TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (7): Liver transplant

Rationale for the potential use of mesenchymal stromal cells in liver transplantation

Morgan Vandermeulen, Céline Grégoire, Alexandra Briquet, Chantal Lechanteur, Yves Beguin, Olivier Detry

Morgan Vandermeulen, Olivier Detry, Department of Abdominal Surgery and Transplantation, CHU Liege, University of Liege, B4000 Liege, Wallonia, Belgium
Céline Grégoire, Yves Beguin, Department of Hematology, CHU Liege, University of Liege, B4000 Liege, Wallonia, Belgium
Alexandra Briquet, Chantal Lechanteur, Yves Beguin, Laboratory of Cell and Gene Therapy, CHU Liege, University of Liege, Sart Tilman B35, B4000 Liege, Wallonia, Belgium
Correspondence to: Olivier Detry, Professor, Department of Abdominal Surgery and Transplantation, CHU Liege, University of Liege, Sart Tilman B35, B4000 Liege, Wallonia, Belgium. olivier.detry@transplantation.be
Telephone: +32-43-667645 Fax: +32-43-667069

Abstract

Mesenchymal stromal cells (MSCs) are multipotent and self-renewing cells that reside essentially in the bone marrow as a non-hematopoietic cell population, but may also be isolated from the connective tissues of most organs. MSCs represent a heterogeneous population of adult, fibroblast-like cells characterized by their ability to differentiate into tissues of mesodermal lineages including adipocytes, chondrocytes and osteocytes. For several years now, MSCs have been evaluated for their *in vivo* and *in vitro* immunomodulatory and 'tissue reconstruction' properties, which could make them interesting in various clinical settings, and particularly in organ transplantation. This paper aims to review current knowledge on the properties of MSCs and their use in pre-clinical and clinical studies in solid organ transplantation, and particularly in the field of liver transplantation. The first available clinical data seem to show that MSCs are safe to use, at least in the medium-term, but more time is needed to evaluate the potential adverse effects of long-term use. Many issues must be resolved on the correct use of MSCs. Intensive *in vitro* and pre-clinical research are the keys to a better understanding of the way that MSCs act, and to eventually lead to clinical success.

Core tip: For several years now, mesenchymal stromal cells (MSC) have been evaluated for their *in vivo* and *in vitro* immunomodulatory and 'tissue reconstruction' properties which could make them interesting in various clinical settings, and particularly in organ transplantation. This paper aims to review current knowledge on the properties of MSCs and their use in pre-clinical and clinical studies, and particularly in the field of liver transplantation.

Vandermeulen M, Grégoire C, Briquet A, Lechanteur C, Beguin Y, Detry O. Rationale for the potential use of mesenchymal stromal cells in liver transplantation. *World J Gastroenterol* 2014; 20(44): 16418-16432 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i44/16418.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i44.16418

INTRODUCTION

Mesenchymal stromal cells (MSCs) are multipotent and self-renewing cells that reside essentially in the bone marrow as a non-hematopoietic cell population. MSCs represent a heterogeneous population of adult, fibroblast-like cells characterized by their ability to differentiate into tissues of mesodermal lineages including adipocytes, chondrocytes and osteocytes. In addition to the bone marrow, MSCs have been isolated from various other tissues such as adipose tissue[1], skin[2], heart and spleen[3], placenta[4], umbilical cord blood[5] as well as lung and liver[6,7], and it appears that MSCs reside in the connective tissues of most organs[8].

No specific marker for MSCs has yet been found. Presently, MSCs are identified using a number of features defined by the International Society for Cellular Therapy which states three minimal criteria[9]: (1) adhesion to plastic in standard culture conditions; (2) expression of CD105, CD73 and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and Human Leukocyte Antigen (HLA)-DR surface molecules; and (3) *in vitro* differentiation into osteoblasts, adipocytes and chondroblasts.

For several years now, MSCs have been evaluated for their *in vivo* and *in vitro* immunomodulatory and "tissue reconstruction" properties that could make them interesting in various clinical settings such as organ transplantation. This paper aims to review current knowledge on the properties of MSCs and their use in pre-clinical and clinical studies in solid organ transplantation, and particularly in the field of liver transplantation.

IMMUNOMODULATORY EFFECTS OF MSCS

A large number of *in vitro* and *in vivo* studies have documented the anti-inflammatory and immunoregulatory properties of MSCs on both the adaptive and innate immune system. However, there is strong evidence that MSCs are not constitutively immunosuppressive, they have to be "activated" or primed by local inflammatory conditions. Tumor necrosis factor (TNF)-α, interleukin (IL)-1β and interferon (IFN)-γ are the key cytokines to allow MSC immunomodulation by regulating their immunophenotype[10,11]. The high dependence on environment settings could also explain conflicting data in some *in vitro* and *in vivo* studies. These settings must be further studied and considered in clinical trials.

MSC immunogenicity
Both human MSCs (hMSCs) and murine MSCs (mMSCs) show low immunogenicity and do not lead to alloreactive T lymphocyte-mediated immune response *in vitro*. Indeed, under normal conditions, MSC membranes express low levels of human leukocyte antigen (HLA) class I molecules and do not express HLA class Ⅱ (major histocompatibility complex (MHC)- Ⅱ) nor co-stimulato-

ry molecules[12,13]. MSCs were thus considered as immune privileged cells. However, more recent data with mMSCs has suggested that MHC-I on MSCs could present antigen to CD8+ T cells[14]. In addition, a narrow window of IFN-γ could induce MSCs to upregulate MHC-I and MHC- Ⅱ and thus, induces an "antigen presenting cell-like" function. This finding has been observed with both mMSCs and hMSCs[10,15-17]. Furthermore, it has been demonstrated in an animal model of bone marrow[18] and skin transplantation[19] that donor-derived MSCs could be immunogenic and could promote graft rejection.

MSC interaction with immune cells
It is important to highlight that, in some experimental conditions, effects of mMSCs and hMSCs have been evaluated on murine immune cells. Results are not always transposable to human clinical conditions, especially as it is well known that tolerance is more easily achieved in animal models than in humans.

It has been demonstrated *in vitro* and *in vivo*, that MSCs may exert their immunomodulatory effects by acting on many types of immune cells including T cells, B cells and natural killer (NK) cells. The ability of MSCs to inhibit T cell proliferation has been shown in various experimental settings both with mMSCs and hMSCs. *In vitro*, hMSCs highly inhibit proliferation and cytokine production[20] as well as the development of human cytotoxic CD8+ T cells in mixed-lymphocyte reactions (MLRs)[21,22]. Moreover, it has been observed that MSCs promote human T cell anergy and inhibit alloreactive T cells through a TH2 pathway[23]. Nevertheless, it appears that the effect of MSCs on T cells is dependent on the dose used. While a high MSC/T cell ratio exert strong inhibitory effects, low MSC/T cell ratios enhance T cell proliferation[24].

MSC-induced T-regulatory (T-reg) cell recruitment and generation probably play an important role in MSC-mediated immunomodulatory effects. This has been observed both *in vitro*[25,26] and *in vivo*[27,28] both on murine and human immune cells. Additionally, previous studies have shown that T-reg induced production requires cell contact and some MSC released factors such as prostaglandin (PG)-E2 and tumor growth factor (TGF)-β1[29] or HLA-G[30,31]. It has been suggested that this effect could also be partially mediated by an interaction between MSC chemokine (c-c motif) ligand 1 (CCL1) and its receptor on T cells, chemokine (c-c motif) receptor 8 (CCR8)[23]. More recently, it has been demonstrated that mMSCs could promote T-reg expansion by their effects on immature dendritic cells[32].

Published results on the effects of hMSCs on B cells and NK cells are contradictory. Some studies have demonstrated that MSCs could inhibit the proliferation and immunoglobulin secretion of B cells[33-35] while others have found no effect of MSCs on human B cell proliferation[11,21]. Some researchers have even found that MSCs could stimulate human B cell proliferation and antibody secretion[36,37]. MSCs have shown an ability to inhibit the proliferation of IL-2 or IL-15 stimulated human NK

cells[38,39] and their IFN-γ production[38]. The effects of MSCs on the cytotoxic activity of NK cells are even more controverted. While some studies failed to find such an effect[40] (especially in freshly isolated NK cells[41]), others have demonstrated that MSCs could inhibit NK-cell cytotoxicity[30,39]. As MSCs express HLA-1 antigens, even at a low level, it appears that they may be vulnerable to activated NK-cell lysis[42].

Many studies have shown that MSCs can prevent the differentiation, maturation and functions of antigen-presenting cells (APCs), such as human or murine dendritic cells (DC)[17,43,44], and thus indirectly modulate T and B cell functions. In addition, it was shown that mMSCs may induce murine mature DC into a Jagged-2-dependent regulatory DC population[45]. MSCs may also exert effects on innate immune cells, for example through increased IL-10 secretion by macrophages in mice[46].

Mechanisms

The mechanisms of immunosuppression by MSCs remain unclear. Whereas MSCs exert their effect by direct cell contact *via* the expression of adhesion molecules, it has also been shown that the immunomodulatory and anti-inflammatory properties of MSCs mainly involve the production of secreted soluble factors. It has been observed that MSCs are still immunosuppressive without cell contact[22]. It should be noted that the mechanisms of MSC-mediated immunosuppression seems to vary from one species to another[47].

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyses the degradation of tryptophan. The resulting depletion of tryptophan and the accumulation of its metabolites have shown strong inhibitory properties on immune cells, including human T cells[48], activated B cells[11] and NK cells[39]. MSCs do not constitutively express IDO, but IDO can be upregulated under inflammatory conditions, for example after exposure to IFN-γ, TNF-α and IL-1[47,48]. IDO could play an important role regarding transplantation given that it has been shown to partially inhibit allo-responses of T cells *in vitro*, and to enhance tolerance towards the graft and allogeneic T cell transfer *in vivo*[49,50]. IDO seems to be predominant in human MSC-mediated immunomodulatory properties[47]. However IDO does not seem to be the only mechanism implicated as in some conditions where MSCs do not express IDO they keep their immunomodulatory properties[51]. A high concentration of nitric oxide (NO) is known to inhibit the immune response in both *in vitro* and *in vivo* studies. It has been shown to inhibit the proliferation of T cells in murine models. NO is synthesized by the inducible NO synthase (iNOS) that is induced in murine MSCs by interaction with CD4+ or CD8+ lymphocytes in inflammatory conditions involving IFN-γ and TNF-α or IL-1[52,53]. As in the case of IDO for human MSCs, iNOS appears to play a major role in murine MSC-mediated immunomodulation[47,52]. Both tryptophan depletion and NO are expected to have an exclusively local action[54,55].

