11.2

Mesenchymal stromal cells in treatment of renal ischemia/reperfusion injury

D.K. de Vries, A.F.M. Schaapherder, M.E.J. Reinders

Adapted version of Front Immunol. 2012;3:162

Introduction

Ischemia/reperfusion (I/R) injury is the exacerbation of tissue damage upon reestablishment of circulation after a period of ischemia. I/R injury is considered a major contributor to tissue damage in multiple clinical situations such as myocardial infarction, stroke and organ transplantation. In many clinical settings, the duration of ischemia is beyond control, and preventive and therapeutical measures are required to reduce the extent of I/R injury. Unfortunately, current treatment is primarily supportive. The pathophysiology of I/R injury is multifactorial and only partially understood. However, the general local reaction to reperfusion is thought to involve an inflammatory response that leads to tissue damage. In the quest for new therapeutical options for renal I/R injury, stem cells have come into play. With their multipotent immune modulating properties they hold promise to lead to improvement in the repair phase after I/R injury is evident.

Renal repair

In recent years, it has become clear that not only fibrotic repair but also restoration of damaged kidney tissue can occur. This has been best established for acute kidney injury, where resident tubular epithelial cells that survive a given damage dedifferentiate and subsequently re-enter the cell cycle and replace the necrotic tubular epithelium. In this process they take up an immature mesenchymal phenotype and re-express transcription factors that are involved in fetal nephrogenesis. More recently, glomerular epithelial repair, involving both local resident cells as well as multipotent progenitor cells has been described.[1]

Dedifferentiated cells outside the injured kidney may also migrate to the site of injury within the kidney. Kidney biopsies in male recipients of a female donor kidney with acute tubular necrosis showed presence of the male Y chromosome in renal tubular cells. No Y chromosome staining was seen in patients without acute tubular necrosis. This provides evidence that extra-renal cells may participate in renal regeneration.[2;3]

The call for better treatment strategies for I/R injury has directed research toward more encompassing cellular-based therapies, particularly aimed at the use of stem cells. The multi-factorial pathophysiology of I/R injury makes a pharmacological agent that has a single mechanistic target less likely to be therapeutically effective. In contrast, stem cells are versatile, and able to target a whole cascade of repair mechanisms simultaneously and successively, thereby improving organ protection and repair.

Mesenchymal stromal cells

Of all bone marrow-derived cells, mesenchymal stromal cells (MSCs) hold special promise in attenuating kidney injury, since nephrons are largely of mesenchymal origin and stromal cells are of crucial importance for signalling, leading to differentiation of both nephrons and collecting ducts. MSCs are characterized by three main criteria; 1) The ability to differentiate into osteoblasts, adipocytes and chondroblasts *in vitro*, 2) the expression of surface makers CD73, CD90 and CD105, and 3) plastic adherence in culture.[4]

MSCs have the ability to secrete numerous growth factors and cytokines that collectively stimulate mitogenesis, inhibit apoptosis and modulate immune responses. MSCs can alter cytokine secretion profiles of naïve and effector T cells,[5] DCs and natural killer cells to induce a more anti-inflammatory or tolerant phenotype.[6;7] It is proposed that in an inflammatory microenvironment, MSCs promote a Th1 to Th2 shift. Furthermore, MSCs have been reported to induce T-cell division arrest,[8] to inhibit differentiation and maturation of DCs[9] and to decrease production of inflammatory cytokines by various immune cell populations.[6] These immune modulating effects could be achieved both with autologous and allogeneic MSCs.

The mechanism of MSC-induced kidney repair has been addressed in numerous studies. There is growing evidence that the process of transdifferentiation is rare and it probably does not have any relevance to renal repair *in vivo*. The primary means of these cells most likely involve paracrine and endocrine effects; including mitogenic, anti-apoptotic, anti-inflammatory, antifibrotic and angiogenic influences (Figure 1).[10] The factors that mediate the paracrine effects are obviously of great interest. Several factors, such as IDO, VEGF, HGF and IGF-1 have been mentioned because they are abundant in MSC-conditioned medium.[11]

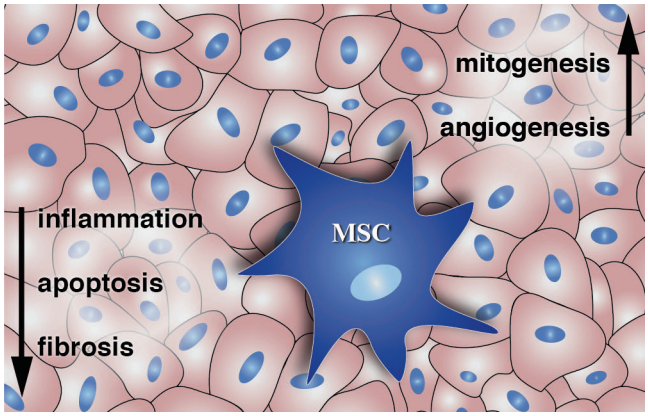

Figure 1: MSCs diminish damage and induce repair. Schematic illustration of the paracrine effects of MSCs on their environment. While stimulating repair by mitogenic and angiogenic effects, MSCs inhibit ongoing inflammation, apoptosis and later fibrosis of injured tissue.

