and allogeneic BM-MSC trials, there does not appear to be significant efficacy in terms of LVEF improvement between cell-treated and control groups.

It would appear that ASCs might be better suited than BM-MSCs for cardiac applications at this early stage, as evidenced by their superior angiogenic properties in vivo [115]. Other studies also suggest that while the 2 cell types are equally adept at modulating an anti-inflammatory environment through secreted factors, ASCs appear to induce significant improvement in infarct area and LV infarct wall thickness, compared with the BM-MSC-treated and control groups [196].

Larger phase II/III trials will be needed to compare the efficacy of BM-MSCs and ASCs in a clinical setting. The therapeutic potential and clinical experience of MSC application for the treatment of cardiovascular (mainly cardiac) diseases are reviewed in greater detail in [198–200], and summarized in Table 3.

Graft-versus-host disease

Acute GvHD is a significant cause of morbidity and mortality after allogeneic HSCT. Treatment with

corticosteroids remains the gold standard for acute GvHD; however, even with prompt initiation, it is suboptimal [118]. Moreover, the response rate to steroid therapy is 30%–50% [200], and 30%–60% of the patients develop steroid-resistant GvHD [201]. Several clinical studies have demonstrated that BM-MSCs are effective in a considerable proportion of patients with acute de novo GvHD (combined with steroid therapy) [118] and steroid-resistant GvHD [200,202–205] with no detectable side effects or ectopic tissue formation. Moreover, both haploidentical and third-party (HLA-mismatched) BM-MSCs can be safely and effectively used to treat de novo and steroid-resistant acute GvHD [118,200,202–205]. Several recent reports indicate that BM-MSCs may also be effective in managing chronic GvHD [206–208].

In contrast, the Osiris-sponsored phase III trial (NCT00366145 at www.clinicaltrials.gov), using allogeneic BM-MSCs (Prochymal) in the treatment of patients ($n=244$) with steroid-resistant acute GvHD, failed to meet the primary end-point (complete response of ≥ 28 days duration) [209], although by day 100, Prochymal showed significantly improved overall response rates (both complete and partial) in steroid-refractory GvHD, involving liver (76% vs. 47%, $P=0.03$) and gastrointestinal (82% vs. 68%, $P=0.03$), but not skin (78 vs. 77%, $P=0.9$) [210]. The treatment of patients with GvHD affecting all 3 organs (skin, liver, and gut) resulted in a 63% overall response rate in the Prochymal group, while none of the placebo-treated patients responded ($P<0.05$). Additionally, patients treated with Prochymal had significantly less progression of liver GvHD compared with placebo (37% vs. 65%, $P=0.05$). There were no significant differences in the incidence of infection, recurrent malignancy, and toxicities between the groups. It should also be noted that there were more patients with severe (grade IV) GvHD in the Prochymal group than in the control group. Collectively, these results suggest that the addition of Prochymal is safe and shows improvement in patients with steroid-resistant acute GvHD involving visceral organs; however, the durability of the response remains questionable.

Similarly, the use of Prochymal appeared to be ineffective, as per the clinical trial (NCT00562497 at www.clinicaltrials.gov) evaluation criteria in treating patients ($n=192$) with de novo GvHD [209].

ASCs (both haploidentical and HLA-mismatched) have been shown to be effective in the salvage therapy of 6 patients with severe steroid-resistant acute GvHD [119]. Complete response was achieved in 5 patients (1 patient did not respond to ASC treatment); 4 of them were alive after a median follow-up of 40 months (1 patient died of leukemia relapse). All 4 survivors were in good clinical condition and free of their hematological malignancy. No side effects were observed after ASC treatment. Two pediatric patients with severe steroid-resistant GvHD were also successfully treated with intravenous infusions of ASCs from HLA-mismatched unrelated donors [211]. A larger, placebo-controlled trial using varying doses of autologous ASCs is currently underway (NCT01056471 at www.clinicaltrials.gov) and will provide some answers on the efficacy of ASCs in treating GvHD.

It should be noted that several studies showed that MSCs are not effective for preventing acute GvHD if they are cotransplanted with HSCs (reviewed in [95] and [212]). This is consistent with results of preclinical studies [148,167,168], implying that MSCs need to be "switched-on" by an evolving allogeneic immune response in the host [168]; conversely, transplanting after the immune response has been fully mounted is also ineffective, suggestive of an optimum window of opportunity [168]. Thus, the conflicting results in the GvHD trials may be explained by considering the cell dose, timing, and duration of treatment.

