# Clinical Applications and Biosafety of Human Adult Mesenchymal Stem Cells

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**Abstract:** Mesenchymal Stem Cells (MSCs) are a population of adherent cells that can differentiate into mesenchymal lineage populations (cartilage, bone and fat tissue). In addition, they seem to be able to differentiate also into a broader type of lineages other than the original mesodermal germ layer.

Bone marrow MSCs are a standard in the field of adult stem cell biology and clinical applications; however adipose-derived MSCs are becoming an attractive alternative due to their minimally invasive accessibility and availability in the body.

MSCs modulate several effector immune functions by interacting both with innate and adoptive immune responses. Several local signals from the tissue microenvironment, together with cytokine and soluble factors released by MSCs influence anti-inflammatory and tissue repair properties of infused MSCs. Therefore, cellular therapies utilizing *ex vivo* expanded MSCs may be an interesting approach for inflammatory and autoimmune diseases.

Biosafety is still one of the most important aspects; therefore the production of clinical-grade MSCs requires the careful identification and control of all the phases of cell manipulation and release.

Many clinical applications of adult MSCs are in progress and are using bone marrow or adipose tissue-derived MSCs for the treatment of Graft Versus Host Disease (GVHD), inflammatory joint diseases and osteocartilagineous defects, digestive tract, cardiovascular and neurological diseases.

**Keywords:** Bone-marrow mesenchymal stem cell, adipose derived stem cell, biological characteristics, clinical applications, biosafety, cell therapy, human, adult.

## INTRODUCTION

More than forty years ago, Friedenstein [1] described a population of mononuclear cells from the bone marrow with clonogenic properties, with the ability to adhere to plastic substrates when cultured *ex vivo*, to develop colony-forming unit fibroblasts (CFU-F) and differentiate into chondrocytes, osteoblasts and adipocytes.

In the following years, different groups described subsets of bone marrow stromal cells with characteristics resembling the population originally described by Friedenstein and many terms have been used to define these cells, such as mesenchymal stem cells/marrow stromal cells (MSCs) [2, 3], bone marrow stromal stem cells (BMSSC) [4], marrow-isolated adult multipotent inducible cells (MIAMI) [5] and mesenchymal adult stem cells (MACs) [6].

The current definition of these cells is either mesenchymal stem cells (MSCs) [2] or stromal cells [7, 8], respectively, due to their ability to differentiate into mesenchymal lineage populations and their belonging to the stroma that is believed to have a physical supporting role to the hematopoietic stem cells niche.

Despite years of intense investigation, the location and role of native MSCs within their tissue of origin *in vivo* are not known, mainly because of the lack of specific markers allowing their distinct identification [9, 10]. MSCs are known to undergo phenotype modulation during *ex vivo* cultures, acquiring expression of new markers while also losing some old ones [11].

The definition of MSCs is based on the characterization of cell populations expanded *in vitro* and no uniformly accepted specific surface markers of MSCs have been identified; instead a mix of *in vitro* aspects, including a combination of phenotype characteristics and functional properties have been utilized.

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular therapy (ISCT) selected three minimal criteria for the identification of MSCs [12, 13]:

a. Adherence to plastic when cultured as isolated cells;
b. Positivity (≥ 95%) for CD73, CD90 and CD105 surface molecules and negativity (≥ 95%) for CD14, CD34, CD45 and human leukocyte antigen-DR (HLA-DR) surface molecules;
c. Ability to differentiate within cartilage, bone and fat.

These criteria allow only a retrospective definition of a cell population containing MSCs but do not allow direct purification of native mesenchymal progenitors from tissues [reviewed by 14-19].

## MESENCHYMAL STEM CELLS FROM BONE MARROW: PHENOTYPE AND DIFFERENTIATION

Bone marrow stromal cells are a standard in the field of adult stem cell biology and clinical applications. Most studies on phenotype and functional activity of bone marrow Mesenchymal Stem Cells (BM- MSCs) have been performed on *in vitro* cultured cells.

In *in vitro* conditions, these cells are adherent to plastic, present a defined set of surface molecules and can be specifically induced to differentiate, within their own germ layer, into cartilage, bone and fat tissue [13, 20].

### 1. Surface Marker Expression

Cultured BM-MSC phenotype is defined by the co-expression or lack of different non-specific surface antigens. Besides those required by ISCT criteria, also CD44 (hyaluronic acid receptor), CD146 (melanoma-cell adhesion molecule, Mel-CAM) and CD200 (OX2) are strongly expressed on BM-MSCs. MSCs are also negative for CD11b (integrin αM) and B cell markers (CD19 or CD79) (Table **1**) [16, 17, 19-26].

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**Table1. Cell-surface Markers Expressed by Human Bone Marrow Mesenchymal Stem Cells (BM-MSCs) and Adipose-derived Stem Cells (ASCs)**

| Markers | Antigen | BM-MSCs | | ASCs | |
|---|---|---|---|---|---|
| | | Native | Cultured | FBS | HS |
| CD9 | Tetraspanin family | n.d. | | | |
| CD10 | CALLA (common acute lymphocytic leukemia antigen) | n.d. | | | |
| CD11b | ITGAM (integrin αM) | | | | |
| CD13 | Aminopeptidase | n.d. | | | |
| CD14 | LPS receptor | n.d. | | | |
| CD19 | B lymphocyte antigen | n.d. | | | |
| CD29 | β1Integrin | n.d. | | | |
| CD31 | PECAM-1 (Platelet endothelial cell adhesion molecule-1) | n.d. | | | |
| CD34 | Sialoprotein | | | | |
| CD44 | Hyaluronic acid receptor | n.d. | | | |
| CD45 | Pan-leukocyte antigen | | | | |
| CD49a | Integrin α1 chain | | | | n.d. |
| CD49b | Integrin α2 chain | n.d. | | n.d. | n.d. |
| CD49c | Integrin α3 chain | n.d. | | n.d. | n.d. |
| CD49d | Integrin α4 chain | n.d. | | | |
| CD49e | Integrin α5 chain | n.d. | | | |
| CD51 | Integrin aV chain | n.d. | | | |
| CD73 | Ecto-5-endonuclease, SH3, SH4 | | | | |
| CD79 | B lymphocyte antigen | n.d. | | | n.d. |
| CD90 | Thy-1 | | | | |
| CD105 | Endoglin | | | | |
| CD106 | VCAM-1 (vascular cell adhesion molecule-1) | | | | |
| CD117 | c-kit | n.d. | | | |
| CD133 | AG133 (prominin) | | | | |
| CD140b | PDGF-Rβ (platelet-derived growth factor receptor-β) | | | n.d. | n.d. |
| CD146 | Mel-CAM (melanoma-cell adhesion molecule) | | | | |
| CD166 | ALCAM (activated lymphocyte cell adhesion molecule) | | | | |
| CD200 | OX-2 | | | n.d. | n.d. |
| CD271 | NGFR (neural growth factor receptor) | | | | n.d. |
| MHC I | Major Histocompatibility Complex Class I | n.d. | | | |
| MHC II | Major Histocompatibility Complex Class II | n.d. | | | |
| Stro-1 | Unknown antigen | | | | |
| Stro-4 | Unknown antigen | | | n.d. | n.d. |
| GD2 | Ganglioside | | | n.d. | n.d. |
| SSEA4 | Stage-specific embryonic antigen | | | n.d. | n.d. |

The table shows the different cell preparations in columns and the surface antigens in rows. The white color codes for negative expression, medium grey codes for low expression and black codes for positive expression.

n.d.: not determined; FBS: fetal bovine serum; HS: human serum.

Based on MSC data from Bernardo *et al.* [17], Pittenger *et al.* [21], Delorme *et al.* [22], da Silva *et al.* [23], Deschaseaux *et al.*[25] and Pontikoglou *et al.* [26].

Based on ASC data from Lindroos *et al.* [72, 116], Mitchell *et al.* [82], Gimble *et al.* [86], Rada *et al.* [88], Daher *et al.* [93], Schaffler *et al.* [94], Katz *et al.* [95], Gronthos *et al.* [97], Kern *et al.* [100], McIntosh *et al* [105], Zannettino *et al.* [113].

Furthermore, intermediate levels of Major Histocompatibility Complex Class I (MHC-I) antigens were present, whereas the expression of Major Histocompatibility Complex Class II (MHC-II) could be evidenced only on Interferon-γ (IFN-γ) activated cells [27, 28].

Conversely, even if some antigens are shared with cultured cells, the phenotype of native MSC precursors is less characterized because of the low frequency of these cells in the bone marrow samples and/or the absence of specific markers allowing their identification (Table **1**) [2].

Nevertheless, different markers have been used in an attempt to purify native MSCs from bone marrow [reviewed by 17, 26, 29]: Stro-1 identified bone marrow stromal cells distinct from hematopoietic stem cells (HSCs) [30]; Stro-4 seemed specific for mesenchymal precursor cells, being able to enrich colony-forming fibroblasts when utilised for MSC isolation from bone marrow [31] and CD140b (platelet derived growth factor receptor β, PDGF-Rβ) has been proposed as a marker for the isolation of clonogenic MSCs [32, 33].

Bone marrow MSCs purified on the basis of CD271 (low affinity receptor of neural growth factor, LNGFR) displayed the ability to differentiate into multiple mesenchymal lineages [34] and are considered to be a subset with immunosuppressive properties, promoting lymphoemopoietic engraftment *in vivo* [35]. The finding of this glycoprotein, (mainly present on neural cells), on BM-MSCs is consistent with a partial origin of MSCs from neuroectodermal germ layer or, at least, with an initial influence of neuroepithelial cells on MSC differentiation [36, 37].

In addition, also GD2 (ganglioside) [38], SSEA4 (stage specific embrionic antigen) [39] and CD49a (integrin α1 chain) [40], CD105 (endoglin) [24], CD146 (Mel-CAM) [26] and CD200 (OX2) [22] have been proposed for selecting native MSCs from bone marrow.

Despite the identification of these other MSC markers, none has been demonstrated to be individually able to identify the true mesenchymal progenitors.

Proteomic approaches and microarray analyses that allow the comparison of expression profiles among MSCs obtained from different tissues, cultured in different conditions and for different periods [41] might better characterize peculiar differences. For example, NOTCH3, JAG2 and ITGA11 transcripts have been observed on MSCs expanded from bone marrow [42, 43].

Although native MSC identity is not clearly defined, various studies have shown a similar phenotype and differentiation pattern, *in vitro*, between MSCs and pericytes and also an origin of MSCs from these cells [29, 44, 45].

The presence of ubiquitous reserves of multilineage progenitor cells in the vascular niche of capillaries and blood vessels may account for the possibility of obtaining MSCs from different tissues and organs [46]. Besides the bone marrow, cells with multilineage differentiation potential have been isolated from adult (dental pulp [47], skeletal muscle [48], synovial membrane [49], circulatory system [50], adipose tissue [51]) as well as fetal tissues (amniotic fluid [52], umbilical cord [53], fetal blood, liver and lung [54, 55]).

### 2. Differentiation Potential

In the last twenty years, the differentiation potential of MSCs has attracted the attention of researchers. Since the 1990s Pitttenger and co workers [21] showed that MSCs could differentiate into mesodermal lineages such as cartilage, bone and fat. In addition, it has been reported that MSCs are able to differentiate into a broader type of other lineages besides the original mesodermal germ layer [56, 57], such as vascular smooth muscle [58, 59], myogenic cells [60], hepatocytes [61], endothelial cells [62], neural cells [63] and cardiomyocytes [64]. Although most of these observations were obtained from *in vitro* experiments, they provide evidence to recognize the differentiation of MSCs *in vivo*. However, the definitive proof of the differentiation of MSCs into tissue other than that of mesodermal origin is still lacking.

Different local signals from the tissue microenvironment regulated the differentiation of engrafted MSCs, *in vivo*, into tissue-specific cells [19], which are required by damaged tissues [65, 66, 67], into cells composing the niche for tissue repair [68] and cells with regulatory functions, contributing to tissue repair and regeneration by means of trophic or immunomodulatory cytokine production [69].

Although mesenchymal stem cells obtained from bone marrow continue to be utilized for cell therapies, the painful harvesting, together with the need for *in vitro* expansion of the low numbers of stem cells present in this tissue, induced the search for alternative tissue options.

Adipose tissue attracted researcher attention due to minimally invasive accessibility and amount of depots in the body.

## ADIPOSE-DERIVED STEM CELLS: PHENOTYPE AND DIFFERENTIATION

Zuk and co-workers [51] were the first to describe the presence of multilineage cells in human adipose tissue [reviewed by 70-73].

Adipose tissue originates from mesodermal germ layer and histologically it belongs to connective tissues. In humans it is one of the most shared tissues, being distributed as subcutaneous and visceral fat [74]. It is also present in the bone marrow as yellow component and in breast tissue [reviewed by 75, 76].

Two types of functionally different fat are described in mammals:

a) The white adipose tissue involved in body energy storage and mobilization (in the form of cholesterol, triglycerides and lipid soluble vitamins) [74, 76];

b) The brown adipose tissue responsible for basal and induced energy dissipation and thermogenesis, converting nutrient into heat [76, 77].

In addition to storing energy, adipose tissue provides mechanical protection, immune and endocrine function and tissue regenerative potential. Adipose tissue surrounds vital organs and is located beneath the skin, where it protects from infections and trauma. Bacterial and fungal infections of fat are uncommon and metastases are unusual, probably related to the innate and adaptive immune cells contained in the tissue, as well as to the potentially high local fatty acid concentrations that are lethal to pathogens and non-adipose cell types [75, 77, 78].

Fat tissue produces different factors (including interleukin 6, IL-6 and angiotensin II), secretes numerous adipokines (such as leptin and resistin) and cytokines (like insulin growth factor -1, IGF-1 and tumor necrosis factor-α, TNF-α) and activates hormones (such as glucocorticoids and sex steroids) [75-77, 79].

Different cytokines and hormones are produced also by the other cellular components of adipose tissues that, in proportion to the cell types present, affect the overall amount and type of soluble factors derived from the tissue [76].

The removal of fat tissues (by liposuction aspirates and for reconstructive surgery) supplies a considerable amount of material (about 100-3000 ml of fat from liposuction aspirates) containing about 300,000 mesechymal stem cells/1ml volume), that are routinely wasted, could be used for research in regenerative medicine applications [51, 80, 81, 82].

In the adipose tissue, adipocytes are supported and surrounded by the stromal vascular fraction (SVF), a heterogeneous set of cell populations. The SVF, isolated by enzymatic collagenase digestion of adipose tissue, contains the stromal cells, ASCs (adipose derived

stem cells) [83], that have the ability of multilineage differentiation into adipocytes, chondrocytes osteoblasts, myocytes, endothelial cells, hematopoietic cells, hepatocytes and neuronal cells [70, 84-91]. Furthermore, the stromal vascular fraction contains vascular endothelial cells and their progenitors, vascular smooth muscle cells and also cells with hematopoietic progenitor activity [92, 93]. The SVF also contains leukocytes that may be localized in the adipose parenchyma [93]. Despite the cellular heterogeneity of the crude SVF, the culture of human adipose-derived cells favours the expansion for a relatively homogeneous cell population expressing a stromal membrane phenotype [reviewed by 71, 72, 86, 88, 93, 94].

A standardized nomenclature was proposed in 2004 during the International Federation of Adipose Therapeutics and Science (IFATS) meeting because a variety of names were used to identify the plastic adherent cell population isolated from adipose tissue that had undergone collagenase digestion. Researchers referred to these cells as lipoblasts, pericytes, preadipocytes, processed lipoaspirate (PLA) cells, adipose-derived stem/stromal cells (ASCs), adipose-derived adult stem (ADAS) cells, adipose-derived adult stromal cells, adipose-derived stromal cells (ADSCs), multipotent adipose-derived stem cells (hMADS) and adipose mesenchymal stem cells (AdMSCs) [70, 85, 95].

According to IFATS's recommendations, the term adipose-derived stem/stromal cells (ASCs or ADSCs) should be used to identify the isolated, plastic adherent, multipotent cell population [93, 96]. ASCs fulfill the characteristics required for the application of stem cells in regenerative medicine: marked amounts of adipose-derived stem/stromal cells (ASCs) can be obtained from adipose tissue in a more easy-to-handle way than other types of MSCs, with less pain for the patient [94].

The main characteristics of ASCs as stem/stromal cells include the ability to develop fibroblast-like clones and plastic adhesion, their wide proliferative capacity and the expression of a panel of membrane antigens [98]. The starting adherent cells grow into spindle- or star-shaped cells after the second passage in culture and assume a fibroblast-like appearance.

They also have the ability to differentiate into many mesodermal lineages, such as cartilage, bone, muscle and epithelium, as well as neural progenitors [98]. Adipose-derived mesenchymal stem cells share a lot of similarities, such as morphology, distribution of surface antigens, multipotency with other stem cells obtained, for example, from bone marrow (Table **2**) or umbilical cord blood. [99-103].

Surface markers and differentiation potential specifically inducible towards mesodermal lineages (cartilage, bone and fat) [22, 84, 98] are used to characterise ASCs, likewise adopted for human MSCs isolated from bone marrow and other mesenchymal tissues.

### 1. Surface Marker Expression

Whereas specific antigens identify embryonic stem cells [104], similarly to BM-MSCs, ASCs lack unique markers. Therefore a panel of surface molecules [99, 105-108], mostly shared with MSCs from bone marrow, must be used to identify these cells. ASCs fulfill the minimal phenotype standard criteria (as defined by ISCT) [12, 13]: they express CD73, CD90 and CD105 and lack CD14, CD34 and CD45 antigens.

In addition, CD13, CD29, CD166 and MHC I are uniformly described to be strongly expressed; a moderate expression has been observed for CD9, and CD133 has been described to be poorly or not expressed on ASCs. Moreover, CD11b, CD19 or CD79, CD31 and MHC-II are also absent on ASCs [72, 82, 86, 88, 93-95, 97, 100, 105] (Table **1**).

However, some differences are described for CD106, cognate receptor of CD49d which are two molecules associated with hematopoietic stem-cell and progenitor-cell homing/mobilization within the bone marrow [82, 99]. ASCs appear positive for CD49d and almost negative for CD106, whereas on BM-MSCs these molecules are reciprocally expressed on MSCs [106].