An important aspect of the effect of MSCs is their ability to home to areas of injury or inflammation. Both animal and human studies have provided evidence that stem cells from hematopoietic tissues are able to engraft in nephrons as cells with tubular phenotype.[12;13] Exogenously administered MSCs can engraft into various injured structures in the kidney.[14-16] Recently, studies have shed light on the exact factors that facilitate homing of MSCs. Amid them, CD44 and hyaluronic acid interactions were shown to be crucial in recruiting exogenous MSCs to injured renal tissue and to enhance renal regeneration. Others have shown that stromal-derived factor-1 (SDF-1) and CXCR4 interactions may play a role in tubular homing.[14;17]

Sources of MSCs

While initially isolated from the bone marrow (bm), MSCs have now been identified within most tissues and are thought to represent a perivascular cell population involved in normal tissue homeostasis.[18] Indeed, MSCs have been isolated from adipose tissue, umbilical cord (uc) blood, placenta and different organs.[19-23] Recently MSCs have also been isolated from the human and mouse kidney. In mice these cells were extensively compared to bm cells.[24] Transcriptome and immunophenotype analysis of the renal MSC-like populations supported strong congruence with bmMSCs. Interestingly, despite this molecular congruence, distinct identities were found within the renal MSC-like population, suggesting organ-specific functions.[24] In addition, it is suggested that the adult mouse kidney contains interstitial mesenchymal cell progenitors that are able to provide paracrine support for surrounding vessels and tubular epithelial cells.[25] Future studies are needed to elucidate whether regeneration and functional repair of damaged kidney epithelium and endothelium can be enhanced via the resident renal stem cells. In the meantime, bmMSCs are the best

characterized population and currently more than 200 clinical trials are ongoing using bmMSCs (www.clinicaltrials.gov).

MSCs ameliorate renal ischemia/reperfusion injury in vivo

Although MSCs most probably do not replace damaged cells, evidence on beneficial effects of MSCs in renal I/R injury is accumulating in animal experiments. Intravenous injection of bm derived lineage-negative pluripotent cells after experimental renal I/R significantly attenuated the creatinine rise.[26] Peripherally administered purified MSCs were quickly present in peritubular capillaries and glomeruli after reperfusion, and functional and histological damage was significantly attenuated by MSC therapy.[27] Even when administered 24 hours after I/R injury, MSCs still were able to ameliorate damage.[27;28]

Different studies have reported beneficial effects of human MSCs on acute repair in the kidney.[29] The therapeutic potential of human bmMSCs was studied in immunodeficient NOD-SCID mice. Infused bmMSCs reduced renal cell apoptosis and increased proliferation after cisplatin-induced acute renal failure. bmMSCs also preserved the integrity of the tubular epithelium and peritubular vessels, and prolonged survival.[30] In search for new sources of MSCs for renal repair, human ucMSCs were shown to ameliorate both renal dysfunction and tubular cell injury, and prolong survival in cisplatin-induced acute kidney injury.[31] Furthermore, IGF-1 gene-silenced MSCs were limited in their protective effects. These findings indicate that MSCs exert beneficial effects on tubular cell repair in acute kidney injury by producing the mitogenic and pro-survival factor IGF-1.[32]

Clinical applications of MSCs in renal disease

There are only limited clinical data concerning MSC therapy in renal disease. The first phase I trial of autologous MSCs in kidney injury enrolls cardiac surgery patients with preexisting renal risk factors and therefore at high risk for developing acute kidney injury.[33] In addition, the first safety and feasibility data of autologous MSC administration in the week after kidney transplantation were recently published.[34] Although data are limited to two patients, MSC infusion appeared feasible and restricted memory T cell expansion while enlarging Treg population. However, both patients showed a transient increase in serum creatinine levels within two weeks after cell infusion. In one patient a renal biopsy was performed, which demonstrated a focal inflammatory infiltrate in the renal interstitium, consisting mainly of granulocytes with very few lymphocytes. It was suggested that the various soluble factors produced by MSCs also include proinflammatory mediators,[35-37] which eventually may have contributed to the intragraft recruitment of granulocytes, and deterioration of renal function. This may suggest that timing of infusion is of particular importance. Indeed, other studies have shown that the therapeutic properties of MSCs may largely depend on