The HLA-G protein is a non-classical human MHC-I molecule. Initially found in trophoblasts, where it plays a crucial role in maternal-fetal tolerance[56], HLA-G has recently been involved in immunomodulation by MSCs[57]. HLA-G has shown tolerogenic properties *inter alia* due to its interactions with inhibitory receptors on dendritic cells, NK, and T cells. Selmani *et al*[30] have demonstrated that hMSCs, by secreting the soluble isoform HLA-G5, are capable of inhibiting human allo-activated T lymphocytes, NK-cell cytolysis and IFN-gamma secretion, and of promoting the expansion of CD4+CD25highFoxP3+ regulatory T cells. Likewise, HLA-G can promote CD3+CD4low and CD3+ CD8low immunosuppressive T cells. It seems that HLA-G expression is IL-10-dependent and needs close cell contact with alloreactive T cells[30]. It has been suggested that co-injection of HLA-G and MSCs could be used to prevent rejection in organ transplantation.

Another candidate mechanism involves the role of PGE2 (Prostaglandin E2) secreted by MSCs. It appears that MSC-derived PGE2 is involved in MSC-mediated immunomodulation by acting on murine and human T cells (in both TH1 and TH2 responses), NK cells and macrophages[46,58]. Prostaglandins have a short half-life. This suggests that they play their role using a paracrine or autocrine action mechanism. Furthermore, it has been observed in human MSCs that IDO and PGE2 have a synergistic inhibitory effect on T cell proliferation, and on the proliferation and cytotoxicity of NK cells[39,59]. However, other studies suggest that PGE2 could in fact have an immunostimulatory role by facilitating TH1 cell differentiation and TH17 cell expansion[60].

IL-10 plays an important role in MSC-mediated immunosuppression through the induction of IL-10 production in APCs[61]. Nevertheless, no direct secretion of IL-10 by MSCs has yet been proven.

Blocking each of these factors alone does not restore immune cell function and proliferation, indicating that multiple factors are involved.

Other factors are also secreted: TGF-β and Hepatocyte growth factor (HGF)[20] (inhibition T-lymphocyte proliferation), IL-1 receptor Antagonist[62] (anti-inflammatory), Peptide LL-37[63] (anti-inflammatory and anti-bacterial), Matrix Metalloproteinase (MMP) 3, MMP9[64] (acting on neoangiogenesis), angiopoietin-1[65] (acting on protein permeability). TNF-α and insulin-like growth factor-binding proteins[51] also seem to be implicated.

On the other hand, MSCs also have the ability to secrete pro-inflammatory chemokines and cytokines, such as monocyte chemo-attractant protein 1 (MCP-1 or CCL2)[66], IL-6, IL-8, soluble ICAM-1, Interferon gamma-induced protein 10 (IP-10 or CXCL10) and MCP-2 (or CCL8). The secretion of these factors is dependent on inflammatory conditions and could enhance immune response *via* immune cell attraction[67]. Therefore, MSCs appear to have a dual immunomodulatory capacity depending on the above-identified secreted factors.

The mechanisms involved in the immunomodula-

tory capacity of MSCs are complex and remain largely unknown. Their properties seem to be highly dependent on many parameters in which local immunologic conditions seem to play a crucial role. Finally, it is important to know that there is currently no single standard method to isolate MSCs. It is thus conceivable that changes in the culture medium used to increase and select MSC population may influence their properties.

TISSUE REPAIR/"ORGAN RECONSTRUCTION" EFFECT

In addition to their ability to differentiate into cells of the mesenchymal lineage, it has been demonstrated that MSCs can also differentiate *in vitro* into other cells such as neurons[68], cardiomyocytes[69], tubular epithelial cells in kidneys and hepatocytes[70-72]. They are also capable of differentiating and engrafting into many tissues, especially if an inflammatory signal is present[73]. These data have motivated further research in the field of MSCs as potential "tissue repairers". Cultured MSCs have shown strong evidence of "tissue repair" properties in response to tissue injury or disease in many animal models with myocardial infarction[74], kidney disease[75,76], lung injury or some neurological disorders[64]. In clinical trials, MSCs have been used successfully to treat bone and cartilage diseases[77] (*e.g.*, osteogenesis imperfecta), as well as acute and chronic myocardial infarction[78-80].

MSCs have shown the ability to home in on injured tissue after intravenous infusion. It has been demonstrated that MSCs can express several chemokine receptors such as CCR1, CCR7, CXCR4, CXCR6, CX3CR1[81], CCR4, CCR10, CXCR5[82], c-Kit, c-Met[83], VEGF receptors[84] and PDGF receptors[85]. This variety of receptors and the chemotactic migration they have shown in response to the stimulating chemokines and cytokines could partially explain their ability to migrate to sites of inflammation. This hypothesis assumes that the injured tissue also expresses specific receptors facilitating the adhesion and migration of MSCs. However, the exact mechanism of homing in on injured tissue remains largely unknown.

Nevertheless, many studies have observed that MSCs are significantly trapped in the lung after intravenous infusion[86,87]. Despite their ability to migrate to inflammation sites and to differentiate into many tissues, MSCs exhibit very low and transient levels of engraftment *in vivo*[86,88]. For example, in a mouse model of acute myocardial infarction, a significant improvement of myocardial function was observed after human MSC injection, while no donor cell could be detected 3 wk after infusion. In a rat model, no MSC could be found in the liver within 7 d after injection of syngeneic rat MSCs in recipient livers through the portal vein[89]. Contradictorily, in a clinical trial treating myocardial infarction with intracoronary injection of MSCs, the MSCs were still viable 3 mo after transplantation[90]. In another study, MSCs were detected in various tissues of baboons 19 mo after intravenous injection[88].

In fact, it is thought that MSCs are likely to act through the secretion of soluble factors and change of the tissue microenvironment with paracrine interactions, rather than through their transdifferentiation capacity[91,92]. However, current *in vivo* data are not sufficient to define the exact mechanism. It has been demonstrated that MSCs could facilitate tissue repair by stimulating angiogenesis[93] and inhibiting apoptosis, as well as fibrosis, in the site of injury[94].

Furthermore, there is much evidence supporting the protective effect of MSCs in acute kidney injury models[95]. It appears that MSCs could increase the proliferation of tubular cells and reduce apoptosis[96,97]. There is a lack of data on the treatment of liver injury with MSCs, but their properties and regenerative potential mentioned above have encouraged researchers and clinicians to investigate further in this field. They could play a therapeutic role in the replacement of diseased hepatocytes, and the stimulation of their regeneration through the action of trophic molecules[98].

In a study on acute liver injury, rats were successfully treated with MSC infusion, with a decrease of biochemical markers of liver injury and an improved survival rate. Hepatocyte replication was enhanced while apoptosis decreased by 90%[98]. Similarly, it has been demonstrated that MSCs are efficient in treating fulminant hepatic failure in rats[99]. Otherwise, it has been suggested that MSCs could only be efficient in a therapeutic window, indicating that higher doses could paradoxically be inefficient or even induce liver fibrosis[98].

Although it is hoped that MSCs could potentially be an alternative to liver transplantation in end-stage liver disease, or a potential temporary solution to maintaining liver conditions of patients waiting for a graft, MSCs have been tried in only a small number of clinical trials to treat cirrhosis.

In a phase I - II trial, 8 patients with end-stage liver cirrhosis were treated with the infusion of autologous MSCs *via* a peripheral or portal vein. The treatment was well tolerated, with no significant adverse effects and the liver function was significantly improved[100]. A randomized placebo-controlled trial using MSCs to treat decompensated cirrhosis has recently been published[101]. Out of 27 patients, 15 received autologous bone marrow MSCs *via* a peripheral vein and 12 received a placebo. The results were evaluated using the Model for End-Stage Liver Disease (MELD) score, Child-Pugh score, liver function tests and liver volume. In this study, there was no beneficial effect of MSC infusion in cirrhotic patients. It is clear that other studies with larger cohorts are necessary to clarify the therapeutic potential of MSCs in cirrhosis.

ANTI-OXIDATIVE EFFECT/TREATMENT OF ISCHEMIA REPERFUSION INJURY

Ischemia reperfusion injury (IRI) is caused by the blood supply returning into a tissue after an ischemic period.

This sudden reperfusion and oxygenation paradoxically impairs the endothelium with a dilatation in arterioles, increased fluid filtration and plasma protein extravasation from post-capillary venules, as well as an increased production of oxygen radicals and a reduction of nitric oxide generation. This imbalance leads to the release of inflammatory mediators (*e.g.*, TNF, platelet activating factor) and the expression of adhesion molecules that cause leukocyte adhesion to the endothelium[102]. This results in the stimulation of both innate and adaptive immune responses with an accumulation of immune cells, followed by organ damage. The release of danger-associated molecular patterns (DAMPs) and the complement system are also implicated[103].

Solid organ transplantation is impacted by IRI, which contributes to acute graft rejection, delayed graft function and enhanced immunogenicity. IRI represents a major concern in liver transplantation, and use of MSCs in IRI has been studied for solid organ transplantation in animal models and in clinical trials.

MSCs seem to be recruited by hypoxic and injured tissues that express adhesion molecules and a SDF-1 gradient stimulating CXCR4 and CXCR7 on these cells[104]. Furthermore, it has been demonstrated that MSCs can transmigrate through TNF-alpha activated endothelium to join the inflamed tissue[105]. Lately, Pan *et al*[106] found that the inactivation of the MEK/ERK signalling pathway by MSCs plays a major role in the improvement of hepatic IRI in rats.

Prevention and treatment of liver IRI in animal models

MSCs have shown therapeutic effects for the treatment of IRI in the kidney, heart and lung in a significant number of studies[107]. Only a few studies have been published for IRI in the liver, and the exact role of MSCs has not yet been defined.

Jin *et al*[108] recently evaluated the effect of allogeneic bone marrow (BM)-derived MSCs to attenuate IRI in rats during the first 24 h after liver reperfusion. In their model partial ischemia was obtained by vascular clamping during 60 min. BM-MSCs were injected through the portal vein. Injury severity, oxidative stress response and apoptosis of the liver was regularly evaluated during the first 24 h and compared to a sham-transplanted control group. The conclusion of this study is that allogeneic BM-MSCs partially protect the liver from IRI when injected *via* the portal vein due to their ability to suppress oxidative stress and to inhibit apoptosis. Another related model using adipose-derived MSC injections *via* a peripheral vein in mice also showed a significant protective effect against liver IRI[109].