In recent studies, CD105 has been shown to be a relatively specific marker for identifying mesenchymal stem cells: adipose-derived stem cells enriched in CD105 positive cells display a potent chondrogenic potential *in vitro*, strong collagen II staining and

**Table 2. Characteristics of Bone Marrow Mesenchymal Stem Cells (BM-MSCs) and Adipose-derived Stem Cells (ASCs)**

| Characteristics | BM-MSCs | ASCs |
|---|---|---|
| Embryological origin | Mesoderm, neural crest | Mesoderm, neural crest |
| Tissue | Bone marrow | White adipose tissue |
| Cell purification process | Without proteolytic digestion | With proteolytic digestion |
| Selection | Plastic adherence | Plastic adherence |
| Antigenic markers | CD49a positive<br>CD73 positive<br>CD90 positive<br>CD105 positive<br>CD271 positive<br>CD34 negative | CD49a positive<br>CD73 positive<br>CD90 positive<br>CD105 positive<br>CD271 positive<br>CD34 positive (early passages) |
| CFU-F | 0.005 | 0.05 |
| Expansion *in vitro* | 20-50 population doublings | 44-80 population doublings |
| Differentiation potential | Mesenchymal lineages (cartilage, bone, adipose tissue) | Mesenchymal lineages (cartilage, bone, adipose tissue) |

Based on data from Dominici *et al.* [13], Pittenger *et al.* [21], Delorme *et al.* [22], Lindroos *et al.* [72], Mitchell *et al.* [82], Zuk *et al.* [84], Gronthos *et al.* [96], De Ugarte *et al.* [98], Kern *et al.* [99], Romanov *et al.* [100], Wagner *et al.* [102], McIntosh *et al.* [105], Bourin *et al.* [208].

higher gene expression of collagen II and aggrecan [109], thus providing an important implication for cartilage regeneration and reconstruction.

Other proposed putative markers for homogeneous stem cell populations are: CD271 [110] and CD146 (moderately expressed on ASCs), which identifies a population of subendothelial cells exhibiting osteogenic, adipogenic and chondrogenic potential and capable of supporting a hemopoietic environment [111]. In addition, the protein Pref-1, first identified on murine 3T3-L1 preadipocytes, was proposed by some authors as a putative ASC marker [112], whereas others have reported the utility of pericytic markers such CD140b and 3G5 [45, 113].

However, the expression of some markers is controversial, because they are described as either positive or negative. For example, Stro-1 (a marker suggested for identifying mesenchymal stem cell populations [30] and proposed for selecting native MSCs from bone marrow [26]) is reported as being either present [85] or absent [97] in ASC cultures.

Similarly, CD34 and CD106 are reported as being present on ASCs [97], absent or expressed on a small population of cells [85, 95].

The expressions of some surface markers changes during culture progression.

Indeed, markers expressed by ASCs or by stromal vascular fraction cells *in vitro* might be occasionally determined/modified by the progressive passages in culture, thus favouring different profiles of antigen expression between *in vivo* unmanipulated and *in vitro* expanded cells [82, 105].

For instance, the expression level of CD29, CD44, CD73, CD90 and CD166 increased from the SVF to the very first passages and subsequently stabilized at a high expression level [82, 106 and Mariani, personal unpublished observations].

The opposite was observed for cell markers such as CD11, CD14, CD34 and CD45 that, expressed on SVF cells, decreased or were lost with consecutive passages, thus suggesting that the adherence to flasks and the subsequent expansions selected a relatively homogeneous cell population [82, 105, 108, 114 and Mariani, personal unpublished observations].

Most of the results were obtained on ASCs grown in medium supplemented with fetal bovine serum (FBS), but the results for ASCs cultured in medium in the presence of human serum derivatives (Table **1**) or in serum-free conditions show a maintained phenotype [72, 115-117] with slight variations particularly in CD14 and CD49 markers.

Some contradictions in the reported ASC results are not unique; in fact, similar differences have been observed for BM-MSCs [96] and may in part be explained by technical reasons (such as sensitivity and sources of antibodies, detection methods) and by the proliferative stage of the cells in culture or donor variability.

Proteomic characterizations, by microarray analysis and Real-Time Polymerase Chain Reaction (RT-PCR), showed that adipose or bone marrow-derived stem cells, share a similar transcriptional profile for stem-related genes [118, 119], thus strengthening the idea that adipose tissue might be a suitable source of MSCs as well as bone marrow. In addition, since the amount of MSCs in the bone marrow is quite low and decreases with age [120], adipose tissue may become a remarkable source of multipotential cells for replacement therapy.

Genomic studies have provided more detailed information, since the differentiation of stem cells is expected to induce significant changes in the gene expression of multipotent populations. ASCs expressed the mesenchymal cell specific markers and molecular markers typical of the embryonic stem cell phenotype: OCT4, Nanog, and Sox2 [121]. The expression of most of them was low in hematopoietic mesenchymal cells [122], therefore the evaluations of the expression of these genes was used to determine the degree of cell differentiation. Further studies on the role of the regulatory factors in the differentiation of ASCs cultured *in vitro* and *in vivo* are expected to explain the molecular mechanisms and highlight some of the transcription pathways involved in the lineage-specific differentiation of these stem cells.

## 2. Differentiation Potential

Stem cells isolated from white and brown adipose tissue differ in number and differentiation potential. In general, adult stem cells isolated from white adipose tissue are more numerous, grow more rapidly and have a higher differentiation potential, than cells isolated from brown tissue [123].

Differences in the ASC population are also described as concerning the isolation from the same type of adipose tissue but in different anatomical regions [124]. However, ASCs are an efficacious source of multipotent cells that have the ability to differentiate into several different cell types under appropriate culture conditions and in the presence of specific inducing factors [71-73].

In addition, ASCs produce various cytokines and growth factors that support angiogenesis, tissue remodeling and antiapoptotic events, (such as vascular endothelial growth factor, VEGF; hepatocyte growth factor, HGF; interleukin 6, IL-6; IL-7; tumor necrosis factor, TNFα; macrophage colony-stimulating factor, M-CSF and transforming growth factor, TGF-β1), potentially influencing cell differentiation and modulating the surrounding cells [125, 126].

Several *in vitro* studies have shown that ASCs differentiate into chondrocytes when cultured in a medium supplemented with insulin growth factor (IGF), bone morphogenetic proteins (BMPs) and transforming growth factor-β (TGF-β) [127, 128]. The chondrogenic differentiation of ASCs was confirmed by their ability to produce cartilage *in vitro* and *in vivo*, in a variety of experimental models [106, 129, 130]

Under osteogenic culture conditions in medium containing dexamethasone, β-glycerophosphate and vitamin D3, ASCs expressed genes and proteins associated with the osteoblastic phenotype. [131-133]. ASCs under osteogenic stimulation adhered to scaffolds, migrated, proliferated and differentiated in order to regenerate damaged bone tissue *in vivo* [106, 134-137].

Obviously, ASCs have an exceptional potential to differentiate into mature adipocytes *in vitro* [106, 131, 132, 138], under the influence of insulin, isobutylmethylxanthine, dexamethasone, rosiglitazone and indomethacin. This type of differentiation is very promising for developing improved techniques to repair soft tissue defects, particularly after oncological surgery [139].

In addition to their ability to differentiate into classical lineages, ASCs differentiate in the presence of dexamethasone and hydrocortisone and display a myogenic phenotype *in vitro* [140] and retain their differentiation potential towards the cardiomyogenic lineage [64, 106, 141]. Furthermore, ASCs can differentiate into endothelial cells and, by produced pro-angiogenic factors, contribute to vessel formation [142-145].

Studies using human adipose stromal-vascular cell fractions for developing an osteogenic and vasculogenic construct in a one step procedure are interesting [146, 147]. Human ASCs, under perfusion flow, in a three-dimensional background were able, when implanted in nude mice, to form bone tissue and blood vessels functionally connected to the mouse vascular network and containing mouse erythrocytes.

It was also suggested that ASCs might have the ability to differentiate into putative neurogenic cells [106, 148, 149], exhibiting a neuronal-like morphology and expressing several proteins consistent with the neuronal lineage and acquire a pancreatic endocrine phenotype (induction of the insulin, glucagon and somatostatin genes) *ex vivo* in response to defined culture conditions [150, 151].

A few studies have reported an epithelial differentiation and, by using these results, a tissue-engineered airway construct, as a prototype vocal fold replacement, was produced with a three-dimensional structure of fibrin and ASCs [140, 152].

The differentiation of ASCs into hepatocyte-like cells has also been investigated [61, 153]. Human ASCs were transplanted into the livers of immunodeficient mice with or without prior hepatocyte differentiation *in vitro*, and it was observed that the predifferentiation of ASCs *in vitro* promotes liver integration *in vivo* [154].

Human ASCs were also found to restore damaged intervertebral disc segments in rats, when transplanted into degenerated disc [155].

## INTERACTIONS BETWEEN MSCS AND IMMUNE RESPONSE

Mesenchymal stem cells modulate several effector immune functions by interacting both with innate and adaptive immune responses either by cell-to-cell contact or soluble factors [reviewed by 16, 26, 156-162].

### 1. MSCs and Innate Immunity

Natural Killer (NK) cells are important effector cells of the innate immunity, playing a key role in antiviral and anti tumor responses [163]. They display spontaneous lytic activity, against cells not expressing MHC class I molecules, that is strictly regulated by a balance of signals transmitted by activating and inhibitory receptors interacting with MHC molecules on target cells [164].

Proliferation of NK cells induced by IL-2 or IL-15 cytokines and the production of IFN-γ were highly susceptible to MSC-mediated inhibition [165] (Table **3**).

MSCs downregulated NKp30 and NKG2D-activating NK receptors, thus abrogating also spontaneous lytic activity [166-168]. Conversely, NK cells activated *in vitro* by cytokines were able to kill both allogenic and autologous MSCs [169].

The susceptibility was dependent on the low surface level of MHC-I molecules and on the expression of ligands for activating NK receptors [166]. Upregulation of MHC-I molecules on MSCs by incubation with IFN-γ partially protected MSC from lysis [170].

Neutrophils are also important mediators of innate immunity responsible for microorganism killing during bacterial infections. After binding to bacterial products neutrophils undergo respiratory burst; MSCs have been shown to impair this metabolic activity significantly and to inhibit apoptosis of both resting and activated neutrophils [171]. This anti-apoptotic effect of MSCs seemed to be mediated by IL-6 [171]. Delayed apoptosis was thought to preserve the pool of neutrophils that will be rapidly recruited in response to infections (Table **3**) [172].

In human macrophages, MSCs inhibited the production of TNFα and IL-12, but increased the production of IL-6 and IL-10 (Table **3**) [173].

Myeloid dendritic cells (DCs) are the most potent antigen-presenting cells, essential in the activation of the immune response and in the induction of tolerance. During maturation DCs progressively upregulated MHC class I and class II antigens and expressed co-stimulatory and other surface molecules (CD11c, CD80, CD83 and CD86) [174].

*In vitro*, MSCs inhibited the maturation of human blood monocytes and CD34+ hematopoietic progenitor cells into DCs [175]. MSCs incubated with mature DCs decreased the expression of MHC-II and other molecules involved in antigen presentation, thus suppressing the ability of DC to stimulate T cell proliferation [157]. In addition, MSCs decreased the production of IL-12 and TNFα, but, when incubated with plasmocytoid DCs (which are specialized for the production of type I IFN in response to microbial stimuli) upregulated IL10 production, thus increasing anti inflammatory response [169] (Table **3**).

### 2. MSCs and Adaptive Immunity

MSCs have been found to inhibit T lymphocyte proliferation and activation in response to alloantigens, polyclonal mitogens, CD3 and CD28 antibodies *in vitro* [176, 177].

Immunosupression concerned all CD3, CD4 and CD8 T subpopulations, and might be mediated by both allogeneic and autologous MSCs, thus indicating that it was not restricted by Major Histocompatibility Complex [178, 179] (Table **3**).

The reduced proliferation was dependent on an MSC-mediated arrest of cell division in the G0-G1 phase of the cell cycle, which was further associated with inhibition of cyclin D2 expression (the first cell cycle protein induced following stimulation) [180].

MSCs did not induce T-cell apoptosis, instead they promoted the survival of resting T cells, and the rescue of T cells from activation-induced cell death by down-regulating the CD95-CD95 ligand on their surface [181]. Moreover, MSCs decreased IFN-γ production by T helper (Th)-1 cells, whereas they increased interleukin (IL)-4 by Th-2 cells, thereby promoting a shift from a proinflammatory to an anti-inflammatory response [169]. MSCs decreased other functions of T-cells, such as the production of IL-2 and TNFα [169] (Table **3**).

MSCs have also been reported to down-modulate MHC restricted lysis of virus-infected or allogenic cells, which is mainly mediated by CD8 cytotoxic T lymphocytes (CTL) [182] and to increase, in mixed lymphocyte reactions, the proportion of CD4+CD25+ FoxP3+ regulatory T cells (T regs), which show potent immune suppressor activity [183, 184].

Finally, MSCs have been reported to promote, both *in vitro* and *in vivo*, the generation of CD8 T regs [185].

Indeed, MSCs can suppress antigen-specific T cell proliferation and cytotoxicity as well as induce anti-inflammatory or T regulatory cells. Soluble HLA-G, has been shown to be directly involved in the MSC-mediated induction of Tregs [186].

Studies analysing the interaction between MSCs and B lymphocytes have shown contrasting results (Table **3**).

B-lymphocyte proliferation activated by anti-immunoglobulin antibodies, cytokines or soluble CD40 ligand [37], as well as B cell maturation and immunoglobilin production, could be inhibited *in vitro* by MSCs, through the release of humoral factors [187, 188].

Nevertheless, activated B cells become susceptible to the suppressive activity of MSCs in the presence of exogenously added IFN-γ [158].

MSCs also modulated the expression of some chemokine receptors (CXCR4, CXCR5, CCR7) on B cells, thus influencing chemotactic responses of these cells to the paired ligand molecules (CXCL12 and CXCL13) [188].

However, other studies applying different experimental approaches obtained opposite results, thus showing that MSCs could foster survival, proliferation and differentiation of transitional and naive B lymphocytes to antibody-secreting cells and strongly enhanced proliferation and differentiation of memory B-cell populations into plasma cells [189, 190].

### 3. Mechanisms of Immunomodulation

Relatively little is known about the underlying mechanisms responsible for the immune-modulatory activities of MSCs.

The initial phases of the interaction between MSCs and immune cells involve direct cell-to-cell contact by surface adhesion molecules, whereas the following crosstalk is mainly mediated by the numerous soluble factors constitutively or subsequently produced

**Table 3. Effect of MSCs on the Cells of Innate and Adaptive Immunity**

| | | |
|---|---|---|
| **Natural Killer (NK) cells** | | |
| | ↓ proliferation induced by IL-2 | |
| | ↓ proliferation induced by IL-15 | |
| | ↓ IFN-γ production | |
| | ↓ spontaneous lytic activity | |
| **Neutrophils** | | |
| | ↓apoptosis of resting and activated cells | |
| | ↓ respiratory burst | |
| **Macrophages** | | |
| | ↓ IL-12 production | |
| | ↓ TNF-α production | |
| | ↑ IL-6 production | |
| | ↑ IL-10 production | |
| **Dendritic cells (DC)** | | |
| | ↓ MHC-II on mature cells | |
| | ↓ CD11c, CD83, co-stimulatory molecules | |
| | ↓ IL-12 production | |
| | ↓ TNF-α production | |
| | ↑ IL-10 production from plasmocytoid DC | |
| **T lymphocytes** | | |
| | Inhibition of proliferative response to | |
| | | polyclonal mitogens |
| | | allogenic cells |
| | | specific antigens |
| | Not MHC-restricted inhibition | |
| | ↑ CD4+ CD25+ FoxP3+ regulatory T lymphocytes (Treg) | |
| | ↑ CD8+ Treg | |
| | ↓ T-cell (CTL) mediated citotoxicity | |
| | ↓ CD95 and CD95 Ligand on T lymphocyte surface | |
| | ↓ IL-2 production | |
| | ↓ IFN-γ production by Th1 lymphocytes | |
| | ↑ IL-4 production by Th2 lymphocytes | |
| **B lymphocytes** | | |
| | Inhibition / activation of proliferative response to | |
| | | anti-immunoglobulin antibodies |
| | | soluble CD40 Ligand |
| | | cytokines |
| | Inhibition/enhancement of maturation | |
| | Inhibition/ enhancement of immunoglobulin production | |
| | Modulation of chemokine receptors | |

Based on data from Salem *et al.* [16], Pontikoglou *et al* [26], Meireilles *et al.* [156], Uccelli *et al.* [157], Ghannam *et al.* [158], Sensebè *et al.* [159], Shi *et al.* [160], Yagi *et al.* [161], Singer *et al.* [162].

by MSCs, after the interactions with target cells [156-158, 160-162].

Nitric oxide (NO) and indoleamine 2,3-dioxygenase (IDO) were released by MSCs after triggering by IFN-γ produced by target cells.

IDO induced the depletion of tryptophan (an essential amino acid for lymphocyte proliferation) from the local environment and was required to inhibit the proliferation of Th1 cells producing IFN-γ and, together with prostaglandin E2 (PGE 2), also NK activity [191].

Furthermore, IFN-γ, alone or in combination with other pro-inflammatory cytokines (TNF, IL-1α or IL-1β), stimulated the production of chemokines attracting T cells, together with the synthesis of inducible nitric-oxide synthase (iNOS) inhibiting T-cell activation, through the production of nitric oxide [192, 193].

MSCs produced constitutively other soluble factors, such as transforming growth factor-β1 (TGF-β1), hepatocyte growth factor (HGF), IL-10, prostaglandin E2 (PGE2), haemoxygenase, IL-6, soluble HLA-G [156, 162].

MSC-produced IL-6 was shown to downregulate the respiratory burst, to delay the apoptosis of human neutrophils and modulate the maturation of DCs [171, 194].

Soluble HLA-G has been shown to suppress T-cell proliferation, as well as NK-cell and T-cell lytic activity and to promote the development of regulatory T cells [186, 195].

The contact between MSCs and activated T cells induced IL-10 production, which, in turn, has an essential role in stimulating the release of soluble HLA-G by MSCs [186, 195].

However, the production of some of these molecules can be increased by cytokines released by target cells through their interaction with MSCs, for example, the constitutive production of PGE2 was up-regulated by TNF and IFN-γ [169].

Some findings highlighted the duality of the interactions with the immune response, thus supporting the transition from immunosuppressive to immunostimulatory activities for MSCs. For example, low numbers of MSCs can render dendritic cells prone to promoting T cell activation, whereas high numbers are required to cause the opposite effect [196].

MSCs have been reported to express major histocompatibility complex (MHC) molecules, thus including MHC class II (MHC-II), and the levels of these molecules were altered by proinflammatory cytokines [27, 197].