the timing of their infusion.[38-40] Importance of timing is probably related to the necessity for the appropriate micro-environment to allow MSCs to acquire their anti-inflammatory properties. In our clinical trial we investigate safety and feasibility of autologous bmMSC treatment in patients with subclinical rejection and/or IF/TA in the renal biopsy at 4 weeks or 6 months after renal transplantation (Clinical trials NCT00734396). Hereby we expect to provide additional information about the importance of timing in the transplant setting.

Autologous versus allogeneic MSCs

Until now, most studies have focused on the use of autologous cells, since allogeneic cell transplantation has been reported to be connected with allograft rejection and possibly sensitization.[41;42] However, the use of autologous MSCs also has disadvantages. The cells need weeks of culture and it is still unclear whether autologous MSCs are affected by the renal disease. A few studies have reported influence of renal failure on MSC behavior. In mice, functional incompetence of MSCs was reported under uremic conditions.[43] In addition, in human MSCs it was shown that uremic serum induced an osteoblast-like phenotype in MSCs accompanied by matrix remodeling and calcification.[44]

MSC number, route of administration, and interaction with immunosuppressives

Alongside the cell source, the number of MSCs and the timing of administration are critical. In clinical trials to date, most investigators have used doses of $0,4x10^6$ to $10x10^6$/kg body weight.[45-47] However, no clear correlations have been made between cell dose and clinical effect. Dose escalation studies to monitor safety and efficacy are one of the major objectives for future studies of MSCs.

In most human trials, MSCs have been administered intravenously. Another possible successful route of administration includes intra-arterial or intrarenal infusion.[48-50] An advantage of these routes may be the direct administration at the place of injury, whereas disadvantages include the complexity and possible side effects such as obstruction of capillaries. To date, there are no reports of these treatment modalities in humans.

Current immunosuppressive drugs cannot be withheld from patients receiving MSC treatment after renal transplantation. Therefore, it is of importance that an optimal concurrent immunosuppressive regimen is chosen in which drugs have no negative impact on MSC function and vice versa. So far, this interaction has mainly been assessed by *in vitro* studies [51;52] and future studies are needed to elucidate their interaction with concurrent immunosuppression *in vivo* in order to facilitate successful translation to the clinic.

Possible hurdles of MSC treatment

Although cell therapy with MSCs holds enormous promise for the treatment of many diseases, unwanted side effects of MSC infusions must be assessed with the greatest care. Experimental studies have demonstrated maldifferentiation after injecting MSC directly into damaged tissue.[50;53] In addition, MSCs may adopt and unwanted, myofibroblast-like phenotype after administration.[54;55] Another important concern is that MSCs may differentiate into neoplastic cells or may cause promotion of tumor cell growth,[56-58] although an increased risk of tumor formation has never been confirmed in humans.[59] Currently, more than 2000 patients have been treated with allogeneic or autologous MSCs worldwide for a variety of diseases and so far no major side effects have been reported. However, still little is known about long-term side effects.

Summary

The pathophysiology of I/R injury is complex and characterized by inflammation, leading to tissue injury and graft dysfunction. Given current shortage of donor organs and usage of marginal donor kidneys for transplantation, novel treatment options to minimize renal I/R injury are urgently needed. Recent developments in stem cell research and derived clinical stem cell therapies have given reason to believe that such cell based treatments will become generally available in the near future. Although substantial additional time for the maturation of these therapies for routine clinical use is needed, the first steps of MSC based therapeutic strategies in the treatment of I/R injury have been taken.