In addition to liver IRI, research has also focused on the potential beneficial effect of MSCs in partial liver transplantation. In a recent study 50% reduced-size liver transplantations in rats were used to examine whether MSC-conditioned medium (MSC-CM) could protect hepatocytes and sinusoidal endothelial cells (SEC) and enhance their regeneration[110]. MSC-CM was injected in rats *via* a peripheral vein directly after orthotopic partial liver transplantation. Compared with the control group, the MSC-CM group showed a significantly lower release of liver injury biomarkers and a clear survival benefit. More proliferating hepatocytes and SECs, and less apoptosis were observed. Many inflammatory cytokine levels and the infiltration by neutrophils and Kupffer cell activation were decreased. VEGF and MMP-9 expression was increased in the graft. All these facts suggest that MSC-CM could have potential in prevention of liver injury, and to enhance its regeneration in partial liver transplant. Kanazawa *et al*[111] also found in a model of IRI with major hepatectomy that MSCs protected the liver from IRI and that liver regeneration was enhanced.

However, it has been demonstrated in a liver IRI model that intravenously injected MSCs are short-lived, that viable MSCs do not go beyond the lungs, and that they remain in the circulation for a very limited period[112]. It has thus been suggested that other cells should be implicated to mediate the powerful immunomodulatory and regenerative properties of MSCs on target organs.

POTENTIAL USE OF MSCS IN LIVER TRANSPLANTATION

Liver transplantation represents the unavoidable treatment of end-stage liver diseases. Despite satisfactory long-term results, transplantation success mostly relies on immunotolerance, *via* acceptable graft-host immune matches and immunosuppressive measures. The latter unfortunately exposes the patient to the classical consequences of a down-regulated immune system, such as opportunistic infections and the typical outbreak of neoplasms. Due to their immunomodulatory properties, MSCs could prove highly effective in obtaining sufficient immunotolerance to reach even higher success rates while avoiding excessive immunosuppression, and thus severe and life-threatening side effects.

MSCs as immunomodulation therapy in transplantation

MSCs for graft-*vs*-host disease after hematopoietic cell transplantation: A clinical success? Graft-*vs*-host disease (GVHD) is a major complication frequently observed after hematopoietic cell transplantation (HCT), resulting from the attack of recipient organs by donor lymphocytes. MSCs might play a role in the treatment of GVHD through their immunomodulatory effects rather than their regenerative properties. Although pre-clinical studies for the prevention or treatment of GVHD by MSCs gave rise to conflicting results, MSCs have shown a clear efficacy in clinical trials, especially in steroid-resistant GVHD[113]. In a phase II study, 68% of patients with acute steroid-resistant GVHD showed a complete response to MSC infusion with a significant decrease in mortality[114]. A series of other studies have shown similar results with varying degrees of GVHD, suggesting that MSCs have a serious potential future in GVHD management[115-117].

MSCs in solid-organ transplantation

Animal models: MSC infusion has shown the ability to prolong graft survival in heart[118-120], skin[121] and kidney[122-124] animal transplantation models. However, one group found no effect of MSCs alone on heart allograft survival in a mouse model[125], and another group found that MSCs infused after kidney transplantation could cause premature graft dysfunction[122].

Only a few studies have been published in liver transplantation models. In one such study, it was demonstrated that adipose-derived MSCs significantly decreased acute rejection after orthotopic liver transplantation in rats[126], based on serum rejection markers and on hepatocyte apoptosis. Serum levels of IL-2 were reduced and those of IL-10 were increased. In this model, MSC were infused intravenously 7 d before and 3 d after liver transplantation as well as during the operation *via* the portal vein. MSCs also played a role in a discordant liver xenotransplant model by alleviating acute rejection[127].

Another group studied the ability of BM-MSC infusion to inhibit acute graft rejection after allogeneic liver transplantation in rats[128]. MSCs were derived from the recipient, the liver donor or a third party, and infused intravenously at the time of surgery as well as once daily for 3 d thereafter. MSC-treated recipients survived significantly longer compared with the control group. Furthermore, there was no significant difference between the 3 groups receiving MSCs from various origins. Histological analysis showed severe acute graft rejection at day 7 in rats without MSC infusion, while acute graft rejection was significantly decreased in the other groups. These observations were associated with a marked increase in the number of T-reg cells in recipients receiving MSCs. This suggests an important role of T-reg cells in MSC-mediated immunosuppression.

Available data in humans (kidney transplantation)

Results of a phase I clinical trial studying the treatment of allograft rejection after kidney transplantation by autologous BM-MSCs, have recently been published[129]. The MSC-based treatment was well-tolerated and no related serious adverse effects were reported. Two MSC infusions were performed after a biopsy-proven rejection or interstitial fibrosis/tubular atrophy (IF/TA). In this study, MSCs showed their ability to reduce IF/TA. In addition, a donor-specific down-regulation of the peripheral blood mononuclear cell proliferation was shown. However, a potentially increased susceptibility to opportunistic infections was observed, with the development of viral infections in 3 out of 6 MSC-treated patients.

In a randomized controlled trial in living donor kidney transplantation, Tan *et al*[130] demonstrated that, in comparison with antibody induction therapy, induction by autologous MSCs significantly correlated with fewer acute rejections, a lower risk of opportunistic infections and a better renal function at 1 mo. Furthermore, fewer adverse effects were seen in both autologous MSC groups compared to the control group. This study was conducted on 156 patients recruited from February 2008 to May 2009 and divided into 3 groups (group 1 and 2 received MSCs at kidney reperfusion and two weeks later, plus a standard dose or low dose of calcineurin inhibitors (CNIs), respectively. The control group received anti-IL-2 receptor antibody plus standard-dose CNIs.

In a pilot study, Perico *et al*[131] injected autologous BM-MSC in 2 living-related kidney transplant recipients at day 7 post-transplant, after induction therapy with basiliximab/low-dose thymoglobulin. The peripheral blood showed a progressive increase of the T-reg population and a strong inhibition of memory/effector CD8 T cell function/expansion, promoting a long-term tolerogenic environment compared with the control group. However, a few days after MSC infusion transient renal dysfunction was observed. A biopsy excluded graft rejection but revealed a focal inflammatory infiltrate with neutrophil and MSC recruitment as well as a complement-C3 deposition.

The same group also investigated pre-transplant infusion of autologous BM-MSCs in 2 living-related kidney transplant recipients[132]. No renal dysfunction was observed while MSC immunomodulatory properties were preserved. In addition, it was observed that the avoidance of basiliximab in induction therapy did not facilitate further T-reg expansion.

In another recent pilot study, six patients transplanted with living-donor related kidneys received 2 donor-derived BM-MSC infusions (the first at the time of transplantation, the second one month later) in combination with sparing doses of tacrolimus[133]. Six other patients were used as a control group and received standard doses of tacrolimus and no MSCs. The MSC-treated group had stable renal function 12 mo post-transplant despite reduced tacrolimus compared with the control group. No acute rejection occurred, except for one in the control group. Significantly increased B cell levels were observed in the MSC-treated group 3 mo after transplantation. No toxic side effects were associated with MSC infusion.

Ongoing clinical trials in liver transplantation

MSC Liege study: Taking advantage of our expertise and experience concerning the use of MSCs in the HCT context[115], and using an already functioning good manufacturing practice (GMP)-compliant laboratory able to produce clinical-grade MSCs, we initiated a first trial in 2011 exploring the safety and tolerability of third-party MSC infusions after kidney or liver transplantation in a prospective phase Ⅰ-Ⅱ study (NCT01429038).

In this study, after successful transplantation, 10 liver and 10 kidney transplant recipients under standard immunosuppressive treatment (tacrolimus, mycophenolate mofetil (MMF) and steroids) receive an intravenous infusion of 1.5×10^6/kg-3×10^6/kg of third-party MSCs on post-operative day 3 ± 2. These patients are prospectively compared to the same number of liver or kidney transplant recipients who meet inclusion criteria but have not not received MSC infusion. Safety is assessed by recording side effects, including opportunistic infections and

cancers. The immunosuppressive potential of MSCs will be evaluated by the rate of rejection episodes, graft/patient survivals, immunohistology of 3-mo (kidney) and 6-mo (liver) graft biopsies and *in vitro* evaluation of patient immune functions. In a second step, reduction (kidney) and progressive weaning (liver) of immunosuppression will be attempted in recipients who received MSCs. Final results are expected by the end of 2014. The next step will be to assert the immunosuppressive potential of MSCs after organ transplantation, and the opportunity to develop larger, randomised and controlled phase III trials.

"Mesenchymal stem cells in solid organ transplantation"-1 study: In a mesenchymal stem cells in solid organ transplantation phase I study (MiSOT-I) started in April 2013, the safety of MultiStem® infusion for immunomodulation after liver transplantation has been evaluated (NCT01841632). MultiStem is a new biological product derived from multipotent adult progenitor cells (MAPCs) which belong to the family of MSCs. Patients, divided into four cohorts, will receive 2 doses of Multi-Stem (first intraportal at liver transplantation, second at day 3 post-transplant) in addition to immunosuppression (calcineurin-inhibitor-free 'bottom-up' immunosuppressive regimen with basiliximab, mycophenolic acid, and steroids). From cohort 1 to 4, an increasing dose escalation is performed (3-6 patients in each group). The primary outcome will be infusional and acute toxicity (intraportal, pulmonary and systemic). The secondary outcomes will be biopsy-proven acute rejection, whether MultiStem promotes malignant transformation nor tumor growth, and the long-term safety of MultiStem administration (up to 6 years). Final results are expected in 2016.

The Beijing study

A third study is ongoing. This phase I study will include a total of 50 patients randomly assigned to two groups; in the first group, patients will receive conventional immunosuppressive agents plus umbilical cord (UC-) MSCs at the day of liver transplantation and then once every 4 wk, at a dose of 1×10^6 UC-MSCs/kg for 12 wk (NCT01690247). In the second group patients will receive conventional treatment plus a placebo. Both groups will be followed for 48 wk. The study will evaluate the incidence of acute rejection and early liver function recovery, as well as patient and graft survival rates, and the prevalence of adverse events as secondary outcomes.