Previous studies have shown that low levels of IFN- γ induced MSCs to express MHC-II as antigen presenting cells (APC); conversely high levels of IFN- γ downregulated the expression of MHC-II [27, 198].

In this context, IFN-γ-stimulated-MSCs can uptake, process and present exogenous antigens, to CD4+ T cells, *via* their upregulated MHC-II molecules, [197, 199].

The roles of MSCs as both immunostimulatory and immunosuppressor cells and the balance between the two opposite actions should be considered for the clinical applications of MSCs.

## *EX VIVO* MANIPULATION OF MSCS FOR CELL-BASED THERAPIES: CONCERNS AND POTENTIAL RISKS

### 1. Regulatory Rules

Current regulations concerning stem cell therapies mirror the criteria adopted for biopharmaceutical industries.

American Food and Drug Administration and the European Medicine Agency (EMEA) and related local regulatory authorities divide procedures into "minimal" and "more than minimal" manipulation based on the degree of manipulation performed to isolate MSC population and the related risk of adverse events [200-204].

Good laboratory practices (GLP), which are adopted by the majority of clinical laboratories, are sufficient for minimal manipulations such as cell cryopreservation procedures, whereas for more than minimal manipulations the required Good Manufacturing Practices (GMP), assure a more stringent degree of control of the laboratory procedures.

In fact, the more-than-minimal manipulations include complex activities, such as cell expansion *in vitro*, activation, combination with scaffolds, use of the cells for other than the tissues normal function, unrelated allogenic transplantation [200, 205].

However, if these rules are well adaptable to the biopharmaceutical processes aimed at extensive production, they are less adaptable to clinical laboratories, which often prepare custom-processed cells for individual patients.

The European Regulation defines the use of mesenchymal stem cells (either derived from bone marrow or from adipose tissue) as advanced therapy medicinal products [201, 206].

MSCs are considered products for tissue-engineering or somatic-cell therapy, depending on the origin, preparation process and proposed clinical uses.

The production of clinical-grade MSCs in agreement with GMP procedures requires the careful identification and control of all the phases of production.

The methods of expansion must be monitored to assure and maintain the functionality of the cells (viability, morphology, phenotype, proliferative rate and differentiation capacities) as well as genomic stability (karyotype and telomerase expression) in the time course of the culture. In addition, bacteriological tests must be performed to assure the sterility of the cell product [reviewed by 159, 207-211].

### 2. Culture Conditions: Media, Serum and Serum Substitutes

Culture medium has an important influence on growing and differentiation capacities of MSCs and ASCs during passages in culture. MSCs and/or ASCs are seeded and expanded in classical media (such as MEM, D-MEM, RPMI-1640 and D-MEM/F-12) [82, 84, 97, 159, 208].

To supply vital nutrients, attachment and proliferative factors for expanding cells, culture medium is usually supplemented with fetal bovine serum (FBS) or human serum (HS), or plasma and growth factors [212].

Nevertheless, serum concentrations and species of origin influence the proliferation of ASCs [213]. For instance, FBS is a classical supplement for cell cultures, it supplies all the factors necessary for optimal cell proliferation *in vitro*, [213], but for the following clinical applications of cultured cells, the presence of FBS can be dangerous for the patient. Animal-derived bio-molecules, (foreign to the human species), present in the culture medium can be transferred with human cells into the recipient subjects and trigger severe immunological responses *in vivo*, such as anaphylactic reactions that might explain some of the failures in the therapeutic use of mesenchymal stem cell [214-216].

Other possible risks include the transfer of bacterial or viral infections, prions, and perhaps not yet identified zoonoses [211, 215]. The European regulatory agencies pay particular attention to the use of FBS as a possible source of diffusion of bovine spongiform encephalopaty (BSE) and for the use of FBS in GMP production, a certificate should be obtained to control the risk of transmission of infectious diseases [208].

In addition, the significant variability of animal serum among the different lots, might influence the reproducibility of the results; moreover, serum composition is largely uncharacterized, containing variable concentrations of cytokines and growth factors [108, 115, 213, 216].

Autologous human serum seems a more suitable option for clinical applications, since it avoids the introduction of allogeneic or xenogeneic molecules into the recipient. However, conflicting results on proliferation rate and differentiation potential have been reported [217].

Higher proliferative rates of BM-MSCs using autologous human serum and results comparable with FCS have been observed, [218-220]. The replacement of animal serum with human autologus or AB serum appeared to be effective as bovine serum if supplemented with fibroblast growth factor- 2 (FGF-2) [221].

Likewise, results are conflicting concerning differentiation using autologous serum compared to FBS, described as improved towards osteogenic and adipogenic lineages [222] or similar for osteogenic differentiation [220].

A further alternative to the use of animal serum is platelet-rich plasma and human platelet lysate. Platelets, respectively activated by thrombin or by a cycle of freezing/thawing, can release cytokines (such as platelet-derived growth factor -PDGF, endothelial growth factor - EGF and vascular endothelial growth factor - VEGF), which induce a great proliferation of mesenchymal stem cells [208, 223, 224].

After this treatment, MSCs retained their immune-modulatory potential and showed a more efficient suppression of allo-antigen-induced lymphocyte proliferation. No apparent genetic abnormalities and no tumorigenic potential when implanted into nude mice were detectable [225].

These substitutes were able to reduce the doubling time of both MSCs and ASCs *in vitro* and maintain the morphology and function of the cells [208].

Another alternative to bovine serum is patient serum collected in concomitance with the tissue sample [211]. However, it appears to be less favorable, since serum derived from aged individuals might interfere with MSC proliferation and/or differentiation capacity [225].

The careful definition of medium is important to maintain homogeneity between processes of cell production. Formulations containing nutrients to reduce serum concentrations (reduced serum media) [115] and completely serum-free media supplemented with proteins [218, 226], are now available for the expansion of BM-MSCs and ASCs.

The development of defined medium compositions completely serum free for the expansion of adult stem cells, able to support the proliferation and maintain the multi-potential capacity of the cells, is in its infancy, but results are encouraging [115, 116, 227].

However, these media lack molecules favoring MSC attachment, therefore plastic surfaces of culture devices must be previously coated with proteins favoring cell adhesion; they do not contain growth factors, which must be added to the medium, which has the disadvantage that they have not been produced with GMP and usually their formulation is not disclosed, which prevents its use in clinical trials [208].

In addition, the medium solution must also support the expansion of the cells multifold in a minimum number of passages, since long-term *in vitro* culture may alter the biology of ASCs [228, 229].

## 3. Donor Characteristics

An age-related decrease in the number or functional abilities of stem cells is described not only *in vivo*, but also *in vitro* in relationship to culture passages. These findings are relevant for regenerative medicine protocols, where cells from elderly donors need to be used for therapy [75, 120].

The age, anatomical region of collection and sex of the tissue donor can influence the quality and functionality of the adipose-derived stem cells, probably because of the different fat distribution between men and women and different blood supplies, cytokine signaling and gene expression profiles of adipose tissue among different depots [211, 230].

In mice it was observed that adipocyte progenitor cells accumulated with age, more in visceral fat than in subcutaneous fat, mostly in females [231].

Ageing may alter the availability of progenitor cells derived from adipose tissue also in humans and ASCs obtained from older donors appears to have a slower rate of proliferation [232- 234].

Not only age, but also the anatomical region influenced the proliferative potential: stromal cells obtained from subcutaneous adipose tissue displayed faster proliferation rates than those observed in the cells from the omental region [235].

In addition, an altered angiogenic potential [234] and a modified potential of differentiation towards osteogenic lineage have been reported [232, 233] even if changes in the number of precursors with osteogenic capacity were not shown. Conversely, adipogenic potential was not related to donor age [233].

It was also found that the differentiation potential varied among fat depots and differences have also been reported between gender [232]. Males presented a more efficient overall differentiation potential than females but dependent on depots, whereas females maintained a degree of osteogenic differentiation of ASCs that was not influenced by abdominal harvesting site, thus suggesting a hierarchy of osteogenic differentiation potential based on gender and anatomic harvest site [236].

Belonging to a defined anatomical depot also influenced susceptibility to apoptosis, with superficial abdomen depot being more resistant than the other compartments [232].

Similar and sometimes controversial data have been reported for BM-MSCs [reviewed by 237].

In general, age-related changes have been reported for the size of the MSC pool, but the results were dependent on mouse strain [238], collection methods, source and site of harvest of bone marrow samples in humans [120, 239-244].

An age related loss of the proliferative capacity of MSC together with telomere shortening, as well as the appearance of a senescent morphology were observed [242, 245]. The osteogenic, chondrogenic and adipogenic differentiation capacities of MSCs in donors of various ages did not change in culture in early passages, whereas in late passages tripotent clones lost their adipogenic potential [243, 246, 247].

## 4. Transforming Risk

The spontaneous transformation of human primary cells during *in vitro* expansion, even if is seldom described, remains a major safety problem [208, 210, 225].

Spontaneous transformation was mainly observed in murine models: murine MSCs, expanded *in vitro* for long periods, underwent chromosomal aberrations and assumed distinctive tumoral features [248] and when implanted or injected into immune-compromised mice, induced the formation of sarcomas [249, 250]. However, it was also observed that murine MSCs were prone to acquire chromosomal abnormalities even after few *in vitro* passages and when systemically injected, they embolized within lung capillaries and rapidly expanded and destroyed lung parenchyma by forming osteosarcoma-like nodules [250].

The development of these tumors was specific for mice MSCs; in fact similar experiments performed with human MSCs obtained from fetal blood, showed that, human cells did not develop chromosomal abnormalities after different passages *in vitro* and the cells were cleared from the lungs, even if the starting engraftment was similar [250].

Human MSC behavior appeared to be greatly different from that of mice. When cultured for long periods, MSCs obtained from

human bone marrow, neither underwent transformation [248, 249], nor presented chromosomal alterations [251].

Bernardo and co-workers [251] performed extensive genetic characterizations of human MSC from bone marrow after long term *in vitro* passages or in the senescence phase. No chromosomal abnormalities, telomerase activity or expression of human telomerase reverse transcriptase (hTERT) transcript were observed and *in vivo* transplantation of long-term cultured human MSCs to nude mice did not result in tumor formation [251, 252].

Similarly, Meza-Zepeda and co-workers [253 ] described the genetic stability of cells cultured to reach senescence and Choumerianou and co-workers [254] showed that MSCs obtained from pediatric bone marrow maintained a stable chromosome content and anchorage-dependent growth and did not express detectable levels of hTERT. In addition, these samples did not induce tumor formation when injected into SCID mice [254].

However, this issue is still a concern because of the first reports describing spontaneous transformation. In these reports modifications in human MSC subpopulations [255] and immortalized MSCs (transduced by human telomerase reverse transcriptase), were shown after very long-term *in vitro* culture [256, 257].

Rubio and co-workers [258, 259] described the spontaneous transformation of ASCs associated with a mesenchymal-epithelial transition and similarly Rosland and co-workers [260] showed that about 46% of bone marrow-derived MSCs underwent malignant transformation after a period ranging between 5 and 106 weeks of culture.

However, these last studies [258, 260] were subsequently retracted because the data referred to as transformation were actually determined by a cross contamination of culture with an epithelial cancer cell line [261, 262].

In addition, the same research group [263] was afterwards unable to obtain new human MSC transformation events in numerous new samples of adipose tissue both of pediatric and adult origin and in various independent sets of cultures.

It has been described that MSCs display a variable level of genome instability with aneuploidy aspects in some *ex vivo* cultures [208, 263], the aneuploidy was transient and donor dependent but it was not influenced by the culture protocol.

However, these cells underwent progressive growth arrest and normally evolved to senescence without acquiring selective growth advantages or transforming potential in culture, and when injected into immune-compromised mice did not develop tumours [264]. However, deletion/mutation analysis revealed that a few strains of MSCs reached senescence without expressing $p16^{INK4A}$ (a regulator of the cell cycle, known to contribute to cellular senescence and also stem cell aging) [210, 265].

A recent study investigating the toxicity and the tumorigenicity of human culture-expanded ASCs, found that they maintained morphology, phenotype and differentiation capacity of MSCs and were genetically stable until at least the twelfth passage in culture [266]. No toxicity or tumorigenicity was observed when these cells were injected in immune-compromised mice; and when they were used for the treatment of patients with spinal cord injury, no serious adverse events were recorded [266].

Our group also evaluated the potential susceptibility of *in vitro*-expanded ASCs to genetic alterations at different *in vitro* culture time points. In particular, we analysed the accumulation of DNA damage *in vitro* by microsatellite instability (MSI) analysis and the replicative potential by telomere length and telomerase activity. No cases of microsatellite instability were observed and allele patterns were maintained throughout the culture period for all the analyzed donors, thus indicating that repeated duplications *in vitro* did not alter genetic stability of short repeated sequences. ASCs showed a low degree of random fluctuation in the telomere dynamics, during *in vitro* culture and telomerase activity was undetectable in the majority of the samples [unpublished observations].

Overall, these data support the bio-safety of MSCs but, the paramount importance of this aspect, also prompted a thorough investigation of these cells, considering also the main molecules involved in senescence and transformation pathways (p16, p21, p53, hTERT and c-myc).

## 5. *In vivo* Interactions Between MSC and Tumors

MSCs can home to the stroma of developing tumors or to metastatic sites when injected either systemically or intra-peritoneally in animal models for different tumors [reviewed by 267-269]. Therefore, another important matter of debate is whether the administration of MSCs, is able to promote the growth of hidden tumors.

Despite various studies in recent years, contradictory results have been obtained showing that MSCs promote either tumor progression or inhibition (Table **4**).

In general, studies in favour of the tumor progression indicated that when MSCs were co-injected with cancer cells in immunocompromised mice, they increased angiogenesis, accelerated tumor growth and increased the number of metastases and tissue necrosis.

The contemporaneous, subcutaneous injection of MSC and colon cancer cells fostered tumor growth by increasing the rates of angiogenesis and tissue necrosis [270]. Both adult and fetal MSCs were injected, showing a similar growth-promoting effect but fetal MSCs fostered less tumor incidence than did adult MSCs (Table **4**).

In addition, MSCs were able to increase the mobility and metastatic growth of otherwise weakly metastatic human breast carcinoma cells [271] and the dimension of colon cancer tumors by decreasing apoptosis [272]. Tumor cells were shown to stimulate the secretion of CCL5 chemokine from mesenchymal stem cells, which acts in a paracrine fashion on the cancer cells to enhance their motility, invasion and metastasis. The enhanced metastatic ability was reversible and dependent on CCL5 signaling through the chemokine receptor CCR5 [271] (Table **4**).

Likewise, bone marrow MSCs, stem cells derived from adipose tissue are reported to exhibit tumor trophism.

In a model of breast cancer, ASCs localized in the tumor *in vivo*, (not only when co-injected locally, but also when injected intravenously), and were able to promote the development of broad and fast tumors [273] and embodied in the tumor vessels to differentiate into endothelial cells [274] (Table **4**).

ASCs transplanted subcutaneously or intracranially into BALB/c nude mice, together with melanoma cells, increased tumor dimension [275] and induced a greater number of living cells, thus influencing apoptosis even when injected into an adjoining site [269].

A low tumor latency and decreased apoptosis as well as modulation of melanoma cell responses to cytotoxic drugs *in vitro*, were also observed [276].

The CXCL12/CXCR4 axis was shown to be involved in the migratory interaction of tumor and mesenchymal cells, whereas it was suggested that FGF-2 and VEGF were modulating molecules favouring angiogenesis (Table **4**) [273, 276, 277].

These results however should be considered with caution because it has been shown that *in vivo* MSCs do not proliferate when implanted alone, but only when implanted with tumors

Other studies disagree with reported tumor-promoting properties (Table **4**). When mixed with tumor cells, MSCs inhibited primary tumor growth and metastases formation in mice transplanted with Lewis lung carcinoma or B16 melanoma. The tumor inhibition was apparently due to soluble factor(s) released by marrow stromal cells. In co-cultures with B16 melanoma cells, adherent bone mar-

**Table 4. MSC Influence on *In vivo* Tumors: Promotion and Inhibition of Tumor Growth**

| MSC Origin | Tumor | Delivery | Evidences | Suggested mechanisms | References |
|---|---|---|---|---|---|
| h fetal and adult BM | Colon cells (SW480, F6) | s.c.co-injected | ↑ incidence | ↑ angiogenesis ↑ proliferation | [270] |
| h BM | Breast cells (MCF/Ras, MDA-MB231, MDA-MB-435, HMLR) | s.c.co-injected | ↑ dimension ↑ metastasis | Secretion of CCL5 chemokine | [271] |
| h BM | Colon cells (KM12SM) | Orthotopic co-injected | ↑ dimension ↑ metastasis | ↑ angiogenesis ↓ apoptosis | [272] |
| h and m AT | Breast carcinoma cells (4T1, MDA-MB-231 | i.v.co-injected or after 24 hours | ↑ dimension | Secretiono of SDF1/CXCR4 | [273] |
| h AT | Lung or glioma cells (H460, U87MG) | s.c. co-injected | ↑ dimension | ↓ apoptosis | [275] |
| h AT | Prostate cells (PC3) | s.c. injected in contro-lateral side after 7 days | ↑ incidence ↑ dimension | ↑ angiogenesis modulation of tumoral CXCR4 | [277] |
| h AT | Melanoma (A475, M4Beu) and glioblastoma cells (8MGBA) | co-injected or i.v.synchronus | ↓ latency ↑ dimension | SDF1/CXCR4 | [276 |
| h AT | Prostate cells | s.c. co-injected | ↑ dimension | ↑ angiogenesis differentiation into endothe-lial cells | [274] |
| h BM | Lewis lung carcinoma and mela-noma (B16) | co-injected | ↓ dimension ↓ metastasis | Anti-proliferative effect | [278] |
| h BM | Kaposi sarcoma | i.v. injected | ↓ dimension | ↓ AKT signaling | [279] |
| h BM | Non Hodgking lymphoma (SKW6.4, BJAB) | i.p. after 4 days | ↓ dimension ↑ survival | Endothelial cell apoptosis observed *in vitro* | [280] |
| h BM | Human myelogenous leukaemia cells (K562) | grown isolation *in vivo* | ↓ proliferation | Secretion of DKK-1 | [281] |
| h AT | Pancreatic cancer cells | i.t. 10000/mm3 of es-tablished tumor | ↓ dimension | Cell cycle arrest in G1 phase | [282] |

Abbreviations: AT, adipose tissue; BM, bone marrow; CCL5, chemokine ligand 5; CXCR4, chemokine receptor type 4; DKK-1, Dickkopf-related protein 1; h, human; ; i.p,intra peritoneum i.t., intra tumor; i.v., intravenous; MSC, mesenchymal stem cells; m.,murine; s.c., sub cutaneous; SDF1, stromal derived factor 1; VEGF, vascular endothelial growth factor.

row cells exerted a significant anti-proliferative effect, which was increased by the previous culture of the bone marrow cells with granulocyte-macrophage colony-stimulating factor (Table **4**) [278].