Clinical aspects of MSC application for the treatment of GvHD are reviewed in greater detail in [212–214], and are summarized in Table 3.

Autoimmune diseases

MSCs are also being investigated in patients with various autoimmune diseases.

Crohn's disease. Autologous BM-MSCs have been used for the treatment of fistulizing Crohn's disease. Ciccocioppo et al. conducted a phase I trial in which autologous BM-MSCs were locally injected to 10 patients with fistulizing Crohn's disease (9 patients with complex perianal fistulas and active rectal disease and 1 patient with multiple enterocutaneous fistulas) [215]. Patients were followed up for 12 months with no adverse effects reported. Seven patients (including the one with multiple enterocutaneous fistulas) had sustained complete fistula closure without signs of fibrosis, and 3 patients showed a partial response. A reduction of Crohn's disease activity index (CDAI) and perianal disease activity index ($P<0.01$ for both) was observed for all patients. Seven of 9 patients with perianal disease underwent lower endoscopy at the end of the follow-up, and complete healing of the rectal mucosa was evident, relative to the inflammation present before BM-MSC treatment. Additionally, the percentage of mucosal and circulating regulatory $FOXP3^+$ T cells significantly increased during the treatment and remained stable until the end of the follow-up. In another study, Duijvestein et al. investigated the role of intravenously infused autologous BM-MSCs for the treatment of patients ($n=9$) with refractory luminal Crohn's disease [216]. Disease remission (ie, CDAI less than 150) was not achieved in any patient. Three of the 9 patients showed a reduction of ≥ 70 points in CDAI 6 weeks post-treatment (baseline median CDAI was 326); however, the disease worsened significantly in 4 patients and required additional treatment. It was also shown that BM-MSC treatment was associated with an increase in $CD4^+$ regulatory T cells and with a decrease in inflammatory cytokine levels in mucosal biopsies. The results of these 2 studies suggest that the local administration of BM-MSCs may be superior to systemic administration, although a phase II study using systemically administered allogeneic BM-MSCs (Prochymal) in 9 patients with refractory moderate-to-severe Crohn's disease also showed promising results [217]. All 9 patients had CDAI scores that significantly decreased by day 28, and 3 patients (33%) achieved a clinical response (reduction in CDAI ≥ 100 points) by day 14. No serious adverse events were observed. Based on these promising results, Osiris had started a phase III clinical trial (NCT00482092 at www.clinicaltrials.gov; see Table 3).

García-Olmo et al. conducted a phase I clinical trial in which they investigated the role of locally administered autologous ASCs (a single dose of $3–30\times10^6$ cells in combination with fibrin glue) for the treatment of fistulizing Crohn's

disease [218]. Four patients with 9 fistulas of different types (rectovaginal, enterocutaneous, and perianal) were enrolled in the study. Eight of the 9 inoculated fistulas were followed weekly for at least 8 weeks. External openings of 6 fistulas (75%) were closed (covered with epithelium) at week 8, while 2 remaining fistulas showed a partial closure with decreased output flow. No adverse effects were observed during the average follow-up of 22 months. Another randomized phase II clinical trial performed by the same group compared the efficacy of intralesionally administered autologous ASCs (20×10^6 cells) plus fibrin glue versus fibrin glue alone (control) for the treatment of complex perianal fistulas associated with Crohn's disease, and of cryptoglandular (non-Crohn's) origin [219]. Fistula healing was evaluated at 8 weeks and 1 year; if healing was not observed at week 8, then an additional dose of ASCs (40×10^6 cells) plus fibrin glue or fibrin glue alone was administered. In the Crohn's disease group (14 patients of 49), complex fistulas were closed in 5 of the 7 (71%) patients treated with ASCs plus fibrin glue (1 patient had recurrence at 1 year follow-up [219,220]), while only 1 of the 7 (14%) patients responded in the control group. Statistical significance was not reached in the Crohn's subgroup (due to small sample size). A phase III trial investigating the safety and efficacy of allogeneic ASCs for the treatment of complex perianal fistulas in patients with Crohn's disease is expected to start in the first half of 2012 [221].