VARIABLES TO BE CONSIDERED/ISSUES TO BE RESOLVED

At present many questions remain unanswered in the field of MSCs therapy in solid-organ transplantation. These issues could explain the conflicting data obtained in previous studies. Further *in vitro* investigations and pre-clinical studies could help to define the settings of future clinical trials through a better understanding of the mechanisms of action of MSCs.

Dosage and sources of MSCs

The ideal amount of MSCs necessary to achieve some clinical effect has not yet been studied, and additionally, the ideal source of MSCs in the setting of organ transplantation has not been determined. Usually isolated from the bone marrow, MSCs can now be isolated from other more easily accessible human tissues such as adipose tissue or cord-blood. Compared with BM-derived MSCs, adipose- and cord- derived MSCs have comparable phenotypic and immunomodulatory properties[134]. Nevertheless, it seems that many genes are differentially expressed in MSCs depending on their tissue origin[135]. These differences could alter the function of MSCs in clinical use.

Although not quite clear, it should be noted that MSCs derived from adipose tissue seem to be more likely to develop chromosomal abnormalities than BM-derived MSCs, after many passages in culture[136,137]. High-passage MSCs should thus be avoided for clinical applications.

Origin of MSCs- autologous vs allogeneic

MSCs can be isolated from the organ recipient (autologous) or from the organ donor, or from a third party (allogeneic).

While some have suggested that allogeneic MSCs may be more efficient as immunosuppressors[138], others have shown in animal models that donor-derived MSCs could be preferable[139]. In a recent study, it has been demonstrated that both autologous and allogeneic MSCs were able to inhibit alloreactivity and had comparable efficacy[22,127].

In terms of alloreactivity, MSCs appear to bear low immunogenicity (see above). In a clinical case of osteogenesis imperfecta, no sign of alloreactivity was observed in the recipient after infusion of fully mismatched allogeneic MSCs[140]. Yet some papers have reported the induction of memory T cell responses and immune rejection after allogeneic MSC infusion[18,141]. One cannot exclude that donor-derived MSCs could induce alloreactivity and accelerate graft rejection. Nevertheless, in the field of kidney transplantation, Crop *et al*[22] have demonstrated that donor-derived MSCs are not immune-rejected and are even able to inhibit alloreactivity in kidney transplant patients when infused before transplantation.

MSC interaction with immunosuppressive drugs

In clinical transplant studies, MSCs are used concomitantly with immunosuppressive drugs. As MSCs and immunosuppressive drugs inhibit the same targets (essentially T cells), it is reasonable to consider that interactions between them can occur. Therefore, it is essential to know which drugs can (positively or negatively) affect MSC function.

In vitro, some have shown that tacrolimus (a calcineurin inhibitor) and rapamycin (a mTOR inhibitor) decrease MSCs immunosuppressive properties[142], and conversely, that MSCs reduce the immunosuppressive capacities of tacrolimus and rapamycin. Such an effect has not been

found with mycophenolic acid (MPA). Moreover, a high dose of tacrolimus seems to be toxic for MSCs, while MPA and rapamycin at a therapeutic dose just inhibit MSC proliferation[143]. Nevertheless, others have shown that cyclosporine A (CsA) (another calcineurin inhibitor) and MSCs exert cumulative effects against all activated lymphocytes[138]. Furthermore, it has been demonstrated that MPA and MSCs have a synergistic immunosuppressive effect[143].

In vivo, MPA and MSCs also synergize to promote long-term allograft tolerance in rat heart transplantation[144]. In contrast to what is observed *in vitro*, rapamycin and MSCs synergize as immunomodulators to promote cardiac allograft long-term survival[119]. Moreover, in a rat renal transplantation model, it has been shown that CsA antagonizes MSC efficacy, and that this combination has no advantage in terms of allograft survival rates compared with CsA alone[122]. Nevertheless, this study has to be contrasted with other studies using various immunosuppressive drug used together with CsA in which MSC efficacy was not altered[19,110]. The choice of concomitant immunosuppressive drugs is an important matter for debate, and more studies are needed to define which are the most effective drugs to use with MSCs.

Timing of administration of MSCs

MSCs can be injected before, during or after transplantation, and with single or repeated injection(s). Timing of administration is another important point for discussion. It has been shown *in vivo* that pre-transplant infusion could be more effective than peri-transplant infusion in preventing graft rejection in a murine heart transplantation model[120]. On the other hand, it has been demonstrated that MSCs are effective in the treatment of steroid-resistant GVHD[113], so at the peak of the disease. In a clinical trial, Perico *et al*[131] observed that early post-transplant infusion of MSCs could induce a transient renal dysfunction. This group is now investigating pre-transplant infusions[132].

Protocols investigating timings of administration will probably have to be defined according to expected effects and drugs used concomitantly. Regarding liver transplantation, our group infuses MSCs at day 3 post-liver transplantation, while the MiSOT group performs 2 injections of MSCs at day 0 (intra operatively) and day 3 post-transplantation. In the Beijing study, an injection is performed on the day of liver transplantation and then once every 4 wk during a 12-wk period.

Administration route

In case of liver transplantation, MSCs can be injected through a peripheral vein or through intraportal infusion during surgery, or a combination of both. Intraportal infusion could be helpful in increasing the amount of MSCs homing to the liver. On the other hand, MSC homing behaviour to the inflammation site[69] could potentially concentrate them in the liver when intravenously infused after hepatic transplantation. However, some studies have observed that MSCs could be trapped in the lung after intravenous infusion[86,87]. Whatever the case, it is clear that to define the best route of administration, it is necessary to better understand the homing capacity of MSCs, and whether MSCs really require close contact with the target organ in order to be effective.

MSC side effects and safety

To date, no major adverse effects have been reported in the mid-term in the significant number of clinical trials using MSC-based therapy, for example in the context of BMT[113-117], solid-organ transplantation[129-133] and in many completed clinical trials for various therapeutic applications[145]. Only some studies have shown mild and transient adverse effects around the time of injection[145]. More experience is needed in order to confirm the long-term safety of MSCs.

To reach a sufficient number of cells for MSC-based therapy, *in vitro* expansion is needed. In this context, one of the major concerns is the potential risk of a neoplastic transformation of MSCs[122]. The occurrence of chromosomal aberrations is not uncommon after *in vitro* culture of mMSC, especially after long-term culture. It has been shown *in vivo* that these chromosomally unstable cells could transform into malignant cells with generation of tumors *in vivo*[146-148].

Contrary to mMSCs, *in vitro* expansion of hMSC seems to be far more stable and does not seem to generate genomic instability in these cells even after long-term culture. They do not transform into malignant cells after transplantation in mice[149,150]. Nevertheless, a French study observed the occurrence *in vitro* of transient chromosomal aberrations (aneuploidy) in twenty preparations of BM-MSCs obtained under GMP with two different culture processes. However these cells showed the same senescence as "normal" MSCs and did not lead to tumoral process after injection in immunocompromised mice[151]. Another study has found a high rate of human MSCs spontaneously transformed in malignant cells *in vivo*[152], but this strongly controvert suggesting a cross-contamination with cancerous cells[153]. Moreover, in two recent reviews analysing numerous studies, no evidence was found to affirm the potential of human MSCs for malignant transformation and so far, no risk of malignant transformation has been found in clinical use of hMSCs[149,154].

As MSCs are used as immunosuppressors, another concern is the potential emergence of opportunistic infections and induced cancers. In the case of solid organ transplantation with MSC-based immunosuppression, no increase risk of viral opportunistic infections has been observed so far-one group having even observed a decrease[130]. Nevertheless, another group reported viral opportunistic infections in three patients[129].

Interestingly, the MiSOT study group recently established a system to objectively score the potential emerging adverse effects related to MSC infusions (intravenous or intraportal infusion) after liver transplantation[155]. This

score is calculated using three parameters (pulmonary toxicity, intraportal-infusional toxicity and systemic toxicity), each of them receiving a score of 0 (no adverse events) to 3 (severe adverse events). It has been retrospectively validated on a cohort of 187 liver-transplanted patients not receiving MSCs as a control population. It has been suggested that this new tool could be helpful in assessing the safety of MSC use in solid organ transplantation.

CONCLUSION

The accumulating evidence shows that MSCs have immunosuppressive and reparative capacities *in vivo* and *in vitro*, as well as a potential beneficial effect in ischemia-reperfusion injury. These three principal properties suggest that MSCs could be interesting in liver transplantation to prevent or treat IRI, allograft dysfunction and graft rejection by inducing a durable tolerogenic environment. Using MSCs, and thereby removing or reducing the need for immunosuppressive drugs could avoid the serious side effects associated with these drugs.

Currently available data in clinic show that MSCs are safe to use, at least in the medium-term, but more time is needed to evaluate their potential adverse effects on the long-term. Caution is therefore recommended. Even if encouraging, the results of MSC use *in vitro* and *in vivo* (animals and humans) are sometimes contradictory. Nevertheless, negative results do not necessarily mean that MSCs are not effective in solid-organ transplantation, but rather that a countless number of still unknown (or poorly known) parameters may influence their effectiveness. At the same time, many issues must be resolved to optimize their use. Intensive *in vitro* and pre-clinical research is certainly the key to a better understanding of the way that MSCs act, and to eventually lead to clinical success.