In an *in vivo* model of Kaposi's sarcoma, intravenously injected MSCs homed to sites of tumorigenesis and potently inhibited tumor growth and this inhibitory effect correlated with their ability to inhibit target cell Akt, a protein-kinase playing a key role in various cell activities (such as cell proliferation and migration, apoptosis and transcription) (Table **4**) [279].

In two *in vivo* models of disseminated non-Hodgkin's lymphomas, the intra-peritoneal injection of MSCs significantly increased the overall survival, tumor masses developed more slowly and, at histopathological observation, exhibited a massive stromal infiltration coupled with extensive intra-tumor necrosis [280].

MSCs also inhibited the proliferation of human myelogenous leukemia cells. This effect was mediated by the secretion of Dickkopf-related protein-1 (DKK-1) (an inhibitor of β-catenine signaling) under the regulation of Nanog stem cell transcription factor (Table **4**) [281].

*In vivo*, a single intra-tumoral injection of ASCs in a model of pancreatic adenocarcinoma induced a strong and long-lasting inhibition of tumor growth. These data indicate that ASCs strongly inhibit pancreatic ductal adenocarcinoma proliferation, both *in vitro* and *in vivo* and induce tumor cell death by altering cell cycle progression (Table **4**) [282].

In general, studies have reported both growth promotion and growth suppression for the same cell type including adult and fetal-derived bone marrow, thus suggesting that the age of the donor does not determine the effect of MSCs on tumor progression.

The reason for this discrepancy in unknown but it may be attributable to the different tumor models, immunological characteristics and angiogenic properties of the tumor, eterogeneity of *ex vivo*

MSCs preparations, dose and timing of intra tumor administration, animal models, and the origin of cancer cells.

The possibility that MSCs promote tumor growth and metastasis raises concerns about the safety of their use as clinical tools. However, no evidence of tumor formation has been reported in over 1000 patients so far treated with MSCs for a variety of indications.

In conclusion, understanding the subtle interactions between MSCs and tumor cells is particularly critical given the therapeutic potential of MSCs.

## CLINICAL USE OF MSCS AND ASCS

Having migrated to inflammatory sites following a gradient of inflammatory cytokines, MSCs modulate the local inflammatory reaction by interacting with both adaptive and innate immune-responses [156-162].

In addition, inside the damaged tissues, MSCs may promote the survival of cells and the repair of tissues by recruiting and supporting local stem cells [19, 65-69, 283].

Therefore, cellular therapies utilizing *ex vivo* expanded MSCs may be an interesting approach for inflammatory and autoimmune diseases, as supported by approximately 3500 trials on adult stem cells, recorded on the "clinicaltrials.gov" website up to April 15, 2011.

The following data on clinical studies were obtained by entering the search terms "adult mesenchymal stem cells" and "adipose stem cells". Most of these studies are Phase 1 and Phase II safety trials still recruiting subjects and utilize both freshly isolated and *ex vivo*-expanded cells.

### 1. Graft Versus Host Disease

Acute Graft Versus Host Disease (GVHD) is a severe complication of allogenic stem cell transplantation, associated with high morbidity and mortality, particularly in subjects that do not respond to corticosteroids, which are the first choice of drug for the initial treatment of acute GVHD [284].

The treatment or prevention of acute GVHD during allogenic hematopoietic stem-cell (HSC) transplantation is the main application for MSCs and has aroused considerable interest [16, 19, 26, reviewed by 285-287].

In a landmark case report, Le Blanc and co-workers first described the successful use of MSCs for GVHD treatment [288]. This application, based on the immunomodulatory properties of MSCs, attracted wide interest and many studies have been published, that, despite supporting the positive effect of the infusion of MSCs on the course of GVHD, reported greatly variable rates of responses [289-293]. The response seemed to be more effective in children compared to adults [290, 293] and not influenced by the presence of fetal calf serum [290] or platelet lysates [291, 292] during *in vitro* expansion.

In 2008, the same group [290], studied 55 patients with acute GVHD of gut and liver refractory to steroid therapy. The multicenter phase II experimental study found improved clinical outcome after MSC administration in more than half of the patients and a superior survival rate without significant adverse effects. No correlation between response rate and donor histocompatibility leukocyte antigen (HLA) match was observed.

In a phase II study (Osiris Therapeutics) on 31 patients, the infusion of non-HLA-matched MSCs added to steroids was well tolerated and determined a 77% complete response after about one month [294].

MSCs may also ameliorate not only liver and gastrointestinal manifestations but also skin disease in refractory chronic GVHD [295, 296].

The reduction of acute GVHD was also described when culture-expanded MSCs and HSCs were co-transplanted [297-299], but the results should be interpreted with caution because of the small number of subjects and lack of control cohorts.

However, the results of the different studies aiming at characterizing the mechanisms of the clinical improvement are still contradictory, thus suggesting the need for a better standardization of timing, frequency and dose of transplanted mesenchymal cells. In fact, in various murine models of lethal GVHD, developed following haploidentical or mismatched hematopoietic graft, MSC or ASC administration either eliminated the severity of reaction [300-302] or (even if useful in prevention), failed to show any therapeutic effect [303, 304].

In 2007, a phase II randomized placebo-controlled trial in 78 patients receiving HSC transplantation and developing acute GVHD resistant to corticosteroid therapy was performed [208]. Only 11 patients were enrolled before trial suspension due to the observation of aneuploid karyotype of clones without transformation. One of these patients received MSCs with the altered karyotype, but no adverse events or potential late deleterious effects, including neoplasia, were observed [208].

Further studies are in progress (http://www.clinicaltrials.gov, inquire April 15, 2011): 15 clinical trials using BM-MSCs for the treatment of acute or chronic GVHD have been registered (for a total of 614 patients) (Table **5**); one Phase I, two Phase II using Prochymal® (expanded allogenic MSCs) and one Phase III are already completed and have enrolled more than 350 patients.

A few cases of severe and acute GVHD of gut and liver, which occurred in children and young adults, were successfully resolved by the administration of ASCs [305-307]. Results are still preliminary and more data are needed to validate the effect of ASCs in GVHD.

Currently only one multicenter, phase I-II, clinical trial for the evaluation of the infusion of allogenic ASCs for the treatment of GVHD is in progress (30 subjects) (CMM/EICH/2008) (http://www.clinicaltrials.gov, inquire April 15, 2011) (Table **5**).

### 2. Inflammatory Joint Diseases and Osteo-cartilagineous Defects

*In vivo* studies in animals showed that a single injection of MSCs prevented cartilage loss and bone destruction in a mouse model of collagen-induced arthritis [308], by down modulating T lymphocyte response and TNF-α inflammatory cytokines, while others described non-beneficial effects [309].

In Rheumatoid Arthritis (RA) the inflammatory response driven by T cells in the synovial membrane led to chronic joint destruction. In this disease the administration of MSCs indifferentiated or differentiated to chondrocytes suppressed the proliferation and the activation of T cells stimulated by collagen type II [19, 310].

MSCs also modified the secretion of cytokines favouring IL-10, restoring IL-4 secretion and inhibiting the production of IFN-γ and TNF-α pro-inflammatory factors [311]. Therefore, the anti-inflammatory and immune-suppressive properties of MSCs indicated a possible use for cartilage and bone repair therapy in RA [reviewed by 73, 312-318].

Similar effects on T-cell proliferation and cytokine synthesis and the production of inflammatory mediators by monocytes and fibroblast-like synoviocytes have been also shown for ASCs [319].

The systemic injection of autologous and allogenic BM-MSCs prevented RA, but did not have curative effects [315]. To date no clinical trials based on MSC or ASC transplantation in RA have been registered.

MSCs loaded in a 3-D scaffold, differentiated into chondrocytes have been used for tissue engineering and applied for cartilage repair in osteoarthritis (OA), a progressive disease of synovial joints

**Table 5. Clinical Trials Using MSCs and ASCs Recorded on the "Clinicaltrials.Gov" Website up to April 15, 2011**

| Clinical Use | Cells | Study Phases | | | | | |
|---|---|---|---|---|---|---|---|
| | | Not Known | I | I-II | II | II-III | III |
| Graft Versus Host Disease | BM-MSCs | ■■ | ■■ | ■■■■ | ■■■■■■ | | ■ |
| | ASCs | | | ■ | | | |
| Osteoarthritis | BM-MSCs | ■ | ■■■ | ■■ | | ■ | ■ |
| | ASCs | | | | | | |
| Crohn's Disease | BM-MSCs | | | ■ | ■ | | ■■■ |
| | ASCs | ■■ | ■ | ■■ | ■ | | |
| Liver Diseases | BM-MSCs | | | ■■■■ | ■■■■■■ | | |
| | ASCs | ■■ | | | | | |
| Diabetes | BM-MSCs | ■ | ■■ | ■■■ | ■ | ■ | |
| | ASCs | | | ■■ | | | |
| Heart Diseases | BM-MSCs | | ■ | ■■■■■■ | ■■■■■■■ | | |
| | ASCs | | ■■ | ■ | | | |
| Limb Ischemia | BM-MSCs | | | ■ | | | |
| | ASCs | | | ■■■ | | | |
| Neurological Diseases | BM-MSCs | ■ | ■■■■ | ■■■■■■■ | ■■■ | | |
| | ASCs | | ■ | ■ | | | |

ultimately resulting in the breakdown of osteocartilagineous tissue and patient functional disability [317-320].

MSCs were also used directly for repairing OA cartilage *in situ*, in phase I-II clinical trials [19], but the direct delivery of MSCs has carried out a few times [321]. MSC transplantation in the knee joint of four OA patients, showed encouraging results [322], but suggest that MSC-based procedures mainly rely on their trophic and immunomodulatory effects that significantly influence the local environment and the regenerative potential of tissue resident progenitor cells [reviewed by 312-318].

Eight clinical trials for testing the efficacy of BM-MSC implantation in the treatment of OA have been registered and are currently recruiting patients for an expected total of 268 (Table **5**). No trial appears to be registered for the use of ASCs (http://www.clinicaltrials.gov, inquire April 15, 2011).

At present, our group is involved in a research project funded by the European Community (Adipose-derived stromal cells for osteoarthritis treatment – ADIPOA, Grant n. 241719) that foresees an open multi dose phase I clinical trial for therapeutic applications of ASCs in human OA and a phase II controlled study in OA comparing hyaluronan alone versus hyaluronan combined with ASCs.

MSC transplantation was also proposed for repairing cartilage defects not related to osteoarthritic diseases, bone defects and non-union [323], on the basis that specific host environment may induce the differentiation of MSCs into chondrocytes or osteoblasts and the secretion of cartilagineous matrix [73].

The osteoblastic potential of MSCs may be utilized for the treatment of bone diseases such as osteogenesis imperfecta (OI) [324], a genetic disorder of mesenchymal stem cells characterized by defective type I collagen, osteopenia, bone fragility, severe bone deformities, and growth retardation. Six children with OI type III were treated with two infusions of MSCs: five of six patients showed engraftment in one or more sites, including bone, skin, and marrow stroma and had an acceleration of growth velocity during the first 6 months postinfusion [324]. At present two clinical trials involving the use of BM-MSCs for the treatment of OI have been completed on 23 subjects (http://www.clinicaltrials.gov, inquire April 15, 2011).

MSCs can be utilized also for repairing bone defects [325] or for improving healing of bone non-unions (frequent complication of long bone fractures) [326, 327]. In this situation, the injection of concentrated bone marrow was effective and healing was related to the number of MSCs in the product [327-329]. One trial using BM-MSCs is in progress on six patients for the treatment of long bone non unions (http://www.clinicaltrials.gov, inquire April 15, 2011).

Although MSCs from bone marrow efficiently contribute to long bone reconstruction, their use for jaw reconstruction [330] did not result in as many positive responses, because the implanted stem cells from bone marrow failed to differentiate into osteoblasts [331]. It was found that the embryonic origin of facial skeleton from neural crest negatively influenced differentiation of mesodermal origin stem cells, thus highlighting the importance of the origin of MSCs used for tissue repair.

Autologous SVF cells combined with milled autologous bone supported by fibrin glue were successfully utilized for treating a calvarial fracture and bone continuity was restored within 3 months.

In this case SVF was applied without manipulation but the capacity to produce angiogenic factors and promote neovascularization was also displayed by ASCs [87, 125, 332, 333].

Autologous *ex vivo*-expanded ASCs combined with calcium salts and bone mophogenetic protein has proven to be a very promising model for reconstruction of human maxillo-facial defects [334, 335]. Cell-based procedures have great therapeutic potential for other applications such as osteonecrosis, ligament, tendon and meniscus lesions and inter-vertebral disc repair [reviewed by 155, 317].

### 3. Digestive Tract Diseases

Accumulated animal and human data support the therapeutic potential of MSCs for diseases of the digestive tract. Crohn's disease, perianal fistulas and liver failure are the main digestive tract diseases for which cell-therapy is under evaluation [reviewed by 336].

The administration of autologous bone marrow-derived MSCs appears to be safe and feasible in the treatment of refractory Crohn's disease; no serious adverse events were detected during bone marrow harvesting and administration [337]. An increased therapeutic efficacy was observed when stem cells were first coated with antibodies that specifically target them to inflamed sites [338].

A phase II trial with Prochymal® (expanded allogenic MSCs) showed that all patients improved and 3 out of 9 reached clinical remission [336]. Other studies were suspended because of a similar response in the placebo group [339].

The use of ASCs in inflammatory bowel diseases (ulcerous colitis and Crohn's disease) is under study. In mice with induced acute and chronic colitis and sepsis, the intraperitoneal injection of human or murine ASCs reduced the severity of colitis (eliminating inflammation, diarrhea and weight loss) and increased survival. ASCs also protected from severe sepsis by reducing the infiltration of inflammatory cells in various target organs and by downregulating the production of various inflammatory mediators [340].

Promising results were obtained from clinical trials for the treatment of fistulizing Crohn's disease with ASCs.

In 2002 the first intralesional inoculation of ASCs was described for treating a recurrent and refractory recto-vaginal fistula. Subsequently, numerous other studies have been published along similar lines, which also compared the intra-lesional application of SVF cells with expanded ASCs [341-344].

Recently, the treatment was also performed with expanded BM-MSCs, which resulted in resolving the fistula and attenuating both the Crohn's and perianal diseases [345].

At present, 5 trials (for a total of 621 patients) are registered for the use of MSCs in the treatment of Crohn's disease, of which two are already completed: one phase II study on 10 patients and one phase III on 200 patients aiming at the extended evaluation of Prochymal® (Table **5**). Six more trials, one Phase I study completed (on 9 patients), three Phase I-II and II and two unknown in progress (for a total of 255 patients) are evaluating the use of ASCs for the treatment of fistulas associated to Crohon's Disease (Table **5**) (http://www.clinicaltrials.gov, inquire April 15, 2011).

The underlying mechanism of the therapeutic potential of MSCs on hepatic cirrhosis, and the long-term destiny of the injected MSCs are far from being clear [reviewed by 61, 336]. However, preclinical studies have shown the differentiation of MSCs into hepatocytes, *in vivo*, in different liver zones, depending on the site of injection [346] and the production of molecules with anti-fibrogenic activity that attenuate hepatic fibrosis [347, 348].

Significant clinical-pathological improvements have been achieved after the application of BM-MSCs and ASCs by systemic injection *via* the hepatic artery or the portal vein [336].

In four patients with decompensated liver cirrhosis, MSC transplantation improved liver functions and increased liver volumes after one year [349].

A randomized controlled trial in patients receiving autologous BM-MSCs or placebo, showed a partial improvement of liver function in the first three months [350] and an improvement in the laboratory scores of hepatic function was obtained in a phase I-II study that used autologous BM-MSCs differentiated to hepatocytes before infusion [351]

Ten studies are currently evaluating the efficacy of MSC administration on liver failure caused by hepatitis B virus and liver cirrhosis in 838 patients. Two phase I-II studies have been completed on 188 patients (Table **5**). Two trials utilizing the infusion of ASCs have been suspended (http://www.clinicaltrials.gov, inquire April 15, 2011).

Studies on diabetic murine models have shown that the intravenous administration of BM-MSCs might localize to the pancreas [336] and significantly reduce the level of glycemia [352]. Co-transplantation of MSCs and bone marrow favored the regeneration of insulin secreting β-cells and blocked the T-cell mediated disruption of the new cells in the pancreas of mice with type I diabetes [353].

Co-transplantation of islets with MSCs had a profound impact on the remodelling process, maintaining islet organisation and improving islet revascularisation. MSCs also improved the ability of islets to reverse hyperglycaemia [354].

Human bone marrow multipotent stromal cell subsets activated endogenous programs for islet regeneration after transplantation in NOD/SCID mice, by stimulating the formation of small β-cell clusters associated with the ductal epithelium [355]. Transplanted MSCs are thought to decrease glycemia through generating new β-cells. They can differentiate directly *in vivo* into new functional β-cells or induce the differentiation of endogenous pancreatic stem cells into new β-cells in response to trophic cytokines [356]. In addition, MSCs produce cytokines and growth factors which might enhance islet survival and function after transplantation [357].

A safe and effective treatment of insulinopenic diabetics using insulin-producing ASCs transfused with unfractionated cultured bone marrow was reported. All patients were successfully infused with bone marrow and ASC without any adverse effects and showed 30–50% decreased insulin requirements with an increase in serum c-peptide levels during a follow-up period of up to 23 months [358, 359].

At present eight studies utilizing MSCs are in progress: six on 224 patients with Type 1 Diabetes and two on 124 patients with type 2 Diabetes (Table **5**). Two more phase I-II studies are evaluating safety and efficacy of autologous ASC transplantation in 30 patients with Type 1 Diabetes and in 34 patients with Type 2 Diabetes (http://www.clinicaltrials.gov, inquire April 15, 2011).

### 4. Cardiovascular Diseases

MSCs have produced functional advantages when used as a therapeutic approach in ischemic heart diseases [reviewed by 360, 361]. They contributed to decreasing infarct dimension and myocardial scars, restoring myocardial perfusion and improving ventricular function [67] Furthermore, they were used with positive results also in models of dilated cardiomyopathy and arhythmia [362, 363].

Therapeutic effects of MSCs are based both on cell differentiation in cardiac tissue [65, 66] and secretion of soluble factors with trophic as well as anti inflammatory properties [364].