References

1. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, Ronconi E, Meini C, Gacci M, Squecco R, Carini M, Gesualdo L, Francini F, Maggi E, Annunziato F, Lasagni L, Serio M, Romagnani S, Romagnani P: Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol* 17:2443-2456, 2006
2. Gupta S, Verfaillie C, Chmielewski D, Kim Y, Rosenberg ME: A role for extrarenal cells in the regeneration following acute renal failure. *Kidney Int* 62:1285-1290, 2002
3. Poulsom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnarasah S, Jeffery R, Hunt T, Alison M, Cook T, Pusey C, Wright NA: Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 195:229-235, 2001
4. Dominici M, Le BK, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E: Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315-317, 2006
5. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F: Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 101:3722-3729, 2003
6. Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105:1815-1822, 2005
7. Stagg J: Immune regulation by mesenchymal stem cells: two sides to the coin. *Tissue Antigens* 69:1-9, 2007
8. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F: Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 105:2821-2827, 2005
9. 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* 177:2080-2087, 2006
10. Ninichuk V, Gross O, Segerer S, Hoffmann R, Radomska E, Buchstaller A, Huss R, Akis N, Schlondorff D, Anders HJ: Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. *Kidney Int* 70:121-129, 2006
11. Togel F, Weiss K, Yang Y, Hu Z, Zhang P, Westenfelder C: Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am J Physiol Renal Physiol* 292:F1626-F1635, 2007
12. Poulsom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnarasah S, Jeffery R, Hunt T, Alison M, Cook T, Pusey C, Wright NA: Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 195:229-235, 2001
13. Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, Igarashi P: Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. *J Am Soc Nephrol* 14:1188-1199, 2003
14. Herrera MB, Bussolati B, Bruno S, Morando L, Mauriello-Romanazzi G, Sanavio F, Stamenkovic I, Biancone L, Camussi G: Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury. *Kidney Int* 72:430-441, 2007
15. Wong CY, Cheong SK, Mok PL, Leong CF: Differentiation of human mesenchymal stem cells into mesangial cells in post-glomerular injury murine model. *Pathology* 40:52-57, 2008
16. Ninichuk V, Gross O, Segerer S, Hoffmann R, Radomska E, Buchstaller A, Huss R, Akis N, Schlondorff D, Anders HJ: Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. *Kidney Int* 70:121-129, 2006
17. Togel F, Isaac J, Hu Z, Weiss K, Westenfelder C: Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. *Kidney Int* 67:1772-1784, 2005
18. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari

L, Huard J, Peault B: A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3:301-313, 2008

19. Toma JG, McKenzie IA, Bagli D, Miller FD: Isolation and characterization of multipotent skin-derived precursors from human skin. *Stem Cells* 23:727-737, 2005
20. 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* 13:4279-4295, 2002
21. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, Alison M, Remuzzi G: Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol* 15:1794-1804, 2004
22. Hoogduijn MJ, Gorjup E, Genever PG: Comparative characterization of hair follicle dermal stem cells and bone marrow mesenchymal stem cells. *Stem Cells Dev* 15:49-60, 2006
23. da Silva ML, Chagastelles PC, Nardi NB: Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci* 119:2204-2213, 2006
24. Pelekanos RA, Li J, Gongora M, Chandrakanthan V, Scown J, Suhaimi N, Brooke G, Christensen ME, Doan T, Rice AM, Osborne GW, Grimmond SM, Harvey RP, Atkinson K, Little MH: Comprehensive transcriptome and immunophenotype analysis of renal and cardiac MSC-like populations supports strong congruence with bone marrow MSC despite maintenance of distinct identities. *Stem Cell Res* 8:58-73, 2012
25. Plotkin MD, Goligorsky MS: Mesenchymal cells from adult kidney support angiogenesis and differentiate into multiple interstitial cell types including erythropoietin-producing fibroblasts. *Am J Physiol Renal Physiol* 291:F902-F912, 2006
26. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV: Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest* 115:1743-1755, 2005
27. Togel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C: Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* 289:F31-F42, 2005
28. Lange C, Togel F, Ittrich H, Clayton F, Nolte-Ernsting C, Zander AR, Westenfelder C: Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int* 68:1613-1617, 2005
29. Morigi M, Benigni A, Remuzzi G, Imberti B: The regenerative potential of stem cells in acute renal failure. *Cell Transplant* 15 Suppl 1:S111-S117, 2006
30. Morigi M, Introna M, Imberti B, Corna D, Abbate M, Rota C, Rottoli D, Benigni A, Perico N, Zoja C, Rambaldi A, Remuzzi A, Remuzzi G: Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. *Stem Cells* 26:2075-2082, 2008
31. Morigi M, Rota C, Montemurro T, Montelatici E, Lo C, V, Imberti B, Abbate M, Zoja C, Cassis P, Longaretti L, Rebulla P, Introna M, Capelli C, Benigni A, Remuzzi G, Lazzari L: Life-sparing effect of human cord blood-mesenchymal stem cells in experimental acute kidney injury. *Stem Cells* 28:513-522, 2010
32. Imberti B, Morigi M, Tomasoni S, Rota C, Corna D, Longaretti L, Rottoli D, Valsecchi F, Benigni A, Wang J, Abbate M, Zoja C, Remuzzi G: Insulin-like growth factor-1 sustains stem cell mediated renal repair. *J Am Soc Nephrol* 18:2921-2928, 2007
33. Humphreys BD, Bonventre JV: Mesenchymal stem cells in acute kidney injury. *Annu Rev Med* 59:311-325, 2008
34. Perico N, Casiraghi F, Introna M, Gotti E, Todeschini M, Cavinato RA, Capelli C, Rambaldi A, Cassis P, Rizzo P, Cortinovis M, Marasa 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* 6:412-422, 2011
35. Huang H, Kim HJ, Chang EJ, Lee ZH, Hwang SJ, Kim HM, Lee Y, Kim HH: IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. *Cell Death Differ* 16:1332-1343, 2009
36. Dahl SR, Kleiveland CR, Kassem M, Lea T, Lundanes E, Greibrokk T: Determination of thromboxanes, leukotrienes and lipoxins using high-temperature capillary liquid