REFERENCES

1   **Zuk PA**, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; **13**: 4279-4295 [PMID: 12475952 DOI: 10.1091/mbc.E02-02-0105]

2   **Toma JG**, Akhavan M, Fernandes KJ, Barnabé-Heider F, Sadikot A, Kaplan DR, Miller FD. Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 2001; **3**: 778-784 [PMID: 11533656 DOI: 10.1038/ncb0901-778]

3   **Hoogduijn MJ**, Crop MJ, Peeters AM, Van Osch GJ, Balk AH, Ijzermans JN, Weimar W, Baan CC. Human heart, spleen, and perirenal fat-derived mesenchymal stem cells have immunomodulatory capacities. *Stem Cells Dev* 2007; **16**: 597-604 [PMID: 17784833 DOI: 10.1089/scd.2006.0110]

4   **Zhang Y**, Li C, Jiang X, Zhang S, Wu Y, Liu B, Tang P, Mao N. Human placenta-derived mesenchymal progenitor cells support culture expansion of long-term culture-initiating cells from cord blood CD34+ cells. *Exp Hematol* 2004; **32**: 657-664 [PMID: 15246162 DOI: 10.1016/j.exphem.2004.04.001]

5   **Erices A**, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* 2000; **109**: 235-242 [PMID: 10848804]

6   **Fan CG**, Tang FW, Zhang QJ, Lu SH, Liu HY, Zhao ZM, Liu B, Han ZB, Han ZC. Characterization and neural differentiation of fetal lung mesenchymal stem cells. *Cell Transplant* 2005; **14**: 311-321 [PMID: 16052912]

7   **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**: 2396-2402 [PMID: 11588036]

8   **Young HE**, Mancini ML, Wright RP, Smith JC, Black AC, Reagan CR, Lucas PA. Mesenchymal stem cells reside within the connective tissues of many organs. *Dev Dyn* 1995; **202**: 137-144 [PMID: 7734732 DOI: 10.1002/aja.1002020205]

9   **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

10 **Marigo I**, Dazzi F. The immunomodulatory properties of mesenchymal stem cells. *Semin Immunopathol* 2011; **33**: 593-602 [PMID: 21499984 DOI: 10.1007/s00281-011-0267-7]

11 **Krampera M**, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, Romagnani P, Maggi E, Romagnani S, Annunziato F. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells* 2006; **24**: 386-398 [PMID: 16123384 DOI: 10.1634/stemcells.2005-0008]

12 **Majumdar MK**, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, Mosca JD. Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 2003; **10**: 228-241 [PMID: 12595759 DOI: 68710]

13 **Le Blanc K**, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003; **31**: 890-896 [PMID: 14550804]

14 **François M**, Romieu-Mourez R, Stock-Martineau S, Boivin MN, Bramson JL, Galipeau J. Mesenchymal stromal cells cross-present soluble exogenous antigens as part of their antigen-presenting cell properties. *Blood* 2009; **114**: 2632-2638 [PMID: 19654411 DOI: 10.1182/blood-2009-02-207795]

15 **Romieu-Mourez R**, François M, Boivin MN, Stagg J, Galipeau J. Regulation of MHC class II expression and antigen processing in murine and human mesenchymal stromal cells by IFN-gamma, TGF-beta, and cell density. *J Immunol* 2007; **179**: 1549-1558 [PMID: 17641021]

16 **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**: 1545-1555 [PMID: 18715686 DOI: 10.1016/j.exphem.2008.06.008]

17 **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**: 2570-2577 [PMID: 16293599 DOI: 10.1182/blood-2005-07-2793]

18 **Nauta AJ**, Westerhuis G, Kruisselbrink AB, Lurvink EG, Willemze R, Fibbe WE. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. *Blood* 2006; **108**: 2114-2120 [PMID: 16690970 DOI: 10.1182/blood-2005-11-011650]

19 **Sbano P**, Cuccia A, Mazzanti B, Urbani S, Giusti B, Lapini I, Rossi L, Abbate R, Marseglia G, Nannetti G, Torricelli F, Miracco C, Bosi A, Fimiani M, Saccardi R. Use of donor bone marrow mesenchymal stem cells for treatment of skin allograft rejection in a preclinical rat model. *Arch Dermatol Res* 2008; **300**: 115-124 [PMID: 18259766 DOI: 10.1007/s00403-007-0827-9]

20 **Di Nicola M**, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone

marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002; **99**: 3838-3843 [PMID: 11986244]

21 **Angoulvant D**, Clerc A, Benchalal S, Galambrun C, Farre A, Bertrand Y, Eljaafari A. Human mesenchymal stem cells suppress induction of cytotoxic response to alloantigens. *Biorheology* 2004; **41**: 469-476 [PMID: 15299278]

22 **Crop MJ**, Baan CC, Korevaar SS, Ijzermans JN, Alwayn IP, Weimar W, Hoogduijn MJ. Donor-derived mesenchymal stem cells suppress alloreactivity of kidney transplant patients. *Transplantation* 2009; **87**: 896-906 [PMID: 19300194 DOI: 10.1097/TP.0b013e31819b3d72]

23 **Batten P**, Sarathchandra P, Antoniw JW, Tay SS, Lowdell MW, Taylor PM, Yacoub MH. Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: relevance to tissue engineering human heart valves. *Tissue Eng* 2006; **12**: 2263-2273 [PMID: 16968166 DOI: 10.1089/ten.2006.12.2263]

24 **Liu XJ**, Zhang JF, Sun B, Peng HS, Kong QF, Bai SS, Liu YM, Wang GY, Wang JH, Li HL. Reciprocal effect of mesenchymal stem cell on experimental autoimmune encephalomyelitis is mediated by transforming growth factor-beta and interleukin-6. *Clin Exp Immunol* 2009; **158**: 37-44 [PMID: 19737229 DOI: 10.1111/j.1365-2249.2009.03995.x]

25 **Di Ianni M**, Del Papa B, De Ioanni M, Moretti L, Bonifacio E, Cecchini D, Sportoletti P, Falzetti F, Tabilio A. Mesenchymal cells recruit and regulate T regulatory cells. *Exp Hematol* 2008; **36**: 309-318 [PMID: 18279718 DOI: 10.1016/j.exphem.2007.11.007]

26 **Ye Z**, Wang Y, Xie HY, Zheng SS. Immunosuppressive effects of rat mesenchymal stem cells: involvement of CD4+CD25+ regulatory T cells. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 608-614 [PMID: 19073406]

27 **Patel SA**, Meyer JR, Greco SJ, Corcoran KE, Bryan M, Rameshwar P. Mesenchymal stem cells protect breast cancer cells through regulatory T cells: role of mesenchymal stem cell-derived TGF-beta. *J Immunol* 2010; **184**: 5885-5894 [PMID: 20382885 DOI: 10.4049/jimmunol.0903143]

28 **Madec AM**, Mallone R, Afonso G, Abou Mrad E, Mesnier A, Eljaafari A, Thivolet C. Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. *Diabetologia* 2009; **52**: 1391-1399 [PMID: 19421731 DOI: 10.1007/s00125-009-1374-z]

29 **English K**, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP. Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells. *Clin Exp Immunol* 2009; **156**: 149-160 [PMID: 19210524 DOI: 10.1111/j.1365-2249.2009.03874.x]

30 **Selmani Z**, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, Borg C, Saas P, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F. 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**: 212-222 [PMID: 17932417 DOI: 10.1634/stemcells.2007-0554]

31 **Rouas-Freiss N**, Naji A, Durrbach A, Carosella ED. Tolerogenic functions of human leukocyte antigen G: from pregnancy to organ and cell transplantation. *Transplantation* 2007; **84**: S21-S25 [PMID: 17632407 DOI: 10.1097/01.tp.0000269117.32179.1c]

32 **Choi YS**, Jeong JA, Lim DS. Mesenchymal stem cell-mediated immature dendritic cells induce regulatory T cell-based immunosuppressive effect. *Immunol Invest* 2012; **41**: 214-229 [PMID: 22017637 DOI: 10.3109/08820139.2011.619022]

33 **Comoli P**, Ginevri F, Maccario R, Avanzini MA, Marconi M, Groff A, Cometa A, Cioni M, Porretti L, Barberi W, Frassoni F, Locatelli F. Human mesenchymal stem cells inhibit antibody production induced in vitro by allostimulation. *Nephrol Dial Transplant* 2008; **23**: 1196-1202 [PMID: 18029377 DOI: 10.1093/ndt/gfm740]

34 **Corcione A**, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006; **107**: 367-372 [PMID: 16141348 DOI: 10.1182/blood-2005-07-2657]

35 **Tabera S**, Pérez-Simón JA, Díez-Campelo M, Sánchez-Abarca LI, Blanco B, López A, Benito A, Ocio E, Sánchez-Guijo FM, Cañizo C, San Miguel JF. The effect of mesenchymal stem cells on the viability, proliferation and differentiation of B-lymphocytes. *Haematologica* 2008; **93**: 1301-1309 [PMID: 18641017 DOI: 10.3324/haematol.12857]

36 **Traggiai E**, Volpi S, Schena F, Gattorno M, Ferlito F, Moretta L, Martini A. 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**: 562-569 [PMID: 18024418 DOI: 10.1634/stemcells.2007-0528]

37 **Rasmusson I**, Le Blanc K, Sundberg B, Ringdén O. Mesenchymal stem cells stimulate antibody secretion in human B cells. *Scand J Immunol* 2007; **65**: 336-343 [PMID: 17386024 DOI: 10.1111/j.1365-3083.2007.01905.x]

38 **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 [PMID: 16099998 DOI: 10.1634/stemcells.2004-0359]

39 **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**: 1327-1333 [PMID: 17951526 DOI: 10.1182/blood-2007-02-074997]

40 **Rasmusson I**, Ringdén 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**: 1208-1213 [PMID: 14578755 DOI: 10.1097/01.TP.0000082540.43730.80]

41 **Sotiropoulou PA**, Perez SA, Salagianni M, Baxevanis CN, Papamichail M. Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells. *Stem Cells* 2006; **24**: 462-471 [PMID: 16109759 DOI: 10.1634/stemcells.2004-0331]

42 **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**: 1484-1490 [PMID: 16239427 DOI: 10.1182/blood-2005-07-2775]

43 **Ramasamy R**, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. *Transplantation* 2007; **83**: 71-76 [PMID: 17220794 DOI: 10.1097/01.tp.0000244572.24780.54]

44 **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**: 2080-2087 [PMID: 16887966]

45 **Zhang B**, Liu R, Shi D, Liu X, Chen Y, Dou X, Zhu X, Lu C, Liang W, Liao L, Zenke M, Zhao RC. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. *Blood* 2009; **113**: 46-57 [PMID: 18832657 DOI: 10.1182/blood-2008-04-154138]

46 **Németh K**, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009; **15**: 42-49 [PMID: 19098906 DOI: 10.1038/nm.1905]

47   **Ren G**, Su J, Zhang L, Zhao X, Ling W, L'huillie A, Zhang J, Lu Y, Roberts AI, Ji W, Zhang H, Rabson AB, Shi Y. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. *Stem Cells* 2009; **27**: 1954-1962 [PMID: 19544427 DOI: 10.1002/stem.118]

48   **Meisel R**, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; **103**: 4619-4621 [PMID: 15001472 DOI: 10.1182/blood-2003-11-3909]

49   **Hainz U**, Jürgens B, Heitger A. The role of indoleamine 2,3-dioxygenase in transplantation. *Transpl Int* 2007; **20**: 118-127 [PMID: 17239019 DOI: 10.1111/j.1432-2277.2006.00370.x]