Several studies have suggested that MSC differentiation into functional cardiomyocytes occurs rarely under physiological conditions; however the observation of specific cardiac and myocyte markers in the MSC-differentiated cells have suggested that this

process occurs [365]. Subjects with acute myocardial infarction treated with intra-coronary injection of autologous BM-MSCs, showed an improvement of regional and global left-ventricular function and improved heart function was also observed when chronic ischemic cardiomiopathy was treated [366-368].

The combined treatment with autologous BM-MSCs and endothelial progenitor cells showed a better perfusion and left ventricular function and scintigraphic imaging revealed the cell repopulation/regeneration of infarct scar [363]. The efficacy was also found for allogenic MSCs (Prochymal®) [369].

Fourteen clinical trials are currently ongoing on the use of MSCs for cardiac cell therapy (818 patients) (Table **5**). One Phase I-II study on 40 patients has been completed.

Three more phase I and I-II studies evaluating ASC injections in 99 patients are active (Table **5**) (http://www.clinicaltrials.gov inquire April 15, 2011).

Peripheral atherosclerosis is the leading cause of limb ischemia and several groups have initiated cell-based therapies for the treatment of this disease [reviewed by 370].

Although the induction of angiogenesis, the increase of blood flow and capillary thickness was repeatedly observed following MSC transplants in animal models with limb ischemia, clinical data showed that local autologous bone marrow stem cell transplantation increased transcutaneous oxygen pressure and exercise tolerance [371], even if a modest decrease in ischemic symptoms was produced [370].

Only one trial involves the use of MSCs for the treatment of limb ischemia, but, at present, it is not recruiting patients (Table **5**), whereas three Phase I-II studies using ASCs are in progress and recruiting 132 patients (http://www.clinicaltrials.gov, inquire April 15, 2011) (Table **5**).

### 5. Neurological Diseases

The use of MSCs to treat neurodegenerative diseases, has aroused great interest [reviewed by 372, 373]. MSCs have been considered a promising therapeutic strategy for acute injury and progressive degenerative diseases of the central nervous system, such as spinal cord injury [374], stroke, Parkinson's Disease [375], amyotrophic lateral sclerosis [376], and multiple system atrophy (MSA) [377].

Regenerative approaches to neurological diseases using MSCs include cell therapies where cells are delivered *via* intracerebral or intrathecal injection. Following transplantation into the brain MSCs regulate inflammation, decrease apoptosis, promote endogenous neuronal growth and promote synaptic connections. Fifteen clinical trials using MSC injections into the central nervous system of 382 patients to treat traumatic spinal cord injury, stroke, amyotrophic lateral sclerosis, multiple sclerosis, MSA and Parkinson's Disease are currently ongoing (Table **5**). A Phase I-II study on 90 patients with spinal cord injury treated with BM-MSCs has already been completed, as well as a Phase I study on 8 patients treated with ASCs. One more Phase I-II study is currently recruiting 30 patients with multiple sclerosis to be treated with ASCs (Table **5**).

## OPEN QUESTIONS AND FUTURE PERSPECTIVES

MSCs have been produced and used in many clinical applications and even if the results so far obtained have raised stimulating hopes and indicated that mesenchymal stem cells are well tolerated and do not induce severe adverse reactions after administration, only long-term surveys will exclude potential late adverse reactions.

Despite the great interest in using MSCs, several potentially critical problems have not been solved. The identification of specific markers, singularly able to identify the mesenchymal progenitors, as well as definitive standards for their production are still lacking. Supplements added to culture media and time of expansion also influence the quality and the safety of the cell product, whatever the cell source. Furthermore the ways MSCs mediate their protective/reparative effects and modulate inflammatory responses *in vivo* have not been fully defined.

*In vitro* and *in vivo* findings support the hypothesis that the differentiation options of MSCs are flexible, since apparently "terminally" differentiated mesenchymal cells can re-gain stem cell properties and subsequently shift their differentiation status, due to modified external conditions [41, 57, 59, 378-382].

Consistent with mesenchyme plasticity [383], besides the multilineage differentiation properties [21, 71-73], human MSCs can simultaneously express osteogenic and adipogenic markers [384] and homogeneous populations derived from single cloned cells can sequentially switch from the adipocytic to the osteoblastic lineage *in vitro* [385], thus suggesting that these cells can express hybrid characteristics of both adipocytes and osteoblasts [380, 384, 386]. Currently there is an ongoing debate on the fundamental dynamics underlying this kind of cell plasticity.

The MSC system may exhibit a pronounced flexibility in order to be capable of instantaneous fate decisions in the course of development and in case of injury.

This differentiation flexibility raises important therapeutic considerations. In fact, the lack of an early commitment to a defined lineage differentiation, allows these cells to transdifferentiate *in vivo*, following their therapeutic administration. Depending on the environment that these cells find in a particular tissue, their final fate can be influenced, but can also probably be induced to a specific drift when required by preventive or interventional therapies.

In addition, historical histological observations have shown that adipocyte number increases with advancing age, concomitantly with a decreased bone formation [387-389]. In agreement, recent studies have shown an age-related decrease in the number of MSCs [242, 245, 390-392] and osteogenic properties of isolated human MSCs cultured in three dimension conditions [393, 394].

These data suggest that in addition to local or systemic factors, also intrinsic modifications of the cells with ageing can influence their differentiation pathway.

The process of ageing is important from the perspective of tissue regeneration and repair because there is evidence that beneficial functions may be negatively influenced by age.

The complexity of cell therapy needs more convincing controls and more accurate methods, than the simple standard ones so far described, to assess safety, reproducibility and quality of *in vitro*-expanded and *in vivo*-infused cells.

Due to the impossibility of real-time tracking *in vivo* in patients receiving MSCs, the optimal timing, dose and schedule of MSC administration have not been completely defined, and it has not been clarified whether the therapeutic effects of MSCs can be up or down modulated by the co-administration of immunosuppressive treatments.

The anti-inflammatory properties of MSCs might be a new therapeutic tool for decreasing inflammatory response in chronic and autoimmune diseases during activity phases of the disease. Finally, the possibility of putting MSCs together with bioactive substrates as well as using MSCs for gene delivering are further important potential clinical applications.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## ABBREVIATIONS

| | | |
|---|---|---|
| ADAS | = | Adipose-derived adult stem cells |
| ADIPOA | = | Adipose derived stromal cells for osteoarthritis treatment |
| AdMSCs | = | Adipose mesenchymal stem cells |
| ADSCs | = | Adipose-derived stromal cells |
| ALCAM | = | Activated lymphocyte cell adhesion molecule |
| APC | = | Antigen presenting cell |
| ASCs | = | Adipose derived stem cells/stromal cells |
| AT | = | Adipose tissue |
| MSCs | = | Mesenchymal Stem Cells |
| BM | = | Bone marrow |
| BMPs | = | Bone morphogenetic proteins |
| BMSSC | = | Bone marrow stromal stem cells |
| BSE | = | Bovine spongiform encephalopaty |
| CALLA | = | Common acute lymphocytic leukemia antigen |
| CCL | = | Chemokine ligand |
| CTL | = | Cytotoxic T lymphocytes |
| CXCR | = | Chemokine receptor |
| DCs | = | Dendritic cells |
| DKK | = | Dickkopf-related protein |
| EGF | = | Endothelial growth factor |
| EMEA | = | European Medicine Agency |
| FBS | = | Fetal bovine serum |
| FGF | = | Fibroblast growth factor |
| GD | = | Ganglioside |
| GLP | = | Good laboratory practices |
| GMP | = | Good manufacturing practices |
| GVHD | = | Graft Versus Host Disease |
| h | = | Human |
| HGF | = | Hepatocyte growth factor |
| HLA-DR | = | Human leukocyte antigen-DR |
| HS | = | Human serum |
| HSCs | = | Hematopoietic stem cells |
| hTERT | = | Human telomerase reverse transcriptase |
| i.p. | = | Intra peritoneum |
| i.t. | = | Intra tumor |
| i.v. | = | Intravenous |
| IDO | = | Indoleamine 2,3-dioxygenase |
| IFATS | = | International Federation of Adipose Therapeutics and Science |
| IFN | = | Interferon |
| IGF | = | Insulin growth factor |
| IL | = | Interleukin |
| iNOS | = | Inducible nitric-oxide synthase |
| ISCT | = | International Society for Cellular Therapy |
| LNGFR | = | Low affinity receptor of neural growth factor |
| m | = | Murine |
| MACs | = | Mesenchymal adult stem cells |
| MADS | = | Multipotent adipose-derived stem cells |
| M-CSF | = | Macrophage-colony stimulating factor |
| Mel-CAM | = | Melanoma-cell adhesion molecule |
| MHC-I | = | Major Histocompatibility Complex Class I |
| MHC-II | = | Major Histocompatibility Complex Class II |
| MIAMI | = | Marrow-isolated adult multipotent inducible cells |
| MSA | = | Multiple system atrophy |
| MSCs | = | Mesenchymal stem cells/marrow stromal cells |
| MSI | = | Microsatellite instability |
| NGFR | = | Neural growth factor receptor |
| NK | = | Natural Killer |
| NO | = | Nitric oxide |
| OA | = | Osteoarthritis |
| OI | = | Osteogenesis imperfecta |
| PDGF | = | Platelet derived growth factor |
| PDGF-R | = | Platelet-derived growth factor receptor |
| PECAM | = | Platelet endothelial cell adhesion molecule |
| PGE | = | Prostaglandin E |
| PLA | = | Processed lipoaspirate cells |
| RA | = | Rheumatoid Arthritis |
| s.c. | = | Sub cutaneous |
| SDF | = | Stromal derived factor |
| SSEA | = | Stage-specific embryonic antigen |
| SVF | = | Stromal vascular fraction |
| T regs | = | regulatory T cells |
| TGF | = | Transforming growth factor |
| Th | = | T helper |
| TNF | = | Tumor necrosis factor |
| VCAM | = | Vascular cell adhesion molecule |
| VEGF | = | Vascular endothelial growth factor |

## REFERENCES

[1] Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968; 6: 230-47.

[2] Caplan AI. Mesenchymal stem cells. J Orthop Res 1991; 9: 641-50.

[3] Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI. Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. Bone Marrow Transplant 1995; 16: 557-64.

[4] Gronthos S, Zannettino AC, Hay SJ, *et al.* Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. J Cell Sci 2003; 116: 1827-35.

[5] d'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC. Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci 2004; 117: 2971-81.

[6] Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T. Efficient homing of multipotent adult mesenchymal stem cells depends

on FROUNT-mediated clustering of CCR2. Cell Stem Cell 2008; 2: 566-75.

[7] Devine SM, Hoffman R. Role of mesenchymal stem cells in hematopoietic stem cell transplantation. Curr Opin Hematol 2000; 7: 358-63.

[8] Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol 2006; 6: 93-106.

[9] Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. Cell Stem Cell 2008; 2: 313-9.

[10] Jones E, McGonagle D. Human bone marrow mesenchymal stem cells *in vivo*. Rheumatology (Oxford) 2008; 47: 126-31.

[11] Jones EA, Kinsey SE, English A, *et al.* Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. Arthritis Rheum 2002; 46: 3349-60.

[12] Horwitz EM, Le Blanc K, Dominici M, *et al.* Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 2005; 7: 393-5.

[13] Dominici M, Le Blanc K, Mueller I, *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315-7.

[14] Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. J Cell Biochem 2009; 106: 984-91.

[15] Augello A, Kurth TB, De Bari C. Mesenchymal stem cells: a perspective from *in vitro* cultures to *in vivo* migration and niches. Eur Cell Mater 2010; 20: 121-33.

[16] Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells 2010; 28: 585-96.

[17] Bernardo ME, Cometa AM, Pagliara D, *et al.* Ex vivo expansion of mesenchymal stromal cells. Best Pract Res Clin Haematol 2011; 24: 73-81.

[18] Nombela-Arrieta C, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. Nat Rev Mol Cell Biol 2011; 12: 126-31.

[19] Si YL, Zhao YL, Hao HJ, Fu XB, Han WD. MSCs: Biological characteristics, clinical applications and their outstanding concerns. Ageing Res Rev 2011; 10: 93-103.

[20] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007; 25: 2739-49.

[21] Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-7.

[22] Delorme B, Ringe J, Gallay N, *et al.* Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. Blood 2008; 111: 2631-5.

[23] da Silva ML, Caplan AI, Nardi NB. In search of the *in vivo* identity of mesenchymal stem cells. Stem Cells 2008; 26: 2287-99.

[24] Kastrinaki MC, Andreakou I, Charbord P, Papadaki HA. Isolation of human bone marrow mesenchymal stem cells using different membrane markers: comparison of colony/cloning efficiency, differentiation potential, and molecular profile. Tissue Eng Part C Methods 2008; 14: 333-9.

[25] Deschaseaux F, Pontikoglou C, Sensebe L. Bone regeneration: the stem/progenitor cells point of view. J Cell Mol Med 2010; 14: 103-15.

[26] Pontikoglou C, Deschaseaux F, Sensebe L, Papadaki HA. Bone Marrow Mesenchymal Stem Cells: Biological Properties and Their Role in Hematopoiesis and Hematopoietic Stem Cell Transplantation. Stem Cell Rev 2011.

[27] Chan JL, Tang KC, Patel AP, *et al.* Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-gamma. Blood 2006; 107: 4817-24.

[28] Stagg J. Immune regulation by mesenchymal stem cells: two sides to the coin. Tissue Antigens 2007; 69: 1-9.

[29] Pontikoglou C, Delorme B, Charbord P. Human bone marrow native mesenchymal stem cells. Regen Med 2008; 3: 731-41.

[30] Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Blood 1991; 78: 55-62.

[31] Gronthos S, McCarty R, Mrozik K, *et al.* Heat shock protein-90 beta is expressed at the surface of multipotential mesenchymal precursor cells: generation of a novel monoclonal antibody, STRO-4, with specificity for mesenchymal precursor cells from human and ovine tissues. Stem Cells Dev 2009; 18: 1253-62.

[32] Buhring HJ, Battula VL, Treml S, Schewe B, Kanz L, Vogel W. Novel markers for the prospective isolation of human MSC. Ann N Y Acad Sci 2007; 1106: 262-71.

[33] Battula VL, Treml S, Bareiss PM, *et al.* Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1. Haematologica 2009; 94: 173-84.

[34] Quirici N, Soligo D, Bossolasco P, Servida F, Lumini C, Deliliers GL. Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies. Exp Hematol 2002; 30: 783-91.

[35] Kuci S, Kuci Z, Kreyenberg H, *et al.* CD271 antigen defines a subset of multipotent stromal cells with immunosuppressive and lymphohematopoietic engraftment-promoting properties. Haematologica 2010; 95: 651-9.

[36] Takashima Y, Era T, Nakao K, *et al.* Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell 2007; 129: 1377-88.

[37] Morikawa S, Mabuchi Y, Niibe K, *et al.* Development of mesenchymal stem cells partially originate from the neural crest. Biochem Biophys Res Commun 2009; 379: 1114-9.

[38] Martinez C, Hofmann TJ, Marino R, Dominici M, Horwitz EM. Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. Blood 2007; 109: 4245-8.

[39] Gang EJ, Bosnakovski D, Figueiredo CA, Visser JW, Perlingeiro RC. SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood 2007; 109: 1743-51.

[40] Deschaseaux F, Charbord P. Human marrow stromal precursors are alpha 1 integrin subunit-positive. J Cell Physiol 2000; 184: 319-25.

[41] Song L, Webb NE, Song Y, Tuan RS. Identification and functional analysis of candidate genes regulating mesenchymal stem cell self-renewal and multipotency. Stem Cells 2006; 24: 1707-18.

[42] Kubo H, Shimizu M, Taya Y, *et al.* Identification of mesenchymal stem cell (MSC)-transcription factors by microarray and knock-down analyses, and signature molecule-marked MSC in bone marrow by immunohistochemistry. Genes Cells 2009; 14: 407-24.

[43] Kaltz N, Ringe J, Holzwarth C, *et al.* Novel markers of mesenchymal stem cells defined by genome-wide gene expression analysis of stromal cells from different sources. Exp Cell Res 2010; 316: 2609-17.

[44] Sacchetti B, Funari A, Michienzi S, *et al.* Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 2007; 131: 324-36.

[45] Crisan M, Yap S, Casteilla L, *et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008; 3: 301-13.

[46] da Silva ML, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006; 119: 2204-13.

[47] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2000; 97: 13625-30.

[48] Williams JT, Southerland SS, Souza J, Calcutt AF, Cartledge RG. Cells isolated from adult human skeletal muscle capable of differentiating into multiple mesodermal phenotypes. Am Surg 1999; 65: 22-6.

[49] De Bari C., Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001; 44: 1928-42.

[50] Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. J Cell Biol 2001; 153: 1133-40.

[51] Zuk PA, Zhu M, Mizuno H, *et al.* Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7: 211-28.

[52] In't Anker PS, Scherjon SA, Kleijburg-van der KC, *et al.* Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood 2003; 102: 1548-9.

[53] Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000; 109: 235-42.

[54] Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001; 98: 2396-402.

[55] Fan CG, Tang FW, Zhang QJ, *et al.* Characterization and neural differentiation of fetal lung mesenchymal stem cells. Cell Transplant 2005; 14: 311-21.

[56] Gimble JM, Guilak F, Nuttall ME, Sathishkumar S, Vidal M, Bunnell BA. *In vitro* Differentiation Potential of Mesenchymal Stem Cells. Transfus Med Hemother 2008; 35: 228-38.

[57] Charbord P. Bone marrow mesenchymal stem cells: historical overview and concepts. Hum Gene Ther 2010; 21: 1045-56.

[58] Remy-Martin JP, Marandin A, Challier B, *et al.* Vascular smooth muscle differentiation of murine stroma: a sequential model. Exp Hematol 1999; 27: 1782-95.

[59] Delorme B, Ringe J, Pontikoglou C, *et al.* Specific lineage-priming of bone marrow mesenchymal stem cells provides the molecular framework for their plasticity. Stem Cells 2009; 27: 1142-51.

[60] Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 1995; 18: 1417-26.

[61] Chagraoui J, Lepage-Noll A, Anjo A, Uzan G, Charbord P. Fetal liver stroma consists of cells in epithelial-to-mesenchymal transition. Blood 2003; 101: 2973-82.

[62] Oswald J, Boxberger S, Jorgensen B, *et al.* Mesenchymal stem cells can be differentiated into endothelial cells *in vitro*. Stem Cells 2004; 22: 377-84.

[63] Bossolasco P, Cova L, Calzarossa C, *et al.* Neuro-glial differentiation of human bone marrow stem cells *in vitro*. Exp Neurol 2005; 193: 312-25.