BM-MSCs isolated from patients with Crohn's disease were shown to have a similar morphology, phenotype, growth potential, and, most importantly, immunomodulatory capacity compared with BM-MSCs isolated from healthy donors [216,222], suggesting that autologous therapy might be appropriate. Given the similar immunomodulatory profiles of BM-MSCs versus ASCs, it would appear that both cell types are promising for this application with ASC being superior due to their easier access, greater amounts and more pronounced immunosuppressive properties as already discussed in this review.

Systemic lupus erythematosus. Sun et al. [223] demonstrated that the infusion of allogeneic BM-MSCs reconstituted the bone marrow osteoblastic compartment and reversed multiorgan dysfunction more effectively than cyclophosphamide-mediated immunosuppression in mice. Based on these promising preclinical results, they treated 4 patients with cyclophosphamide and glucocorticoid therapy-refractory systemic lupus erythematosus (SLE) using allogeneic BM-MSCs and showed a stable 12–18 month disease remision without any side effects. They also found a significant increase in the peripheral blood $CD4^+FOXP3^+$ T-cell population 3 months post BM-MSC transplantation [223].

In a separate study, Liang et al. treated 15 patients with persistently active, treatment-refractory SLE using allogeneic BM-MSCs [224]. Decreased SLE Disease Activity Index (SLEDAI) score and reduced 24 h proteinuria were seen in all patients after the treatment with concurrent reduction in doses of prednisolone and immunosuppressants. At a 12-month follow-up the mean SLEDAI was significantly decreased (12.2 ± 3.3 vs. 3.2 ± 2.8 at baseline, $n=12$, $P<0.05$); moreover, it remained less than 8 points in 12 of 13 patients, and 4 patients showed complete remission in disease activity (SLEDAI score was 0), which lasted for another 24 months in 1 of these 4 patients. At a 12-month follow-up, mean 24 h proteinuria was also significantly decreased (858.0 ± 800.7 mg vs. 2505.0 ± 1323.9 mg at baseline, $n=12$, $P<0.01$); it significantly decreased in 7 patients and normalized in 5 patients. Anti-dsDNR antibodies were significantly decreased in 11 of 15 (73%) patients at 1 and 3 months post-transplantation. Treatment was also associated with a significant increase in peripheral blood $CD4^+FOXP3^+$ Treg cells.

Interestingly, Carrion et al. treated 2 SLE patients with autologous BM-MSCs and found that there was no positive effect on disease activity, despite the fact that circulating Treg cells were markedly increased in peripheral blood and BM-MSCs suppressed activation and proliferation of peripheral blood lymphocytes in vitro [225]. These results are consistent with preclinical studies showing impaired BM-MSC function in mice with SLE [223]. Collectively, these initial results suggest that allogeneic BM-MSCs may be superior to autologous ones in the treatment of SLE patients.

To our knowledge, the role of ASCs in the treatment of SLE patients has not been investigated, although a recent preclinical study showed that long-term serial administration (28 times) of human ASCs effectively ameliorated SLE (especially early-stage) and restored immune homeostasis in mice [226].

Multiple sclerosis. Mohyeddin-Bonab et al. reported the use of intrathecal injections of autologous BM-MSCs for the treatment of 10 patients with refractory progressive multiple sclerosis (MS) [227]. Their Expanded Disability Status Scale (EDSS) score ranged from 3.5 to 6 over a mean 19-month follow-up. One patient improved (EDSS score decreased from 5 to 2.5), 4 patients showed no change in EDSS, and 5 patients showed progressive disease (their EDSS score increased in the range from 0.5 to 2.5). In the monthly neurological functional system assessment, 6 patients showed some degree of improvement in their sensory, pyramidal, and cerebellar functions; 1 patient showed no difference in clinical assessment; and 3 deteriorated. The results of MRI assessment after 12 months were also mixed: 7 patients with no difference, 2 showed an extra plaque, and 1 patient showed a decrease in the number of plaques.

Riordan et al. presented 3 case reports of a physician-initiated compassionate-use MS treatment using 2 intravenous infusions of autologous SVF cells and multiple intravenous and intrathecal infusions of allogeneic $CD34^+$ cells and BM-MSCs [116]. BM-MSCs were third-party unmatched, and $CD34^+$ cells were matched by mixed lymphocyte reactions. Infusions were performed within a 9–10 day period and were very well tolerated without any significant side effects. There was a marked improvement in patient-reported clinical results.