50   **Bauer TM**, Jiga LP, Chuang JJ, Randazzo M, Opelz G, Terness P. Studying the immunosuppressive role of indoleamine 2,3-dioxygenase: tryptophan metabolites suppress rat allogeneic T-cell responses in vitro and in vivo. *Transpl Int* 2005; **18**: 95-100 [PMID: 15612990 DOI: 10.1111/j.1432-2277.2004.00031.x]

51   **Gieseke F**, Schütt B, Viebahn S, Koscielniak E, Friedrich W, Handgretinger R, Müller I. Human multipotent mesenchymal stromal cells inhibit proliferation of PBMCs independently of IFNgammaR1 signaling and IDO expression. *Blood* 2007; **110**: 2197-2200 [PMID: 17522338 DOI: 10.1182/blood-2007-04-083162]

52   **Sato K**, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, Muroi K, Ozawa K. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. *Blood* 2007; **109**: 228-234 [PMID: 16985180 DOI: 10.1182/blood-2006-02-002246]

53   **Ren G**, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008; **2**: 141-150 [PMID: 18371435 DOI: 10.1016/j.stem.2007.11.014]

54   **Mellor AL**, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004; **4**: 762-774 [PMID: 15459668 DOI: 10.1038/nri1457]

55   **Ignarro LJ**, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987; **84**: 9265-9269 [PMID: 2827174]

56   **Carosella ED**, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. *Adv Immunol* 2003; **81**: 199-252 [PMID: 14711057]

57   **Nasef A**, Mathieu N, Chapel A, Frick J, François S, Mazurier C, Boutarfa A, Bouchet S, Gorin NC, Thierry D, Fouillard L. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. *Transplantation* 2007; **84**: 231-237 [PMID: 17667815 DOI: 10.1097/01.tp.0000267918.07906.08]

58   **Aggarwal S**, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; **105**: 1815-1822 [PMID: 15494428 DOI: 10.1182/blood-2004-04-1559]

59   **Matysiak M**, Orlowski W, Fortak-Michalska M, Jurewicz A, Selmaj K. Immunoregulatory function of bone marrow mesenchymal stem cells in EAE depends on their differentiation state and secretion of PGE2. *J Neuroimmunol* 2011; **233**: 106-111 [PMID: 21354631 DOI: 10.1016/j.jneuroim.2010.12.004]

60   **Yao C**, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K, Sugimoto Y, Narumiya S. Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. *Nat Med* 2009; **15**: 633-640 [PMID: 19465928 DOI: 10.1038/nm.1968]

61   **Yang SH**, Park MJ, Yoon IH, Kim SY, Hong SH, Shin JY, Nam HY, Kim YH, Kim B, Park CG. Soluble mediators from mesenchymal stem cells suppress T cell proliferation by inducing IL-10. *Exp Mol Med* 2009; **41**: 315-324 [PMID: 19307751 DOI: 10.3858/emm.2009.41.5.035]

62   **Ortiz LA**, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci USA* 2007; **104**: 11002-11007 [PMID: 17569781 DOI: 10.1073/pnas.0704421104]

63   **Krasnodembskaya A**, Song Y, Fang X, Gupta N, Serikov V, Lee JW, Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem Cells* 2010; **28**: 2229-2238 [PMID: 20945332 DOI: 10.1002/stem.544]

64   **Kim Y**, Kim H, Cho H, Bae Y, Suh K, Jung J. Direct comparison of human mesenchymal stem cells derived from adipose tissues and bone marrow in mediating neovascularization in response to vascular ischemia. *Cell Physiol Biochem* 2007; **20**: 867-876 [PMID: 17982269 DOI: 10.1159/000110447]

65   **Fang X**, Neyrinck AP, Matthay MA, Lee JW. Allogeneic human mesenchymal stem cells restore epithelial protein permeability in cultured human alveolar type II cells by secretion of angiopoietin-1. *J Biol Chem* 2010; **285**: 26211-26222 [PMID: 20554518 DOI: 10.1074/jbc.M110.119917]

66   **Shi C**, Jia T, Mendez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, Leiner I, Li MO, Frenette PS, Pamer EG. Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. *Immunity* 2011; **34**: 590-601 [PMID: 21458307 DOI: 10.1016/j.immuni.2011.02.016]

67   **Hoogduijn MJ**, Popp F, Verbeek R, Masoodi M, Nicolaou A, Baan C, Dahlke MH. The immunomodulatory properties of mesenchymal stem cells and their use for immunotherapy. *Int Immunopharmacol* 2010; **10**: 1496-1500 [PMID: 20619384 DOI: 10.1016/j.intimp.2010.06.019]

68   **Tropel P**, Platet N, Platel JC, Noël D, Albrieux M, Benabid AL, Berger F. Functional neuronal differentiation of bone marrow-derived mesenchymal stem cells. *Stem Cells* 2006; **24**: 2868-2876 [PMID: 16902198 DOI: 10.1634/stemcells.2005-0636]

69   **Rose RA**, Keating A, Backx PH. Do mesenchymal stromal cells transdifferentiate into functional cardiomyocytes? *Circ Res* 2008; **103**: e120 [PMID: 18948624 DOI: 10.1161/CIRCRESAHA.108.186908]

70   **Taléns-Visconti R**, Bonora A, Jover R, Mirabet V, Carbonell F, Castell JV, Gómez-Lechón MJ. Human mesenchymal stem cells from adipose tissue: Differentiation into hepatic lineage. *Toxicol In Vitro* 2007; **21**: 324-329 [PMID: 17045453 DOI: 10.1016/j.tiv.2006.08.009]

71   **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**: 258-264 [PMID: 15670778 DOI: 10.1016/j.bbrc.2004.12.158]

72   **Schwartz RE**, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002; **109**: 1291-1302 [PMID: 12021244 DOI: 10.1172/JCI15182]

73   **Sordi V**. Mesenchymal stem cell homing capacity. *Transplantation* 2009; **87**: S42-S45 [PMID: 19424004 DOI: 10.1097/TP.0b013e3181a28533]

74   **Minguell JJ**, Erices A. Mesenchymal stem cells and the treatment of cardiac disease. *Exp Biol Med (Maywood)* 2006; **231**: 39-49 [PMID: 16380643]

75   **Kunter U**, Rong S, Djuric Z, Boor P, Müller-Newen G, Yu D, Floege J. Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. *J Am Soc Nephrol* 2006; **17**: 2202-2212 [PMID: 16790513 DOI: 10.1681/ASN.2005080815]

76   **Herrera MB**, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the

renal repair of acute tubular epithelial injury. *Int J Mol Med* 2004; **14**: 1035-1041 [PMID: 15547670]

77 **Horwitz EM**, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* 1999; **5**: 309-313 [PMID: 10086387 DOI: 10.1038/6529]

78 **Zeinaloo A**, Zanjani KS, Bagheri MM, Mohyeddin-Bonab M, Monajemzadeh M, Arjmandnia MH. Intracoronary administration of autologous mesenchymal stem cells in a critically ill patient with dilated cardiomyopathy. *Pediatr Transplant* 2011; **15**: E183-E186 [PMID: 20880092 DOI: 10.1111/j.1399-3046.2010.01366.x]

79 **Yang Z**, Zhang F, Ma W, Chen B, Zhou F, Xu Z, Zhang Y, Zhang D, Zhu T, Wang L, Wang H, Ding Z, Zhang Y. A novel approach to transplanting bone marrow stem cells to repair human myocardial infarction: delivery via a noninfarct-relative artery. *Cardiovasc Ther* 2010; **28**: 380-385 [PMID: 20337639 DOI: 10.1111/j.1755-5922.2009.00116.x]

80 **Katritsis DG**, Sotiropoulou PA, Karvouni E, Karabinos I, Korovesis S, Perez SA, Voridis EM, Papamichail M. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv* 2005; **65**: 321-329 [PMID: 15954106 DOI: 10.1002/ccd.20406]

81 **Sordi V**, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, Belmonte N, Ferrari G, Leone BE, Bertuzzi F, Zerbini G, Allavena P, Bonifacio E, Piemonti L. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* 2005; **106**: 419-427 [PMID: 15784733 DOI: 10.1182/blood-2004-09-3507]

82 **Von Lüttichau I**, Notohamiprodjo M, Wechselberger A, Peters C, Henger A, Seliger C, Djafarzadeh R, Huss R, Nelson PJ. Human adult CD34- progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4. *Stem Cells Dev* 2005; **14**: 329-336 [PMID: 15969628 DOI: 10.1089/scd.2005.14.329]

83 **Forte G**, Minieri M, Cossa P, Antenucci D, Sala M, Gnocchi V, Fiaccavento R, Carotenuto F, De Vito P, Baldini PM, Prat M, Di Nardo P. Hepatocyte growth factor effects on mesenchymal stem cells: proliferation, migration, and differentiation. *Stem Cells* 2006; **24**: 23-33 [PMID: 16100005 DOI: 10.1634/stemcells.2004-0176]

84 **Ball SG**, Shuttleworth CA, Kielty CM. Vascular endothelial growth factor can signal through platelet-derived growth factor receptors. *J Cell Biol* 2007; **177**: 489-500 [PMID: 17470632 DOI: 10.1083/jcb.200608093]

85 **Fiedler J**, Röderer G, Günther KP, Brenner RE. BMP-2, BMP-4, and PDGF-bb stimulate chemotactic migration of primary human mesenchymal progenitor cells. *J Cell Biochem* 2002; **87**: 305-312 [PMID: 12397612 DOI: 10.1002/jcb.10309]

86 **Barbash IM**, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation* 2003; **108**: 863-868 [PMID: 12900340 DOI: 10.1161/01.CIR.0000084828.50310.6A]

87 **Mahmood A**, Lu D, Lu M, Chopp M. Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells. *Neurosurgery* 2003; **53**: 697-702; discussion 702-3 [PMID: 12943585]

88 **Devine SM**, Bartholomew AM, Mahmud N, Nelson M, Patil S, Hardy W, Sturgeon C, Hewett T, Chung T, Stock W, Sher D, Weissman S, Ferrer K, Mosca J, Deans R, Moseley A, Hoffman R. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol* 2001; **29**: 244-255 [PMID: 11166464]

89 **Popp FC**, Slowik P, Eggenhofer E, Renner P, Lang SA, Stoeltzing O, Geissler EK, Piso P, Schlitt HJ, Dahlke MH. No contribution of multipotent mesenchymal stromal cells to liver regeneration in a rat model of prolonged hepatic injury. *Stem Cells* 2007; **25**: 639-645 [PMID: 17110617 DOI: 10.1634/stemcells.2006-0515]