[64] Makino S, Fukuda K, Miyoshi S, *et al.* Cardiomyocytes can be generated from marrow stromal cells *in vitro*. J Clin Invest 1999; 103: 697-705.

[65] Gojo S, Gojo N, Takeda Y, *et al. In vivo* cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res 2003; 288: 51-9.

[66] Barbash IM, Chouraqui P, Baron J, *et al.* Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation 2003; 108: 863-8.

[67] Psaltis PJ, Zannettino AC, Worthley SG, Gronthos S. Concise review: mesenchymal stromal cells: potential for cardiovascular repair. Stem Cells 2008; 26: 2201-10.

[68] Petrie Aronin CE, Tuan RS. Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells. Birth Defects Res C Embryo Today 2010; 90: 67-74.

[69] Ankrum J, Karp JM. Mesenchymal stem cell therapy: Two steps forward, one step back. Trends Mol Med 2010; 16: 203-9.

[70] Zuk PA. The adipose-derived stem cell: looking back and looking ahead. Mol Biol Cell 2010; 21: 1783-7.

[71] Witkowska-Zimny M, Walenko K. Stem cells from adipose tissue. Cell Mol Biol Lett 2011; 16: 236-57.

[72] Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. Stem Cell Rev 2011; 7: 269-91.

[73] Locke M, Feisst V, Dunbar PR. Concise Review: Human Adipose-Derived Stem Cells (ASC): Separating Promise from Clinical Need. Stem Cells 2011; 29: 404-11.

[74] Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell 2007; 131: 242-56.

[75] Tchkonia T, Morbeck DE, Von Zglinicki T., *et al.* Fat tissue, aging, and cellular senescence. Aging Cell 2010; 9: 667-84.

[76] Berryman DE, List EO, Sackmann-Sala L, Lubbers E, Munn R, Kopchick JJ. Growth hormone and adipose tissue: Beyond the adipocyte. Growth Horm IGF Res 2011; e-pub.

[77] Klaus S. Adipose tissue as a regulator of energy balance. Curr Drug Targets 2004; 5: 241-50.

[78] Guo W, Pirtskhalava T, Tchkonia T, *et al.* Aging results in paradoxical susceptibility of fat cell progenitors to lipotoxicity. Am J Physiol Endocrinol Metab 2007; 292: E1041-E1051.

[79] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89: 2548-56.

[80] Katz AJ, Llull R, Hedrick MH, Futrell JW. Emerging approaches to the tissue engineering of fat. Clin Plast Surg 1999; 26: 587-603.

[81] Zhu Y, Liu T, Song K, Fan X, Ma X, Cui Z. Adipose-derived stem cell: a better stem cell than BMSC. Cell Biochem Funct 2008; 26: 664-75.

[82] Mitchell JB, McIntosh K, Zvonic S, *et al.* Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 2006; 24: 376-85.

[83] Williams SK, McKenney S, Jarrell BE. Collagenase lot selection and purification for adipose tissue digestion. Cell Transplant 1995; 4: 281-9.

[84] Gimble J, Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy 2003; 5: 362-9.

[85] Zuk PA, Zhu M, Ashjian P, *et al.* Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-95.

[86] Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res 2007; 100: 1249-60.

[87] Planat-Benard V, Silvestre JS, Cousin B, *et al.* Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation 2004; 109: 656-63.

[88] Rada T, Reis RL, Gomes ME. Adipose tissue-derived stem cells and their application in bone and cartilage tissue engineering. Tissue Eng Part B Rev 2009; 15: 113-25.

[89] Safford KM, Hicok KC, Safford SD, *et al.* Neurogenic differentiation of murine and human adipose-derived stromal cells. Biochem Biophys Res Commun 2002; 294: 371-9.

[90] Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. Biochem Biophys Res Commun 2005; 328: 258-64.

[91] Timper K, Seboek D, Eberhardt M, *et al.* Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. Biochem Biophys Res Commun 2006; 341: 1135-40.

[92] Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol 2006; 24: 150-4.

[93] Daher SR, Johnstone BH, Phinney DG, March KL. Adipose stromal/stem cells: basic and translational advances: the IFATS collection. Stem Cells 2008; 26: 2664-5.

[94] Schaffler A, Buchler C. Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies. Stem Cells 2007; 25: 818-27.

[95] Katz AJ, Tholpady A, Tholpady SS, Shang H, Ogle RC. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells 2005; 23: 412-23.

[96] Nakagami H, Morishita R, Maeda K, Kikuchi Y, Ogihara T, Kaneda Y. Adipose tissue-derived stromal cells as a novel option for regenerative cell therapy. J Atheroscler Thromb 2006; 13: 77-81.

[97] Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001; 189: 54-63.

[98] Dawn B, Bolli R. Adult bone marrow-derived cells: regenerative potential, plasticity, and tissue commitment. Basic Res Cardiol 2005; 100: 494-503.

[99] De Ugarte DA, Alfonso Z, Zuk PA, *et al.* Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. Immunol Lett 2003; 89: 267-70.

[100] Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006; 24: 1294-301.

[101] Romanov YA, Darevskaya AN, Merzlikina NV, Buravkova LB. Mesenchymal stem cells from human bone marrow and adipose tissue: isolation, characterization, and differentiation potentialities. Bull Exp Biol Med 2005; 140: 138-43.

[102] Puissant B, Barreau C, Bourin P, *et al.* Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. Br J Haematol 2005; 129: 118-29.

[103] Wagner W, Wein F, Seckinger A, *et al.* Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005; 33: 1402-16.

[104] Brivanlou AH, Gage FH, Jaenisch R, Jessell T, Melton D, Rossant J. Stem cells. Setting standards for human embryonic stem cells. Science 2003; 300: 913-6.

[105] McIntosh K, Zvonic S, Garrett S, *et al.* The immunogenicity of human adipose-derived cells: temporal changes *in vitro*. Stem Cells 2006; 24: 1246-53.

[106] Strem BM, Hicok KC, Zhu M, *et al.* Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005; 54: 132-41.

[107] Garcia-Castro J, Trigueros C, Madrenas J, Perez-Simon JA, Rodriguez R, Menendez P. Mesenchymal stem cells and their use as cell replacement therapy and disease modelling tool. J Cell Mol Med 2008; 12: 2552-65.

[108] Tapp H, Hanley EN, Patt JC, Gruber HE. Adipose-derived stem cells: characterization and current application in orthopaedic tissue repair. Exp Biol Med (Maywood ) 2008.

[109] Jiang T, Liu W, Lv X, *et al.* Potent *in vitro* chondrogenesis of CD105 enriched human adipose-derived stem cells. Biomaterials 2010; 31: 3564-71.

[110] Quirici N, Scavullo C, de Girolamo L, *et al.* Anti-L-NGFR and -CD34 monoclonal antibodies identify multipotent mesenchymal stem cells in human adipose tissue. Stem Cells Dev 2010; 19: 915-25.

[111] Zimmerlin L, Donnenberg VS, Pfeifer ME, *et al.* Stromal vascular progenitors in adult human adipose tissue. Cytometry A 2010; 77: 22-30.

[112] Smas CM, Sul HS. Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. Cell 1993; 73: 725-34.

[113] Zannettino AC, Paton S, Arthur A, *et al.* Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype *in vitro* and *in vivo*. J Cell Physiol 2008; 214: 413-21.

[114] Maumus M, Peyrafitte JA, D'Angelo R, *et al.* Native human adipose stromal cells: localization, morphology and phenotype. Int J Obes (Lond) 2011; e-pub.

[115] Parker AM, Shang H, Khurgel M, Katz AJ. Low serum and serum-free culture of multipotential human adipose stem cells. Cytotherapy 2007; 9: 637-46.

[116] Lindroos B, Boucher S, Chase L, *et al.* Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells *in vitro*. Cytotherapy 2009; 11: 958-72.

[117] Lindroos B, Aho KL, Kuokkanen H, *et al.* Differential gene expression in adipose stem cells cultured in allogeneic human serum versus fetal bovine serum. Tissue Eng Part A 2010; 16: 2281-94.

[118] Peroni D, Scambi I, Pasini A, *et al.* Stem molecular signature of adipose-derived stromal cells. Exp Cell Res 2008; 314: 603-15.

[119] Egusa H, Iida K, Kobayashi M, *et al.* Downregulation of extracellular matrix-related gene clusters during osteogenic differentiation of human bone marrow- and adipose tissue-derived stromal cells. Tissue Eng 2007; 13: 2589-600.

[120] Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 2008; 129: 163-73.

[121] Rodda DJ, Chew JL, Lim LH, *et al.* Transcriptional regulation of nanog by OCT4 and SOX2. J Biol Chem 2005; 280: 24731-7.

[122] Liedtke S, Enczmann J, Waclawczyk S, Wernet P, Kogler G. Oct4 and its pseudogenes confuse stem cell research. Cell Stem Cell 2007; 1: 364-6.

[123] Prunet-Marcassus B, Cousin B, Caton D, Andre M, Penicaud L, Casteilla L. From heterogeneity to plasticity in adipose tissues: site-specific differences. Exp Cell Res 2006; 312: 727-36.

[124] Fraser JK, Wulur I, Alfonso Z, Zhu M, Wheeler ES. Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots. Cytotherapy 2007; 9: 459-67.

[125] Kilroy GE, Foster SJ, Wu X, *et al.* Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. J Cell Physiol 2007; 212: 702-9.

[126] Salgado AJ, Reis RL, Sousa NJ, Gimble JM. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. Curr Stem Cell Res Ther 2010; 5: 103-10.

[127] Hennig T, Lorenz H, Thiel A, *et al.* Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J Cell Physiol 2007; 211: 682-91.

[128] Awad HA, Halvorsen YD, Gimble JM, Guilak F. Effects of transforming growth factor beta1 and dexamethasone on the growth and chondrogenic differentiation of adipose-derived stromal cells. Tissue Eng 2003; 9: 1301-12.

[129] Mahmoudifar N, Doran PM. Chondrogenic differentiation of human adipose-derived stem cells in polyglycolic acid mesh scaffolds under dynamic culture conditions. Biomaterials 2010; 31: 3858-67.

[130] Jin X, Sun Y, Zhang K, *et al.* Ectopic neocartilage formation from predifferentiated human adipose derived stem cells induced by adenoviral-mediated transfer of hTGF beta2. Biomaterials 2007; 28: 2994-3003.

[131] Zhao Y, Lin H, Zhang J, *et al.* Crosslinked three-dimensional demineralized bone matrix for the adipose-derived stromal cell proliferation and differentiation. Tissue Eng Part A 2009; 15: 13-21.

[132] Hong L, Colpan A, Peptan IA, Daw J, George A, Evans CA. 17-Beta estradiol enhances osteogenic and adipogenic differentiation of human adipose-derived stromal cells. Tissue Eng 2007; 13: 1197-203.

[133] Lee JH, Rhie JW, Oh DY, Ahn ST. Osteogenic differentiation of human adipose tissue-derived stromal cells (hASCs) in a porous three-dimensional scaffold. Biochem Biophys Res Commun 2008; 370: 456-60.

[134] Jeon O, Rhie JW, Kwon IK, Kim JH, Kim BS, Lee SH. *In vivo* bone formation following transplantation of human adipose-derived stromal cells that are not differentiated osteogenically. Tissue Eng Part A 2008; 14: 1285-94.

[135] Lin Y, Wang T, Wu L, *et al.* Ectopic and in situ bone formation of adipose tissue-derived stromal cells in biphasic calcium phosphate nanocomposite. J Biomed Mater Res A 2007; 81: 900-10.

[136] Li X, Yao J, Wu L, *et al.* Osteogenic induction of adipose-derived stromal cells: not a requirement for bone formation *in vivo*. Artif Organs 2010; 34: 46-54.

[137] Gastaldi G, Asti A, Scaffino MF, *et al.* Human adipose-derived stem cells (hASCs) proliferate and differentiate in osteoblast-like cells on trabecular titanium scaffolds. J Biomed Mater Res A 2010; 94: 790-9.

[138] Mauney JR, Nguyen T, Gillen K, Kirker-Head C, Gimble JM, Kaplan DL. Engineering adipose-like tissue *in vitro* and *in vivo* utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds. Biomaterials 2007; 28: 5280-90.

[139] Brayfield C, Marra K, Rubin JP. Adipose stem cells for soft tissue regeneration. Handchir Mikrochir Plast Chir 2010; 42: 124-8.

[140] Brzoska M, Geiger H, Gauer S, Baer P. Epithelial differentiation of human adipose tissue-derived adult stem cells. Biochem Biophys Res Commun 2005; 330: 142-50.

[141] Rodriguez-Serrano F, Alvarez P, Caba O, *et al.* Promotion of human adipose-derived stem cell proliferation mediated by exogenous nucleosides. Cell Biol Int 2010; 34: 917-24.

[142] Froehlich H, Gulati R, Boilson B, *et al.* Carotid repair using autologous adipose-derived endothelial cells. Stroke 2009; 40: 1886-91.

[143] Madonna R, De Caterina CR. *In vitro* neovasculogenic potential of resident adipose tissue precursors. Am J Physiol Cell Physiol 2008; 295: C1271-C1280.

[144] Heydarkhan-Hagvall S, Schenke-Layland K, Yang JQ, *et al.* Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. Cells Tissues Organs 2008; 187: 263-74.

[145] Verseijden F, Posthumus-van Sluijs SJ, Pavljasevic P, Hofer SO, van Osch GJ, Farrell E. Adult human bone marrow- and adipose tissue-derived stromal cells support the formation of prevascular-like structures from endothelial cells *in vitro*. Tissue Eng Part A 2010; 16: 101-14.

[146] Scherberich A, Galli R, Jaquiery C, Farhadi J, Martin I. Three-dimensional perfusion culture of human adipose tissue-derived endothelial and osteoblastic progenitors generates osteogenic constructs with intrinsic vascularization capacity. Stem Cells 2007; 25: 1823-9.

[147] Muller AM, Mehrkens A, Schafer DJ, *et al.* Towards an intraoperative engineering of osteogenic and vasculogenic grafts from the stromal vascular fraction of human adipose tissue. Eur Cell Mater 2010; 19: 127-35.

[148] Nakada A, Fukuda S, Ichihara S, *et al.* Regeneration of central nervous tissue using a collagen scaffold and adipose-derived stromal cells. Cells Tissues Organs 2009; 190: 326-35.

[149] Erba P, Terenghi G, Kingham PJ. Neural differentiation and therapeutic potential of adipose tissue derived stem cells. Curr Stem Cell Res Ther 2010; 5: 153-60.

[150] Okura H, Komoda H, Fumimoto Y, *et al.* Transdifferentiation of human adipose tissue-derived stromal cells into insulin-producing clusters. J Artif Organs 2009; 12: 123-30.

[151] Kajiyama H, Hamazaki TS, Tokuhara M, *et al.* Pdx1-transfected adipose tissue-derived stem cells differentiate into insulin-producing cells *in vivo* and reduce hyperglycemia in diabetic mice. Int J Dev Biol 2010; 54: 699-705.

[152] Long JL, Zuk P, Berke GS, Chhetri DK. Epithelial differentiation of adipose-derived stem cells for laryngeal tissue engineering. Laryngoscope 2010; 120: 125-31.

[153] Banas A, Teratani T, Yamamoto Y, *et al.* Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology 2007; 46: 219-28.

[154] Aurich H, Sgodda M, Kaltwasser P, *et al.* Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue *in vitro* promotes hepatic integration *in vivo*. Gut 2009; 58: 570-81.

[155] Jeong JH, Lee JH, Jin ES, Min JK, Jeon SR, Choi KH. Regeneration of intervertebral discs in a rat disc degeneration model by implanted adipose-tissue-derived stromal cells. Acta Neurochir (Wien ) 2010; 152: 1771-7.

[156] da Silva M.L., Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009; 20: 419-27.

[157] Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008; 8: 726-36.

[158] Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noel D. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res Ther 2010; 1: 2.

[159] Sensebe L, Krampera M, Schrezenmeier H, Bourin P, Giordano R. Mesenchymal stem cells for clinical application. Vox Sang 2010; 98: 93-107.

[160] Shi Y, Hu G, Su J, *et al.* Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. Cell Res 2010; 20: 510-8.

[161] Yagi H, Soto-Gutierrez A, Parekkadan B, *et al.* Mesenchymal stem cells: Mechanisms of immunomodulation and homing. Cell Transplant 2010; 19: 667-79.

[162] Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. Annu Rev Pathol 2011; 6: 457-78.

[163] Solana R, Mariani E. NK and NK/T cells in human senescence. Vaccine 2000; 18: 1613-20.

[164] Moretta A, Bottino C, Vitale M, *et al.* Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu Rev Immunol 2001; 19: 197-223.

[165] Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006; 24: 74-85.

[166] Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006; 107: 1484-90.

[167] Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 2008; 111: 1327-33.

[168] Poggi A, Prevosto C, Massaro AM, *et al.* Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. J Immunol 2005; 175: 6352-60.

[169] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005; 105: 1815-22.

[170] Gotherstrom C, Lundqvist A, Duprez IR, Childs R, Berg L, Le Blanc K. Fetal and adult multipotent mesenchymal stromal cells are killed by different pathways. Cytotherapy 2011; 13: 269-78.

[171] Raffaghello L, Bianchi G, Bertolotto M, *et al.* Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche. Stem Cells 2008; 26: 151-62.

[172] Fadeel B, Ahlin A, Henter JI, Orrenius S, Hampton MB. Involvement of caspases in neutrophil apoptosis: regulation by reactive oxygen species. Blood 1998; 92: 4808-18.

[173] Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. Exp Hematol 2009; 37: 1445-53.

[174] Jiang XX, Zhang Y, Liu B, *et al.* Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005; 105: 4120-6.

[175] Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells. J Immunol 2006; 177: 2080-7.

[176] Di Nicola M., Carlo-Stella C, Magni M, *et al.* Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002; 99: 3838-43.

[177] Bartholomew A, Sturgeon C, Siatskas M, *et al.* Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. Exp Hematol 2002; 30: 42-8.

[178] Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 2003; 75: 389-97.

[179] Rasmusson I, Ringden O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp Cell Res 2005; 305: 33-41.

[180] Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 2005; 105: 2821-7.

[181] Benvenuto F, Ferrari S, Gerdoni E, *et al.* Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells 2007; 25: 1753-60.

[182] Rasmusson I, Ringden O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation 2003; 76: 1208-13.

[183] Maccario R, Podesta M, Moretta A, *et al.* Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica 2005; 90: 516-25.

[184] Di Ianni M, Del Papa B, De Ioanni M, *et al.* Mesenchymal cells recruit and regulate T regulatory cells. Exp Hematol 2008; 36: 309-18.