Given the autoimmune involvement of MS, it is anticipated that the immunomodulatory properties of both ASCs and BM-MSCs may be effective, although trials controlling for the route of administration, source of cell, cell preparation, and dose are needed to properly answer efficacy questions.

Type I diabetes mellitus. Twenty-five patients with type I diabetes receiving intra-pancreatic injections of autologous BM-MSCs and hyperbaric oxygen treatment showed improved metabolic control and reduced insulin requirements 12 months later [228].

A similar pilot study using ASCs has demonstrated the feasibility and safety of this approach. Vanikar et al. intraportally injected allogeneic ASCs along with cultured

bone marrow (isolated from a related donor) in 11 insulin-dependent diabetes mellitus patients [120]. The mean follow-up was 7.3 months. Clinical parameters significantly improved as shown by (1) decreased mean exogenous insulin requirement (1.14 U/kg/day vs. 0.63 U/kg/day at baseline, $P = 0.009$); (2) decreased mean levels of glycosylated hemoglobin (8.47% vs. 7.39%, $P = 0.03$); (3) raised serum c-peptide levels (0.1 ng/mL vs. 0.37 ng/mL, $P = 0.05$); and (4) disappeared diabetic ketoacidosis events with a mean 2.5 kg weight gain on a normal vegetarian diet and physical activities. No side effects were observed. Questions on whether ASCs or BM-MSCs of an autologous or allogeneic nature are better suited for type I diabetes, a fundamentally metabolic disorder, remain to be answered in larger, controlled clinical trials.

Ischemic central nervous system injury

Several preclinical animal studies have clearly demonstrated the beneficial effects of MSCs after an ischemic stroke [229,230], although clinical experience is still relatively nascent. Lee et al. conducted a long-term (up to 5 years) observer-blinded follow-up study that evaluated the safety and efficacy of intravenous BM-MSC infusion in patients with severe middle cerebral artery ischemic stroke [231]. They found that the mortality rate in the MSC group ($n = 16$) was significantly lower than in the control group ($n = 36$). There was a tendency for more patients with an improved outcome in the MSC group [evaluated by modified Ranking Scale (mRS)]. Moreover, the proportion of patients with an improved mRS value significantly increased in the MSC group, but not in the control group. There was no difference in comorbidities during the follow-up period, as well as no significant adverse effects were observed after BM-MSC treatment. Clinical improvement in the MSC group significantly correlated with (1) the serum levels of stromal cell-derived factor-1α at the time of MSC treatment, and (2) the degree of involvement of the subventricular region of the lateral ventricle. The authors concluded that BM-MSCs therapy may improve outcomes after ischemic stroke depending on the specific characteristics of an individual patient.

To our knowledge, no clinical trials investigating the potential of ASCs/SVF in the treatment of ischemic CNS injury have been reported to date, although a recent preclinical study demonstrated that ASCs are superior to BM-MSCs in the treatment of ischemic stroke in a mouse model [232], perhaps due to the superior angiogenic potential of ASCs [115].

The current status of MSC application progress and possibilities for the treatment of ischemic stroke are concisely reviewed in [233].

Liver diseases

Kharaziha et al. performed a phase I/II clinical study, in which 8 patients with end-stage liver disease (cirrhosis of different ethiologies) were treated with autologous BM-MSCs [234]. The cells were induced to differentiate into hepatocyte progenitors (as defined by the expression of albumin and α-fetoprotein) and were then injected into either the portal vein or peripheral vein. Liver function and clinical parameters evaluated at baseline and 1, 2, 4, 8, and 24 weeks after an injection of predifferentiated BM-MSCs showed significant improvement (as verified by the Model for End-Stage Liver Disease Score and appropriate serum parameters for liver function). No adverse effects were noted. All patients had an improved general condition and quality of life, which was noted 2 months after the injection. Other clinical studies investigating the use of BM-MSCs or BM mononuclear cells for the treatment of liver diseases are also briefly discussed in [234]. Significantly, there are some concerns that in liver pathology, MSCs may show undesirable effects or even worsen the disease because of their profibrogenetic potential [235]. Two clinical trials (NCT00913289, NCT01062750 at www.clinicaltrials.gov) investigating the role of SVF in the treatment of liver cirrhosis were suspended (reasons were not specified).