90 **Chen SL**, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. 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**: 92-95 [PMID: 15219514 DOI: 10.1016/j.amjcard.2004.03.034]

91 **Prockop DJ**. "Stemness" does not explain the repair of many tissues by mesenchymal stem/multipotent stromal cells (MSCs). *Clin Pharmacol Ther* 2007; **82**: 241-243 [PMID: 17700588 DOI: 10.1038/sj.clpt.6100313]

92 **Iso Y**, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, Sobel BE, Delafontaine P, Prockop DJ. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. *Biochem Biophys Res Commun* 2007; **354**: 700-706 [PMID: 17257581 DOI: 10.1016/j.bbrc.2007.01.045]

93 **Kachgal S**, Putnam AJ. Mesenchymal stem cells from adipose and bone marrow promote angiogenesis via distinct cytokine and protease expression mechanisms. *Angiogenesis* 2011; **14**: 47-59 [PMID: 21104120 DOI: 10.1007/s10456-010-9194-9]

94 **Caplan AI**. Why are MSCs therapeutic? New data: new insight. *J Pathol* 2009; **217**: 318-324 [PMID: 19023885 DOI: 10.1002/path.2469]

95 **Erpicum P**, Detry O, Weekers L, Bonvoisin C, Lechanteur C, Briquet A, Beguin Y, Krzesinski JM, Jouret F. Mesenchymal stromal cell therapy in conditions of renal ischaemia/reperfusion. *Nephrol Dial Transplant* 2014; **29**: 1487-1493 [PMID: 24516234 DOI: 10.1093/ndt/gft538]

96 **Tögel FE**, Westenfelder C. Mesenchymal stem cells: a new therapeutic tool for AKI. *Nat Rev Nephrol* 2010; **6**: 179-183 [PMID: 20186233 DOI: 10.1038/nrneph.2009.229]

97 **Donizetti-Oliveira C**, Semedo P, Burgos-Silva M, Cenedeze MA, Malheiros DM, Reis MA, Pacheco-Silva A, Câmara NO. Adipose tissue-derived stem cell treatment prevents renal disease progression. *Cell Transplant* 2012; **21**: 1727-1741 [PMID: 22305061 DOI: 10.3727/096368911X623925]

98 **van Poll D**, Parekkadan B, Cho CH, Berthiaume F, Nahmias Y, Tilles AW, Yarmush ML. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. *Hepatology* 2008; **47**: 1634-1643 [PMID: 18395843 DOI: 10.1002/hep.22236]

99 **Parekkadan B**, van Poll D, Suganuma K, Carter EA, Berthiaume F, Tilles AW, Yarmush ML. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. *PLoS One* 2007; **2**: e941 [PMID: 17895982 DOI: 10.1371/journal.pone.0000941]

100 **Kharaziha P**, Hellström PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, Telkabadi M, Atashi A, Honardoost M, Zali MR, Soleimani M. 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**: 1199-1205 [PMID: 19455046 DOI: 10.1097/MEG.0b013e32832a1f6c]

101 **Mohamadnejad M**, Alimoghaddam K, Bagheri M, Ashrafi M, Abdollahzadeh L, Akhlaghpoor S, Bashtar M, Ghavamzadeh A, Malekzadeh R. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. *Liver Int* 2013; **33**: 1490-1496 [PMID: 23763455 DOI: 10.1111/liv.12228]

102 **Carden DL**, Granger DN. Pathophysiology of ischaemia-reperfusion injury. *J Pathol* 2000; **190**: 255-266 [PMID: 10685060 DOI: 10.1002/(SICI)1096-9896(200002)190]

103 **Eltzschig HK**, Eckle T. Ischemia and reperfusion--from

mechanism to translation. *Nat Med* 2011; **17**: 1391-1401 [PMID: 22064429 DOI: 10.1038/nm.2507]

104 **Liu H**, Liu S, Li Y, Wang X, Xue W, Ge G, Luo X. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. *PLoS One* 2012; **7**: e34608 [PMID: 22511954 DOI: 10.1371/journal.pone.0034608]

105 **Teo GS**, Ankrum JA, Martinelli R, Boetto SE, Simms K, Sciuto TE, Dvorak AM, Karp JM, Carman CV. Mesenchymal stem cells transmigrate between and directly through tumor necrosis factor-α-activated endothelial cells via both leukocyte-like and novel mechanisms. *Stem Cells* 2012; **30**: 2472-2486 [PMID: 22887987 DOI: 10.1002/stem.1198]

106 **Pan GZ**, Yang Y, Zhang J, Liu W, Wang GY, Zhang YC, Yang Q, Zhai FX, Tai Y, Liu JR, Zhang Q, Chen GH. Bone marrow mesenchymal stem cells ameliorate hepatic ischemia/reperfusion injuries via inactivation of the MEK/ERK signaling pathway in rats. *J Surg Res* 2012; **178**: 935-948 [PMID: 22658855 DOI: 10.1016/j.jss.2012.04.070]

107 **Souidi N**, Stolk M, Seifert M. Ischemia-reperfusion injury: beneficial effects of mesenchymal stromal cells. *Curr Opin Organ Transplant* 2013; **18**: 34-43 [PMID: 23254704 DOI: 10.1097/MOT.0b013e32835c2a05]

108 **Jin G**, Qiu G, Wu D, Hu Y, Qiao P, Fan C, Gao F. Allogeneic bone marrow-derived mesenchymal stem cells attenuate hepatic ischemia-reperfusion injury by suppressing oxidative stress and inhibiting apoptosis in rats. *Int J Mol Med* 2013; **31**: 1395-1401 [PMID: 23589072 DOI: 10.3892/ijmm.2013.1340]

109 **Sun CK**, Chang CL, Lin YC, Kao YH, Chang LT, Yen CH, Shao PL, Chen CH, Leu S, Yip HK. Systemic administration of autologous adipose-derived mesenchymal stem cells alleviates hepatic ischemia-reperfusion injury in rats. *Crit Care Med* 2012; **40**: 1279-1290 [PMID: 22336724 DOI: 10.1097/CCM.0b013e31823dae23]

110 **Du Z**, Wei C, Cheng K, Han B, Yan J, Zhang M, Peng C, Liu Y. Mesenchymal stem cell-conditioned medium reduces liver injury and enhances regeneration in reduced-size rat liver transplantation. *J Surg Res* 2013; **183**: 907-915 [PMID: 23522455 DOI: 10.1016/j.jss.2013.02.009]

111 **Kanazawa H**, Fujimoto Y, Teratani T, Iwasaki J, Kasahara N, Negishi K, Tsuruyama T, Uemoto S, Kobayashi E. Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model. *PLoS One* 2011; **6**: e19195 [PMID: 21559442 DOI: 10.1371/journal.pone.0019195]

112 **Eggenhofer E**, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, Baan CC, Dahlke MH, Hoogduijn MJ. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front Immunol* 2012; **3**: 297 [PMID: 23056000 DOI: 10.3389/fimmu.2012.00297]

113 **Baron F**, Storb R. Mesenchymal stromal cells: a new tool against graft-versus-host disease? *Biol Blood Marrow Transplant* 2012; **18**: 822-840 [PMID: 21963621 DOI: 10.1016/j.bbmt.2011.09.003]

114 **Le Blanc K**, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringdén O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008; **371**: 1579-1586 [PMID: 18468541 DOI: 10.1016/S0140-6736(08)60690-X]

115 **Baron F**, Lechanteur C, Willems E, Bruck F, Baudoux E, Seidel L, Vanbellinghen JF, Hafraoui K, Lejeune M, Gothot A, Fillet G, Beguin Y. Cotransplantation of mesenchymal stem cells might prevent death from graft-versus-host disease (GVHD) without abrogating graft-versus-tumor effects after HLA-mismatched allogeneic transplantation following nonmyeloablative conditioning. *Biol Blood Marrow Transplant* 2010; **16**: 838-847 [PMID: 20109568 DOI: 10.1016/j.bbmt.2010.01.011]

116 **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**: 3358-3362 [PMID: 18089385 DOI: 10.1016/j.transproceed.2007.08.103]

117 **Prasad VK**, Lucas KG, Kleiner GI, Talano JA, Jacobsohn D, Broadwater G, Monroy R, Kurtzberg J. Efficacy and safety of ex vivo cultured adult human mesenchymal stem cells (Prochymal™) in pediatric patients with severe refractory acute graft-versus-host disease in a compassionate use study. *Biol Blood Marrow Transplant* 2011; **17**: 534-541 [PMID: 20457269 DOI: 10.1016/j.bbmt.2010.04.014]

118 **Zhou HP**, Yi DH, Yu SQ, Sun GC, Cui Q, Zhu HL, Liu JC, Zhang JZ, Wu TJ. Administration of donor-derived mesenchymal stem cells can prolong the survival of rat cardiac allograft. *Transplant Proc* 2006; **38**: 3046-3051 [PMID: 17112896 DOI: 10.1016/j.transproceed.2006.10.002]

119 **Ge W**, Jiang J, Baroja ML, Arp J, Zassoko R, Liu W, Bartholomew A, Garcia B, Wang H. Infusion of mesenchymal stem cells and rapamycin synergize to attenuate alloimmune responses and promote cardiac allograft tolerance. *Am J Transplant* 2009; **9**: 1760-1772 [PMID: 19563344 DOI: 10.1111/j.1600-6143.2009.02721.x]

120 **Casiraghi F**, Azzollini N, Cassis P, Imberti B, Morigi M, Cugini D, Cavinato RA, Todeschini M, Solini S, Sonzogni A, Perico N, Remuzzi G, Noris M. Pretransplant infusion of mesenchymal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells. *J Immunol* 2008; **181**: 3933-3946 [PMID: 18768848]

121 **Bartholomew A**, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 2002; **30**: 42-48 [PMID: 11823036]

122 **Casiraghi F**, Azzollini N, Todeschini M, Cavinato RA, Cassis P, Solini S, Rota C, Morigi M, Introna M, Maranta R, Perico N, Remuzzi G, Noris M. Localization of mesenchymal stromal cells dictates their immune or proinflammatory effects in kidney transplantation. *Am J Transplant* 2012; **12**: 2373-2383 [PMID: 22642544 DOI: 10.1111/j.1600-6143.2012.04115.x]