[185] Prevosto C, Zancolli M, Canevali P, Zocchi MR, Poggi A. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. Haematologica 2007; 92: 881-8.

[186] Selmani Z, Naji A, Zidi I, *et al.* Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells 2008; 26: 212-22.

[187] Augello A, Tasso R, Negrini SM, *et al.* Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 2005; 35: 1482-90.

[188] Corcione A, Benvenuto F, Ferretti E, *et al.* Human mesenchymal stem cells modulate B-cell functions. Blood 2006; 107: 367-72.

[189] Rasmusson I, Le Blanc K., Sundberg B, Ringden O. Mesenchymal stem cells stimulate antibody secretion in human B cells. Scand J Immunol 2007; 65: 336-43.

[190] Traggiai E, Volpi S, Schena F, *et al.* Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. Stem Cells 2008; 26: 562-9.

[191] Krampera M, Cosmi L, Angeli R, *et al.* Role for interferon-g in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006; 24: 386-98.

[192] Sato K, Ozaki K, Oh I, *et al.* Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood 2007; 109: 228-34.

[193] Ren G, Zhang L, Zhao X, *et al.* Mesenchymal stem cell-mediated immunosuppression occurs *via* concerted action of chemokines and nitric oxide. Cell Stem Cell 2008; 2: 141-50.

[194] Djouad F, Charbonnier LM, Bouffi C, *et al.* Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells 2007; 25: 2025-32.

[195] Selmani Z, Naji A, Gaiffe E, *et al.* HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. Transplantation 2009; 87: S62-S66.

[196] Le Blanc K., Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 2003; 57: 11-20.

[197] Chan WK, Lau AS, Li JC, Law HK, Lau YL, Chan GC. MHC expression kinetics and immunogenicity of mesenchymal stromal cells after short-term IFN-gamma challenge. Exp Hematol 2008; 36: 1545-55.

[198] Ryan JM, Barry F, Murphy JM, Mahon BP. Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol 2007; 149: 353-63.

[199] Stagg J, Pommey S, Eliopoulos N, Galipeau J. Interferon-gamma-stimulated marrow stromal cells: a new type of nonhematopoietic antigen-presenting cell. Blood 2006; 107: 2570-7.

[200] Harvath L. Food and Drug Administration's proposed approach to regulation of hematopoietic stem/progenitor cell products for therapeutic use. Transfus Med Rev 2000; 14: 104-11.

[201] DIRECTIVE 2001/83/EC of the European Parliament and of the Council on the Community Code Relating to Medicinal Products for Human Use. The European Parliament and of the Council of the Community. 83 [2001] Available from URL: http://www.emea.europa.eu/pdfs/human/pmf/2001-83-EC.pdf.

[202] Guideline on Human Cell-Based Medicinal Products. EMEA, Committee for Human Medicinal Products. [2008] Available from URL: http://www.emea.europa.eu/pdfs/human/cpwp/41086906enfin.pdf.

[203] Rhemann W, Morgan G. The regulation of advanced therapies: perspectives from the EU Regulatory Feature. [2009] Available from URL: http://www.taylorwessing.com/uploads/tx_siruplawyermanagement/The_Regulation_of_Advanced_Therapies.pdf

[204] Halme DG, Kessler DA. FDA regulation of stem-cell-based therapies. N Engl J Med 2006; 355: 1730-5.

[205] Burger SR. Design and operation of a current good manufacturing practices cell-engineering laboratory. Cytotherapy 2000; 2: 111-22.

[206] Regulation (EC) no 1394/2007 of the European Parliament and of the Council. Official Journal of the European Union. [2007] Available from URL: http://eur-lex.europa.eu/LexUriServ/LexUriServ.

[207] Bieback K, Kinzebach S, Karagianni M. Translating research into clinical scale manufacturing of mesenchymal stromal cells. Stem Cells Int 2011; 2010: 193519.

[208] Bourin P, Sensebe L, Planat-Benard V, Roncalli J, Bura-Riviere A, Casteilla L. Culture and Use of Mesenchymal Stromal Cells in Phase I and II Clinical Trials. Stem Cells Int 2010; 2010: 503593.

[209] Liras A. Future research and therapeutic applications of human stem cells: general, regulatory, and bioethical aspects. J Transl Med 2010; 8: 131.

[210] Sensebe L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. Hum Gene Ther 2011; 22: 19-26.

[211] Gimble JM, Bunnell BA, Chiu ES, Guilak F. Concise Review: Adipose-Derived Stromal Vascular Fraction Cells and Stem Cells: Let's Not Get Lost in Translation. Stem Cells 2011; 29: 749-54.

[212] Bieback K, Hecker A, Kocaomer A, *et al.* Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. Stem Cells 2009; 27: 2331-41.

[213] Herrera B, Inman GJ. A rapid and sensitive bioassay for the simultaneous measurement of multiple bone morphogenetic proteins. Identification and quantification of BMP4, BMP6 and BMP9 in bovine and human serum. BMC Cell Biol 2009; 10: 20.

[214] Mackensen A, Drager R, Schlesier M, Mertelsmann R, Lindemann A. Presence of IgE antibodies to bovine serum albumin in a patient developing anaphylaxis after vaccination with human peptide-pulsed dendritic cells. Cancer Immunol Immunother 2000; 49: 152-6.

[215] Selvaggi TA, Walker RE, Fleisher TA. Development of antibodies to fetal calf serum with arthus-like reactions in human immunodeficiency virus-infected patients given syngeneic lymphocyte infusions. Blood 1997; 89: 776-9.

[216] Heiskanen A, Satomaa T, Tiitinen S, *et al.* N-glycolylneuraminic acid xenoantigen contamination of human embryonic and mesenchymal stem cells is substantially reversible. Stem Cells 2007; 25: 197-202.

[217] Stute N, Holtz K, Bubenheim M, Lange C, Blake F, Zander AR. Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. Exp Hematol 2004; 32: 1212-25.

[218] Koller MR, Maher RJ, Manchel I, Oxender M, Smith AK. Alternatives to animal sera for human bone marrow cell expansion: human serum and serum-free media. J Hematother 1998; 7: 413-23.

[219] Nimura A, Muneta T, Koga H, *et al.* Increased proliferation of human synovial mesenchymal stem cells with autologous human serum: comparisons with bone marrow mesenchymal stem cells and with fetal bovine serum. Arthritis Rheum 2008; 58: 501-10.

[220] Yamamoto N, Isobe M, Negishi A, *et al.* Effects of autologous serum on osteoblastic differentiation in human bone marrow cells. J Med Dent Sci 2003; 50: 63-9.

[221] Yamaguchi M, Hirayama F, Wakamoto S, *et al.* Bone marrow stromal cells prepared using AB serum and bFGF for hematopoietic stem cells expansion. Transfusion 2002; 42: 921-7.

[222] Oreffo RO, Triffitt JT. Future potentials for using osteogenic stem cells and biomaterials in orthopedics. Bone 1999; 25: 5S-9S.

[223] Johansson L, Klinth J, Holmqvist O, Ohlson S. Platelet lysate: a replacement for fetal bovine serum in animal cell culture? Cytotechnology 2003; 42: 67-74.

[224] Salvade A, Della MP, Gaddi D, *et al.* Characterization of platelet lysate cultured mesenchymal stromal cells and their potential use in tissue-engineered osteogenic devices for the treatment of bone defects. Tissue Eng Part C Methods 2010; 16: 201-14.

[225] Lepperdinger G, Brunauer R, Jamnig A, Laschober G, Kassem M. Controversial issue: is it safe to employ mesenchymal stem cells in cell-based therapies? Exp Gerontol 2008; 43: 1018-23.

[226] Meuleman N, Tondreau T, Delforge A, *et al.* Human marrow mesenchymal stem cell culture: serum-free medium allows better expansion than classical alpha-MEM medium. Eur J Haematol 2006; 76: 309-16.

[227] Rajala K, Lindroos B, Hussein SM, *et al.* A defined and xeno-free culture method enabling the establishment of clinical-grade human embryonic, induced pluripotent and adipose stem cells. PLoS One 2010; 5: e10246.

[228] Boquest AC, Shahdadfar A, Fronsdal K, *et al.* Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after *in vitro* cell culture. Mol Biol Cell 2005; 16: 1131-41.

[229] Izadpanah R, Kaushal D, Kriedt C, *et al.* Long-term *in vitro* expansion alters the biology of adult mesenchymal stem cells. Cancer Res 2008; 68: 4229-38.

[230] Sepe A, Tchkonia T, Thomou T, Zamboni M, Kirkland JL. Aging and regional differences in fat cell progenitors - a mini-review. Gerontology 2011; 57: 66-75.

[231] Majka SM, Fox KE, Psilas JC, *et al.* De novo generation of white adipocytes from the myeloid lineage *via* mesenchymal intermediates is age, adipose depot, and gender specific. Proc Natl Acad Sci U S A 2010; 107: 14781-6.

[232] Schipper BM, Marra KG, Zhang W, Donnenberg AD, Rubin JP. Regional anatomic and age effects on cell function of human adipose-derived stem cells. Ann Plast Surg 2008; 60: 538-44.

[233] Zhu M, Kohan E, Bradley J, Hedrick M, Benhaim P, Zuk P. The effect of age on osteogenic, adipogenic and proliferative potential of female adipose-derived stem cells. J Tissue Eng Regen Med 2009; 3: 290-301.

[234] Madonna R, Renna FV, Cellini C, *et al.* Age-dependent impairment of number and angiogenic potential of adipose tissue-derived progenitor cells. Eur J Clin Invest 2011; 41: 126-33.

[235] van Harmelen V, Skurk T, Rohrig K, *et al.* Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. Int J Obes Relat Metab Disord 2003; 27: 889-95.

[236] Aksu AE, Rubin JP, Dudas JR, Marra KG. Role of gender and anatomical region on induction of osteogenic differentiation of human adipose-derived stem cells. Ann Plast Surg 2008; 60: 306-22.

[237] Bellantuono I, Aldahmash A, Kassem M. Aging of marrow stromal (skeletal) stem cells and their contribution to age-related bone loss. Biochim Biophys Acta 2009; 1792: 364-70.

[238] Ertl R.P., Chen J, Astle CM, Duffy TM, Harrison DE. Effects of dietary restriction on hematopoietic stem-cell aging are genetically regulated. Blood 2008; 111: 1709-16.

[239] Egrise D, Martin D, Vienne A, Neve P, Schoutens A. The number of fibroblastic colonies formed from bone marrow is decreased and the *in vitro* proliferation rate of trabecular bone cells increased in aged rats. Bone 1992; 13: 355-61.

[240] Quarto R, Thomas D, Liang CT. Bone progenitor cell deficits and the age-associated decline in bone repair capacity. Calcif Tissue Int 1995; 56: 123-9.

[241] Oreffo RO, Bord S, Triffitt JT. Skeletal progenitor cells and ageing human populations. Clin Sci (Lond) 1998; 94: 549-55.

[242] Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following *in vitro* expansion. Stem Cells 2004; 22: 675-82.

[243] Stenderup K, Justesen J, Eriksen EF, Rattan SI, Kassem M. Number and proliferative capacity of osteogenic stem cells are maintained during aging and in patients with osteoporosis. J Bone Miner Res 2001; 16: 1120-9.

[244] Bellantuono I, Keith WN. Stem cell ageing: does it happen and can we intervene? Expert Rev Mol Med 2007; 9: 1-20.

[245] Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 2003; 33: 919-26.

[246] Justesen J, Stenderup K, Eriksen EF, Kassem M. Maintenance of osteoblastic and adipocytic differentiation potential with age and osteoporosis in human marrow stromal cell cultures. Calcif Tissue Int 2002; 71: 36-44.

[247] Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate *in vitro* according to a hierarchical model. J Cell Sci 2000; 113 ( Pt 7): 1161-6.

[248] Zhou YF, Bosch-Marce M, Okuyama H, *et al.* Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. Cancer Res 2006; 66: 10849-54.

[249] Miura M, Miura Y, Padilla-Nash HM, *et al.* Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. Stem Cells 2006; 24: 1095-103.

[250] Aguilar S, Nye E, Chan J, *et al.* Murine but not human mesenchymal stem cells generate osteosarcoma-like lesions in the lung. Stem Cells 2007; 25: 1586-94.

[251] Bernardo ME, Zaffaroni N, Novara F, *et al.* Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term *in vitro* culture and do not exhibit telomere maintenance mechanisms. Cancer Res 2007; 67: 9142-9.

[252] Kim J, Kang JW, Park JH, *et al.* Biological characterization of long-term cultured human mesenchymal stem cells. Arch Pharm Res 2009; 32: 117-26.

[253] Meza-Zepeda LA, Noer A, Dahl JA, Micci F, Myklebost O, Collas P. High-resolution analysis of genetic stability of human adipose tissue stem cells cultured to senescence. J Cell Mol Med 2008; 12: 553-63.

[254] Choumerianou DM, Dimitriou H, Perdikogianni C, Martimianaki G, Riminucci M, Kalmanti M. Study of oncogenic transformation in ex vivo expanded mesenchymal cells, from paediatric bone marrow. Cell Prolif 2008; 41: 909-22.

[255] Wang Y, Huso DL, Harrington J, *et al.* Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. Cytotherapy 2005; 7: 509-19.

[256] Serakinci N, Guldberg P, Burns JS, *et al.* Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 2004; 23: 5095-8.

[257] Burns JS, Abdallah BM, Guldberg P, Rygaard J, Schroder HD, Kassem M. Tumorigenic heterogeneity in cancer stem cells evolved from long-term cultures of telomerase-immortalized human mesenchymal stem cells. Cancer Res 2005; 65: 3126-35.

[258] Rubio D, Garcia-Castro J, Martin MC, *et al.* Spontaneous human adult stem cell transformation. Cancer Res 2005; 65: 3035-9.

[259] Rubio D, Garcia S, De la Cueva T, *et al.* Human mesenchymal stem cell transformation is associated with a mesenchymal-epithelial transition. Exp Cell Res 2008; 314: 691-8.

[260] Rosland GV, Svendsen A, Torsvik A, *et al.* Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Res 2009; 69: 5331-9.

[261] de la Fuente R, Bernad A, Garcia-Castro J, Martin MC, Cigudosa JC. Retraction: Spontaneous human adult stem cell transformation. Cancer Res 2010; 70: 6682.

[262] Torsvik A, Rosland GV, Svendsen A, *et al.* Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track - letter. Cancer Res 2010; 70: 6393-6.

[263] Garcia S, Bernad A, Martin MC, Cigudosa JC, Garcia-Castro J, de la Fuente R. Pitfalls in spontaneous *in vitro* transformation of human mesenchymal stem cells. Exp Cell Res 2010; 316: 1648-50.

[264] Tarte K, Gaillard J, Lataillade JJ, *et al.* Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. Blood 2010; 115: 1549-53.

[265] Shibata KR, Aoyama T, Shima Y, *et al.* Expression of the p16INK4A gene is associated closely with senescence of human mesenchymal stem cells and is potentially silenced by DNA methylation during *in vitro* expansion. Stem Cells 2007; 25: 2371-82.

[266] Ra JC, Shin IS, Kim SH, *et al.* Safety of Intravenous Infusion of Human Adipose Tissue-Derived Mesenchymal Stem Cells in Animals and Humans. Stem Cells Dev 2011.

[267] Lazennec G, Jorgensen C. Concise review: adult multipotent stromal cells and cancer: risk or benefit? Stem Cells 2008; 26: 1387-94.

[268] Helmy KY, Patel SA, Silverio K, Pliner L, Rameshwar P. Stem cells and regenerative medicine: accomplishments to date and future promise. Ther Deliv 2010; 1: 693-705.

[269] Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F, III. Concise review: Dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? Stem Cells 2011; 29: 11-9.

[270] Zhu W, Xu W, Jiang R, *et al.* Mesenchymal stem cells derived from bone marrow favor tumor cell growth *in vivo*. Exp Mol Pathol 2006; 80: 267-74.

[271] Karnoub AE, Dash AB, Vo AP, *et al.* Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007; 449: 557-63.

[272] Shinagawa K, Kitadai Y, Tanaka M, *et al.* Mesenchymal stem cells enhance growth and metastasis of colon cancer. Int J Cancer 2010; 127: 2323-33.

[273] Muehlberg FL, Song YH, Krohn A, *et al.* Tissue-resident stem cells promote breast cancer growth and metastasis. Carcinogenesis 2009; 30: 589-97.

[274] Prantl L, Muehlberg F, Navone NM, *et al.* Adipose tissue-derived stem cells promote prostate tumor growth. Prostate 2010; 70: 1709-15.

[275] Yu JM, Jun ES, Bae YC, Jung JS. Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth *in vivo*. Stem Cells Dev 2008; 17: 463-73.

[276] Kucerova L, Matuskova M, Hlubinova K, Altanerova V, Altaner C. Tumor cell behaviour modulation by mesenchymal stromal cells. Mol Cancer 2010; 9: 129.

[277] Lin G, Yang R, Banie L, *et al.* Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. Prostate 2010; 70: 1066-73.

[278] Maestroni GJ, Hertens E, Galli P. Factor(s) from nonmacrophage bone marrow stromal cells inhibit Lewis lung carcinoma and B16 melanoma growth in mice. Cell Mol Life Sci 1999; 55: 663-7.

[279] Khakoo AY, Pati S, Anderson SA, *et al.* Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med 2006; 203: 1235-47.

[280] Secchiero P, Zorzet S, Tripodo C, *et al.* Human bone marrow mesenchymal stem cells display anti-cancer activity in SCID mice bearing disseminated non-Hodgkin's lymphoma xenografts. PLoS One 2010; 5: e11140.

[281] Zhu Y, Sun Z, Han Q, *et al.* Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1. Leukemia 2009; 23: 925-33.

[282] Cousin B, Ravet E, Poglio S, *et al.* Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both *in vitro* and *in vivo*. PLoS One 2009; 4: e6278.

[283] Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. Stem Cells 2007; 25: 2896-902.

[284] Deeg HJ. How I treat refractory acute GVHD. Blood 2007; 109: 4119-26.

[285] Newman RE, Yoo D, LeRoux MA, Nilkovitch-Miagkova A. Treatment of inflammatory diseases with mesenchymal stem cells. Inflamm Allergy Drug Targets 2009; 8: 110-23.

[286] Sato K, Ozaki K, Mori M, Muroi K, Ozawa K. Mesenchymal stromal cells for graft-versus-host disease : basic aspects and clinical outcomes. J Clin Exp Hematop 2010; 50: 79-89.

[287] Tolar J, Villeneuve P, Keating A. Mesenchymal stromal cells for graft-versus-host disease. Hum Gene Ther 2011; 22: 257-62.