Future Perspectives of Actual Practical Clinical Aspects

Many issues remain to be elucidated in understanding MSC biology, mechanisms of action, and clinical application; however, here, we would like to focus on 3 practical clinical aspects.

First, in the majority of cases, MSCs are traditionally expanded using 10% FCS. Although FCS batches are routinely prescreened for biosafety, theoretically FCS may be responsible for the transmission of prions and still unidentified zoonoses; additionally, there is always the risk of an immune reaction in the host to the xenogeneic materials, especially if repeated infusions are needed [134]. Thus, an FCS-free medium should be introduced into routine clinical practice as soon as possible. This is feasible, as several clinical studies have already successfully used human autologous serum and/or platelet lysate [119,120,191,203,234]. Moreover, it was shown that both BM-MSCs [236] and ASCs [237] expanded in either human autologous serum (or platelet lysate) or FCS had a comparable morphology, immunophenotype, and proliferative and differentiation capacity, although functionality needs to be more fully assessed. Allogeneic human serum may be used as an alternative, and may prove to be superior to autologous serum in some cases [238]. Further investigation is needed, as it has been shown that the use of human serum versus FCS is associated with a differential gene expression profile in MSCs [238,239], and it will be necessary to correlate the possible influences of these differences on potential clinical implications, if any. Another alternative would be the use of serum-free and xeno-free culture medium for the cultivation of MSCs, and this approach appears to be feasible, as already demonstrated [240,241], although the addition of growth factors may promote proliferation to these cells, and raise tumorogenic concerns.

Second, the timing (as well as dose and route) of MSCs infusion should be clearly determined in various pathologies. As discussed in this review, and also previously reviewed in [85], the timing of MSC infusion seems to be one of the crucial factors determining the therapeutic efficacy of MSCs in GvHD. Similarly, in an acute MI setting, the administration of MSCs may be effective before the development of fibrosis. However, the administration of MSCs very early after the acute MI may be ineffective, because there is significant inflammation and cellular necrosis at this time, and MSCs may compete for nutrients in an ischemic environment and, thus, negatively impact cardiomyocyte survival in the infarct zone [197].

Third, various studies report contradictory results regarding the MSC effect on tumor formation—some studies show evidence of tumor promotion by MSCs, whereas others demonstrate apparent tumor-suppressive activity of MSCs (reviewed in [242]). Due to their ambiguous role and still unresolved impact on tumor growth, MSCs should not be used for patients with cancer (although at least 2 clinical trials already investigate their use in cancer patients, albeit not directly for cancer treatment, see Table 3). On the other hand, properly modified or conditioned MSCs may serve as an invaluable tool in anticancer therapy; owing to their tropism for tumors, MSCs may be used as cellular delivery vehicles for the targeted delivery of various antitumor agents (reviewed in [243] and [244]). Although to date no tumor formation was reported in human recipients of MSCs, more clinical experience, sensitive karyotype analysis, and long-term follow-ups are needed to be sure that MSCs from various sources do not form de novo tumors through transformation when administered to patients.

Concluding Remarks

MSCs are emerging as a novel powerful tool for the treatment of various diseases, some of which have limited treatment options. Although MSCs are found in virtually all organs, BM-MSCs and ASCs/SVF are probably the best characterized and most commonly used in clinical practice. Although there are some differences between BM-MSCs and ASCs, in their gene expression profile, their angiogenic potential, and secretion of factors, preclinical and clinical data support the use of both MSC populations in various clinical applications; safety in pilot trials has largely been established, and efficacy is being evaluated in a few Phase III trials (eg, for Crohn's disease). In general, ASCs may appear to be a better choice for clinical application compared with BM-MSCs, as they can be obtained in substantially greater amounts (up to 500-fold). In addition, adipose tissue is more abundant, more easily accessible, and harvesting is associated with lower morbidity as compared with the bone marrow. However in certain pathologies, there may be the advantage of using one cell population over another, and this finding will emerge along with further clinical investigations.

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Address correspondence to:
Dr. Marius Strioga
Department of Immunology
Center of Oncosurgery
Institute of Oncology
Vilnius University
P. Baublio. St. 3b-321
LT-08406, Vilnius
Lithuania

E-mail: marius.strioga@vuoi.lt; strioga@gmail.com

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