123 **Zhang W**, Qin C, Zhou ZM. Mesenchymal stem cells modulate immune responses combined with cyclosporine in a rat renal transplantation model. *Transplant Proc* 2007; **39**: 3404-3408 [PMID: 18089393 DOI: 10.1016/j.transproceed.2007.06.092]

124 **De Martino M**, Zonta S, Rampino T, Gregorini M, Frassoni F, Piotti G, Bedino G, Cobianchi L, Dal Canton A, Dionigi P, Alessiani M. Mesenchymal stem cells infusion prevents acute cellular rejection in rat kidney transplantation. *Transplant Proc* 2010; **42**: 1331-1335 [PMID: 20534294 DOI: 10.1016/j.transproceed.2010.03.079]

125 **Eggenhofer E**, Renner P, Soeder Y, Popp FC, Hoogduijn MJ, Geissler EK, Schlitt HJ, Dahlke MH. Features of synergism between mesenchymal stem cells and immunosuppressive drugs in a murine heart transplantation model. *Transpl Immunol* 2011; **25**: 141-147 [PMID: 21704160 DOI: 10.1016/j.trim.2011.06.002]

126 **Wan CD**, Cheng R, Wang HB, Liu T. Immunomodulatory effects of mesenchymal stem cells derived from adipose tissues in a rat orthotopic liver transplantation model. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 29-33 [PMID: 18234635]

127 **Wang JW**, Liu YB, Xu B, Li JT, Qian HR, Zhang M, Peng SY. [The study on immunomodulation of donor mesenchymal stem cells on discordant liver xenotransplantation]. *Zhonghua Wai Ke Zazhi* 2005; **43**: 1254-1258 [PMID: 16271223]

128 **Wang Y**, Zhang A, Ye Z, Xie H, Zheng S. Bone marrow-derived mesenchymal stem cells inhibit acute rejection of rat liver allografts in association with regulatory T-cell expansion. *Transplant Proc* 2009; **41**: 4352-4356 [PMID: 20005397 DOI: 10.1016/j.transproceed.2009.08.072]

129 **Reinders ME**, de Fijter JW, Roelofs H, Bajema IM, de Vries

DK, Schaapherder AF, Claas FH, van Miert PP, Roelen DL, van Kooten C, Fibbe WE, Rabelink TJ. Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. *Stem Cells Transl Med* 2013; **2**: 107-111 [PMID: 23349326 DOI: 10.5966/sctm.2012-0114]

130 **Tan J**, Wu W, Xu X, Liao L, Zheng F, Messinger S, Sun X, Chen J, Yang S, Cai J, Gao X, Pileggi A, Ricordi C. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA* 2012; **307**: 1169-1177 [PMID: 22436957 DOI: 10.1001/jama.2012.316]

131 **Perico N**, Casiraghi F, Introna M, Gotti E, Todeschini M, Cavinato RA, Capelli C, Rambaldi A, Cassis P, Rizzo P, Cortinovis M, Marasà M, Golay J, Noris M, Remuzzi G. Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. *Clin J Am Soc Nephrol* 2011; **6**: 412-422 [PMID: 20930086 DOI: 10.2215/CJN.04950610]

132 **Perico N**, Casiraghi F, Gotti E, Introna M, Todeschini M, Cavinato RA, Capelli C, Rambaldi A, Cassis P, Rizzo P, Cortinovis M, Noris M, Remuzzi G. Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. *Transpl Int* 2013; **26**: 867-878 [PMID: 23738760 DOI: 10.1111/tri.12132]

133 **Peng Y**, Ke M, Xu L, Liu L, Chen X, Xia W, Li X, Chen Z, Ma J, Liao D, Li G, Fang J, Pan G, Xiang AP. Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. *Transplantation* 2013; **95**: 161-168 [PMID: 23263506 DOI: 10.1097/TP.0b013e3182754c53]

134 **Kern S**, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 2006; **24**: 1294-1301 [PMID: 16410387 DOI: 10.1634/stemcells.2005-0342]

135 **Wagner W**, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, Ho AD. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Exp Hematol* 2005; **33**: 1402-1416 [PMID: 16263424 DOI: 10.1016/j.exphem.2005.07.003]

136 **Rubio D**, Garcia-Castro J, Martín MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. *Cancer Res* 2005; **65**: 3035-3039 [PMID: 15833829 DOI: 10.1158/0008-5472.CAN-04-4194]

137 **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**: 553-563 [PMID: 18419597 DOI: 10.1111/j.1582-4934.2007.00146.x]

138 **Maccario R**, Moretta A, Cometa A, Montagna D, Comoli P, Locatelli F, Podestà M, Frassoni F. Human mesenchymal stem cells and cyclosporin a exert a synergistic suppressive effect on in vitro activation of alloantigen-specific cytotoxic lymphocytes. *Biol Blood Marrow Transplant* 2005; **11**: 1031-1032 [PMID: 16338626 DOI: 10.1016/j.bbmt.2005.08.039]

139 **Inoue S**, Popp FC, Koehl GE, Piso P, Schlitt HJ, Geissler EK, Dahlke MH. Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. *Transplantation* 2006; **81**: 1589-1595 [PMID: 16770249 DOI: 10.1097/01.tp.0000209919.90630.7b]

140 **Le Blanc K**, Götherström C, Ringdén O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Nordén-Lindeberg S, Jansson M, Dalton A, Aström E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 2005; **79**: 1607-1614 [PMID: 15940052]

141 **Eliopoulos N**, Stagg J, Lejeune L, Pommey S, Galipeau J. Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. *Blood* 2005; **106**: 4057-4065 [PMID: 16118325 DOI: 10.1182/blood-2005-03-1004]

142 **Buron F**, Perrin H, Malcus C, Héquet O, Thaunat O, Kholopp-Sarda MN, Moulin FT, Morelon E. Human mesenchymal stem cells and immunosuppressive drug interactions in allogeneic responses: an in vitro study using human cells. *Transplant Proc* 2009; **41**: 3347-3352 [PMID: 19857747 DOI: 10.1016/j.transproceed.2009.08.030]

143 **Hoogduijn MJ**, Crop MJ, Korevaar SS, Peeters AM, Eijken M, Maat LP, Balk AH, Weimar W, Baan CC. Susceptibility of human mesenchymal stem cells to tacrolimus, mycophenolic acid, and rapamycin. *Transplantation* 2008; **86**: 1283-1291 [PMID: 19005411 DOI: 10.1097/TP.0b013e31818aa536]

144 **Popp FC**, Eggenhofer E, Renner P, Slowik P, Lang SA, Kaspar H, Geissler EK, Piso P, Schlitt HJ, Dahlke MH. Mesenchymal stem cells can induce long-term acceptance of solid organ allografts in synergy with low-dose mycophenolate. *Transpl Immunol* 2008; **20**: 55-60 [PMID: 18762258 DOI: 10.1016/j.trim.2008.08.004]

145 **Otto WR**, Wright NA. Mesenchymal stem cells: from experiment to clinic. *Fibrogenesis Tissue Repair* 2011; **4**: 20 [PMID: 21902837 DOI: 10.1186/1755-1536-4-20]

146 **Zhou YF**, Bosch-Marce M, Okuyama H, Krishnamachary B, Kimura H, Zhang L, Huso DL, Semenza GL. Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. *Cancer Res* 2006; **66**: 10849-10854 [PMID: 17108121 DOI: 10.1158/0008-5472.CAN-06-2146]

147 **Miura M**, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, Patel V, Seo BM, Sonoyama W, Zheng JJ, Baker CC, Chen W, Ried T, Shi S. Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. *Stem Cells* 2006; **24**: 1095-1103 [PMID: 16282438 DOI: 10.1634/stemcells.2005-0403]

148 **Jeong JO**, Han JW, Kim JM, Cho HJ, Park C, Lee N, Kim DW, Yoon YS. Malignant tumor formation after transplantation of short-term cultured bone marrow mesenchymal stem cells in experimental myocardial infarction and diabetic neuropathy. *Circ Res* 2011; **108**: 1340-1347 [PMID: 21493893 DOI: 10.1161/CIRCRESAHA.110.239848]

149 **Casiraghi F**, Remuzzi G, Abbate M, Perico N. Multipotent mesenchymal stromal cell therapy and risk of malignancies. *Stem Cell Rev* 2013; **9**: 65-79 [PMID: 22237468 DOI: 10.1007/s12015-011-9345-4]

150 **Bernardo ME**, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. 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**: 9142-9149 [PMID: 17909019 DOI: 10.1158/0008-5472.CAN-06-4690]

151 **Tarte K**, Gaillard J, Lataillade JJ, Fouillard L, Becker M, Mossafa H, Tchirkov A, Rouard H, Henry C, Splingard M, Dulong J, Monnier D, Gourmelon P, Gorin NC, Sensebé L. Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. *Blood* 2010; **115**: 1549-1553 [PMID: 20032501 DOI: 10.1182/blood-2009-05-219907]

152 **Røsland GV**, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, Mysliwietz J, Tonn JC, Goldbrunner R, Lønning PE, Bjerkvig R, Schichor C. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res* 2009; **69**: 5331-5339 [PMID: 19509230 DOI: 10.1158/0008-5472.CAN-08-4630]

153 **Torsvik A**, Røsland GV, Svendsen A, Molven A, Immervoll H, McCormack E, Lønning PE, Primon M, Sobala E, Tonn JC, Goldbrunner R, Schichor C, Mysliwietz J, Lah TT, Mo-

taln H, Knappskog S, Bjerkvig R. Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track - letter. *Cancer Res* 2010; **70**: 6393-6396 [PMID: 20631079 DOI: 10.1158/0008-5472.CAN-10-1305]

154   **Prockop DJ**, Brenner M, Fibbe WE, Horwitz E, Le Blanc K, Phinney DG, Simmons PJ, Sensebe L, Keating A. Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy* 2010; **12**: 576-578 [PMID: 20735162 DOI: 10.3109/14653249.2010.507330]

155   **Dillmann J**, Popp FC, Fillenberg B, Zeman F, Eggenhofer E, Farkas S, Scherer MN, Koller M, Geissler EK, Deans R, Ladenheim D, Loss M, Schlitt HJ, Dahlke MH. Treatment-emergent adverse events after infusion of adherent stem cells: the MiSOT-I score for solid organ transplantation. *Trials* 2012; **13**: 211 [PMID: 23151227 DOI: 10.1186/1745-6215-13-211]