[288] Le Blanc K., Rasmusson I, Sundberg B, *et al.* Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004; 363: 1439-41.

[289] Ringden O, Uzunel M, Rasmusson I, *et al.* Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation 2006; 81: 1390-7.

[290] Le Blanc K., Frassoni F, Ball L, *et al.* Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 2008; 371: 1579-86.

[291] Muller I, Kordowich S, Holzwarth C, *et al.* Application of multipotent mesenchymal stromal cells in pediatric patients following allogeneic stem cell transplantation. Blood Cells Mol Dis 2008; 40: 25-32.

[292] von Bonin M, Stolzel F, Goedecke A, *et al.* Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. Bone Marrow Transplant 2009; 43: 245-51.

[293] Ringden O, Le Blanc K. Mesenchymal stem cells for treatment of acute and chronic graft-versus-host disease, tissue toxicity and hemorrhages. Best Pract Res Clin Haematol 2011; 24: 65-72.

[294] Kebriaei P, Isola L, Bahceci E, *et al.* Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant 2009; 15: 804-11.

[295] Zhou H, Guo M, Bian C, *et al.* Efficacy of bone marrow-derived mesenchymal stem cells in the treatment of sclerodermatous chronic graft-versus-host disease: clinical report. Biol Blood Marrow Transplant 2010; 16: 403-12.

[296] Weng JY, Du X, Geng SX, *et al.* Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. Bone Marrow Transplant 2010; 45: 1732-40.

[297] Lazarus HM, Koc ON, Devine SM, *et al.* Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 2005; 11: 389-98.

[298] Ning H, Yang F, Jiang M, *et al.* The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. Leukemia 2008; 22: 593-9.

[299] Baron F, Lechanteur C, Willems E, *et al.* Cotransplantation of mesenchymal stem cells might prevent death from graft-versus-host disease (GVHD) without abrogating graft-versus-tumor effects after HLA-mismatched allogeneic transplantation following non-myeloablative conditioning. Biol Blood Marrow Transplant 2010; 16: 838-47.

[300] Yanez R, Lamana ML, Garcia-Castro J, Colmenero I, Ramirez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have *in vivo* immunosuppressive properties applicable for the control of the graft-versus-host disease. Stem Cells 2006; 24: 2582-91.

[301] Li H, Guo Z, Jiang X, Zhu H, Li X, Mao N. Mesenchymal stem cells alter migratory property of T and dendritic cells to delay the development of murine lethal acute graft-versus-host disease. Stem Cells 2008; 26: 2531-41.

[302] Li H, Guo Z, Zhu H, *et al.* Transplanted mesenchymal stem cells can inhibit the three developmental stages of murine acute graft-versus-host disease. *In vivo* 2010; 24: 659-66.

[303] Sudres M, Norol F, Trenado A, *et al.* Bone marrow mesenchymal stem cells suppress lymphocyte proliferation *in vitro* but fail to prevent graft-versus-host disease in mice. J Immunol 2006; 176: 7761-7.

[304] Badillo AT, Peranteau WH, Heaton TE, Quinn C, Flake AW. Murine bone marrow derived stromal progenitor cells fail to prevent or treat acute graft-versus-host disease. Br J Haematol 2008; 141: 224-34.

[305] Fang B, Song Y, Zhao RC, Han Q, Lin Q. Using human adipose tissue-derived mesenchymal stem cells as salvage therapy for hepatic graft-versus-host disease resembling acute hepatitis. Transplant Proc 2007; 39: 1710-3.

[306] Fang B, Song Y, Lin Q, *et al.* Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant 2007; 11: 814-7.

[307] Fang B, Song Y, Liao L, Zhang Y, Zhao RC. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. Transplant Proc 2007; 39: 3358-62.

[308] Augello A, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum 2007; 56: 1175-86.

[309] Djouad F, Fritz V, Apparailly F, *et al.* Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. Arthritis Rheum 2005; 52: 1595-603.

[310] Zheng ZH, Li XY, Ding J, Jia JF, Zhu P. Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. Rheumatology 2008; 47: 22-30.

[311] Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. Arthritis Res Ther 2008; 10: 223.

[312] Jorgensen C, Djouad F, Fritz V, Apparailly F, Plence P, Noel D. Mesenchymal stem cells and rheumatoid arthritis. Joint Bone Spine 2003; 70: 483-5.

[313] Jorgensen C, Djouad F, Bouffi C, Mrugala D, Noel D. Multipotent mesenchymal stromal cells in articular diseases. Best Pract Res Clin Rheumatol 2008; 22: 269-84.

[314] Djouad F, Bouffi C, Ghannam S, Noel D, Jorgensen C. Mesenchymal stem cells: innovative therapeutic tools for rheumatic diseases. Nat Rev Rheumatol 2009; 5: 392-9.

[315] Bouffi C, Djouad F, Mathieu M, Noel D, Jorgensen C. Multipotent mesenchymal stromal cells and rheumatoid arthritis: risk or benefit? Rheumatology (Oxford) 2009; 48: 1185-9.

[316] Kastrinaki MC, Papadaki HA. Mesenchymal stromal cells in rheumatoid arthritis: biological properties and clinical applications. Curr Stem Cell Res Ther 2009; 4: 61-9.

[317] Noth U, Rackwitz L, Steinert AF, Tuan RS. Cell delivery therapeutics for musculoskeletal regeneration. Adv Drug Deliv Rev 2010; 62: 765-83.

[318] Maumus M, Guerit D, Toupet K, Jorgensen C, Noel D. Mesenchymal stem cell-based therapies in regenerative medicine: applications in rheumatology. Stem Cell Res Ther 2011; 2: 14.

[319] Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum 2009; 60: 1006-19.

[320] Grigolo B, Lisignoli G, Desando G, *et al.* Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. Tissue Eng Part C Methods 2009; 15: 647-58.

[321] Noth U, Steinert AF, Tuan RS. Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. Nat Clin Pract Rheumatol 2008; 4: 371-80.

[322] Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. Int J Rheum Dis 2011; 14: 211-5.

[323] Jorgensen C, Gordeladze J, Noel D. Tissue engineering through autologous mesenchymal stem cells. Curr Opin Biotechnol 2004; 15: 406-10.

[324] Horwitz EM, Gordon PL, Koo WK, *et al.* Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci U S A 2002; 99: 8932-7.

[325] Quarto R, Mastrogiacomo M, Cancedda R, *et al.* Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 2001; 344: 385-6.

[326] Lisignoli G, Fini M, Giavaresi G, Nicoli AN, Toneguzzi S, Facchini A. Osteogenesis of large segmental radius defects enhanced by basic fibroblast growth factor activated bone marrow stromal cells grown on non-woven hyaluronic acid-based polymer scaffold. Biomaterials 2002; 23: 1043-51.

[327] Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005; 87: 1430-7.

[328] Marcacci M, Kon E, Moukhachev V, *et al.* Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng 2007; 13: 947-55.

[329] Berner A, Reichert JC, Muller MB, *et al.* Treatment of long bone defects and non-unions: from research to clinical practice. Cell Tissue Res 2011.

[330] Meijer GJ, de Bruijn JD, Koole R, van Blitterswijk CA. Cell based bone tissue engineering in jaw defects. Biomaterials 2008; 29: 3053-61.

[331] Leucht P, Kim JB, Amasha R, James AW, Girod S, Helms JA. Embryonic origin and Hox status determine progenitor cell fate during adult bone regeneration. Development 2008; 135: 2845-54.

[332] Rehman J, Traktuev D, Li J, *et al.* Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004; 109: 1292-8.

[333] De Francesco F, Tirino V, Desiderio V, *et al.* Human CD34/CD90 ASCs are capable of growing as sphere clusters, producing high levels of VEGF and forming capillaries. PLoS One 2009; 4: e6537.

[334] Mesimaki K, Lindroos B, Tornwall J, *et al.* Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg 2009; 38: 201-9.

[335] Thesleff T, Lehtimaki K, Niskakangas T, *et al.* Cranioplasty with adipose-derived stem cells and biomaterial. A novel method for cranial reconstruction. Neurosurgery 2011.

[336] Trebol LJ, Georgiev HT, Garcia-Arranz M, Garcia-Olmo D. Stem cell therapy for digestive tract diseases: current state and future perspectives. Stem Cells Dev 2011, e-pub.

[337] Duijvestein M, Vos AC, Roelofs H, *et al.* Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut 2010; 59: 1662-9.

[338] Ko IK, Kim BG, Awadallah A, *et al.* Targeting improves MSC treatment of inflammatory bowel disease. Mol Ther 2010; 18: 1365-72.

[339] Allison M. Genzyme backs Osiris, despite Prochymal flop. Nat Biotechnol 2009; 27: 966-7.

[340] Gonzalez-Rey E, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 2009; 58: 929-39.

[341] Garcia-Olmo D, Garcia-Arranz M, Garcia LG , *et al.* Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. Int J Colorectal Dis 2003; 18: 451-4.

[342] Garcia-Olmo D, Herreros D, Pascual M, *et al.* Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion. Int J Colorectal Dis 2009; 24: 27-30.

[343] Garcia-Olmo D, Garcia-Arranz M, Herreros D. Expanded adipose-derived stem cells for the treatment of complex perianal fistula including Crohn's disease. Expert Opin Biol Ther 2008; 8: 1417-23.

[344] Taxonera C, Schwartz DA, Garcia-Olmo D. Emerging treatments for complex perianal fistula in Crohn's disease. World J Gastroenterol 2009; 15: 4263-72.

[345] Ciccocioppo R, Bernardo ME, Sgarella A, *et al.* Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut 2011; 60: 788-98.

[346] Chamberlain J, Yamagami T, Colletti E, *et al.* Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. Hepatology 2007; 46: 1935-45.

[347] Wang J, Bian C, Liao L, *et al.* Inhibition of hepatic stellate cells proliferation by mesenchymal stem cells and the possible mechanisms. Hepatol Res 2009; 39: 1219-28.

[348] Li TZ, Kim JH, Cho HH, *et al.* Therapeutic potential of bone-marrow-derived mesenchymal stem cells differentiated with growth-factor-free coculture method in liver-injured rats. Tissue Eng Part A 2010; 16: 2649-59.

[349] Mohamadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, *et al.* Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. Arch Iran Med 2007; 10: 459-66.

[350] Lyra AC, Soares MB, da Silva LF, *et al.* Infusion of autologous bone marrow mononuclear cells through hepatic artery results in a short-term improvement of liver function in patients with chronic liver disease: a pilot randomized controlled study. Eur J Gastroenterol Hepatol 2010; 22: 33-42.

[351] Kharaziha P, Hellstrom PM, Noorinayer B, *et al.* Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. Eur J Gastroenterol Hepatol 2009; 21: 1199-205.

[352] Ezquer FE, Ezquer ME, Parrau DB, Carpio D, Yanez AJ, Conget PA. Systemic administration of multipotent mesenchymal stromal cells reverts hyperglycemia and prevents nephropathy in type 1 diabetic mice. Biol Blood Marrow Transplant 2008; 14: 631-40.

[353] Urban VS, Kiss J, Kovacs J, *et al.* Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. Stem Cells 2008; 26: 244-53.

[354] Rackham CL, Chagastelles PC, Nardi NB, Hauge-Evans AC, Jones PM, King AJ. Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. Diabetologia 2011; 54: 1127-35.

[355] Bell GI, Broughton HC, Levac KD, Allan DA, Xenocostas A, Hess DA. Transplanted human bone marrow progenitor subtypes stimulate endogenous islet regeneration and revascularization. Stem Cells Dev 2011.

[356] Xie QP, Huang H, Xu B, *et al.* Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation *in vitro*. Differentiation 2009; 77: 483-91.

[357] Park KS, Kim YS, Kim JH, *et al.* Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation. Transplantation 2010; 89: 509-17.

[358] Trivedi HL, Vanikar AV, Thakker U, *et al.* Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin. Transplant Proc 2008; 40: 1135-9.

[359] Vanikar AV, Dave SD, Thakkar UG, Trivedi HL. Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: a novel therapy for insulin-dependent diabetes mellitus. Stem Cells Int 2010; 2010: 582382.

[360] Sanz-Ruiz R, Gutierrez IE, Arranz AV, Fernandez Santos ME, Fernandez PL, Fernandez-Aviles F. Phases I-III Clinical Trials Using Adult Stem Cells. Stem Cells Int 2010; 2010: 579142.

[361] Choi YH, Kurtz A, Stamm C. Mesenchymal stem cells for cardiac cell therapy. Hum Gene Ther 2011; 22: 3-17.

[362] Chin SP, Poey AC, Wong CY, *et al.* Intramyocardial and intracoronary autologous bone marrow-derived mesenchymal stromal cell treatment in chronic severe dilated cardiomyopathy. Cytotherapy 2011, e-pub..

[363] Katritsis DG, Sotiropoulou P, Giazitzoglou E, Karvouni E, Papamichail M. Electrophysiological effects of intracoronary transplantation of autologous mesenchymal and endothelial progenitor cells. Europace 2007; 9: 167-71.

[364] Guo J, Lin GS, Bao CY, Hu ZM, Hu MY. Anti-inflammation role for mesenchymal stem cells transplantation in myocardial infarction. Inflammation 2007; 30: 97-104.

[365] Peran M, Marchal JA, Lopez E, *et al.* Human cardiac tissue induces transdifferentiation of adult stem cells towards cardiomyocytes. Cytotherapy 2010; 12: 332-7.

[366] Chen SL, Fang WW, Ye F, *et al.* Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 2004; 94: 92-5.

[367] Chen S, Liu Z, Tian N, *et al.* Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery. J Invasive Cardiol 2006; 18: 552-6.

[368] Yang Z, Zhang F, Ma W, *et al.* A novel approach to transplanting bone marrow stem cells to repair human myocardial infarction: delivery *via* a noninfarct-relative artery. Cardiovasc Ther 2010; 28: 380-5.

[369] Hare JM, Traverse JH, Henry TD, *et al.* A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 2009; 54: 2277-86.

[370] Lasala GP, Minguell JJ. Vascular disease and stem cell therapies. Br Med Bull 2011; 98: 187-97.

[371] Prochazka V, Gumulec J, Jaluvka F, *et al.* Cell therapy, a new standard in management of chronic critical limb ischemia and foot ulcer. Cell Transplant 2010; 19: 1413-24.

[372] Sadan O, Melamed E, Offen D. Bone-marrow-derived mesenchymal stem cell therapy for neurodegenerative diseases. Expert Opin Biol Ther 2009; 9: 1487-97.

[373] Joyce N, Annett G, Wirthlin L, Olson S, Bauer G, Nolta JA. Mesenchymal stem cells for the treatment of neurodegenerative disease. Regen Med 2010; 5: 933-46.

[374] Wright KT, El Masri W., Osman A, Chowdhury J, Johnson WE. Bone Marrow for the Treatment of Spinal Cord Injury: Mechanisms and Clinical Application. Stem Cells 2011; 29: 169-78.

[375] Park HJ, Lee PH, Bang OY, Lee G, Ahn YH. Mesenchymal stem cells therapy exerts neuroprotection in a progressive animal model of Parkinson's disease. J Neurochem 2008; 107: 141-51.

[376] Choi MR, Kim HY, Park JY, *et al.* Selection of optimal passage of bone marrow-derived mesenchymal stem cells for stem cell therapy in patients with amyotrophic lateral sclerosis. Neurosci Lett 2010; 472: 94-8.

[377] Lee PH, Park HJ. Bone marrow-derived mesenchymal stem cell therapy as a candidate disease-modifying strategy in Parkinson's disease and multiple system atrophy. J Clin Neurol 2009; 5: 1-10.

[378] Bianco P, Gehron RP. Marrow stromal stem cells. J Clin Invest 2000; 105: 1663-8.

[379] Zipori D. The stem state: plasticity is essential, whereas self-renewal and hierarchy are optional. Stem Cells 2005; 23: 719-26.

[380] Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall ME. Playing with bone and fat. J Cell Biochem 2006; 98: 251-66.

[381] Krinner A, Hoffmann M, Loeffler M, Drasdo D, Galle J. Individual fates of mesenchymal stem cells *in vitro*. BMC Syst Biol 2010; 4: 73.

[382] Charbord P, Livne E, Gross G, *et al.* Human bone marrow mesenchymal stem cells: a systematic reappraisal *via* the genostem experience. Stem Cell Rev 2011; 7: 32-42.

[383] Pevsner-Fischer M, Levin S, Zipori D. The origins of mesenchymal stromal cell heterogeneity. Stem Cell Rev 2011; 7: 560-8.

[384] Ponce ML, Koelling S, Kluever A, *et al.* Coexpression of osteogenic and adipogenic differentiation markers in selected subpopulations of primary human mesenchymal progenitor cells. J Cell Biochem 2008; 104: 1342-55.

[385] Song L, Tuan RS. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. FASEB J 2004; 18: 980-2.

[386] Oreffo RO, Cooper C, Mason C, Clements M. Mesenchymal stem cells: lineage, plasticity, and skeletal therapeutic potential. Stem Cell Rev 2005; 1: 169-78.

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[387] Custer RP, Ahfeldt FE. Studies on the structure and function of bone marrow. II. Variations in cellularity in various bones with advancing years of life and their relative response to stimuli. J Lab Clin Med 1932; 17: 960-2.

[388] Vost A. Osteoporosis: a necropsy study of vertebrae and iliac crests. Am J Pathol 1963; 43: 143-51.

[389] Hartsock RJ, Smith EB, Petty CS. Normal variations with aging of the amount of hematopoietic tissue in bone marrow from the anterior iliac crest. A study made from 177 cases of sudden death examined by necropsy. Am J Clin Pathol 1965; 43: 326-31.

[390] Rubin H. Promise and problems in relating cellular senescence *in vitro* to aging *in vivo*. Arch Gerontol Geriatr 2002; 34: 275-86.

[391] Mareschi K, Ferrero I, Rustichelli D, *et al.* Expansion of mesenchymal stem cells isolated from pediatric and adult donor bone marrow. J Cell Biochem 2006; 97: 744-54.

[392] Wilson A, Shehadeh LA, Yu H, Webster KA. Age-related molecular genetic changes of murine bone marrow mesenchymal stem cells. BMC Genomics 2010; 11: 229.

[393] Makhluf HA, Mueller SM, Mizuno S, Glowacki J. Age-related decline in osteoprotegerin expression by human bone marrow cells cultured in three-dimensional collagen sponges. Biochem.Biophys.Res.Commun. 2000; 268: 669-672.

[394] Mueller SM, Glowacki J. Age-related decline in the osteogenic potential of human bone marrow cells cultured in three-dimensional collagen sponges. J Cell Biochem 2001; 82: 583-90.