chromatography-tandem mass spectrometry and on-line sample preparation. *J Chromatogr A* 1216:4648-4654, 2009

37. Bischoff DS, Zhu JH, Makhijani NS, Yamaguchi DT: Acidic pH stimulates the production of the angiogenic CXC chemokine, CXCL8 (interleukin-8), in human adult mesenchymal stem cells via the extracellular signal-regulated kinase, p38 mitogen-activated protein kinase, and NF-kappaB pathways. *J Cell Biochem* 104:1378-1392, 2008
38. Lange C, Togel F, Ittrich H, Clayton F, Nolte-Ernsting C, Zander AR, Westenfelder C: Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int* 68:1613-1617, 2005
39. Fang B, Shi M, Liao L, Yang S, Liu Y, Zhao RC: Systemic infusion of FLK1(+) mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice. *Transplantation* 78:83-88, 2004
40. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG: Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A* 100:8407-8411, 2003
41. 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* 108:2114-2120, 2006
42. Stagg J, Pommey S, Eliopoulos N, Galipeau J: Interferon-gamma-stimulated marrow stromal cells: a new type of nonhematopoietic antigen-presenting cell. *Blood* 107:2570-2577, 2006
43. Noh H, Yu MR, Kim HJ, Jeon JS, Kwon SH, Jin SY, Lee J, Jang J, Park JO, Ziyadeh F, Han DC, Lee HB: Uremia induces functional incompetence of bone marrow-derived stromal cells. *Nephrol Dial Transplant* 27:218-225, 2012
44. Kramann R, Couson SK, Neuss S, Kunter U, Bovi M, Bornemann J, Knuchel R, Jahnen-Dechent W, Floege J, Schneider RK: Exposure to uremic serum induces a procalcific phenotype in human mesenchymal stem cells. *Arterioscler Thromb Vasc Biol* 31:e45-e54, 2011
45. Le BK, 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, Ringden O: Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 371:1579-1586, 2008
46. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR, Jr., Moseley AB, Bacigalupo A: Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant* 11:389-398, 2005
47. Macmillan ML, Blazar BR, DeFor TE, Wagner JE: Transplantation of ex-vivo culture-expanded parental haploidentical mesenchymal stem cells to promote engraftment in pediatric recipients of unrelated donor umbilical cord blood: results of a phase I-II clinical trial. *Bone Marrow Transplant* 43:447-454, 2009
48. Ding Y, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ: Mesenchymal stem cells prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. *Diabetes* 58:1797-1806, 2009
49. Kunter U, Rong S, Djuric Z, Boor P, Muller-Newen G, Yu D, Floege J: Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. *J Am Soc Nephrol* 17:2202-2212, 2006
50. Kunter U, Rong S, Boor P, Eitner F, Muller-Newen G, Djuric Z, van Roeyen CR, Konieczny A, Ostendorf T, Villa L, Milovanceva-Popovska M, Kerjaschki D, Floege J: Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *J Am Soc Nephrol* 18:1754-1764, 2007
51. 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* 92:881-888, 2007
52. Maccario R, Podesta M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S, Frassoni F, Locatelli F: 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* 90:516-525, 2005

53. Breitbach M, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, Fries JW, Tiemann K, Bohlen H, Hescheler J, Welz A, Bloch W, Jacobsen SE, Fleischmann BK: Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood* 110:1362-1369, 2007

54. di Bonzo LV, Ferrero I, Cravanzola C, Mareschi K, Rustichell D, Novo E, Sanavio F, Cannito S, Zamara E, Bertero M, Davit A, Francica S, Novelli F, Colombatto S, Fagioli F, Parola M: Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. *Gut* 57:223-231, 2008

55. Wu GD, Nolta JA, Jin YS, Barr ML, Yu H, Starnes VA, Cramer DV: Migration of mesenchymal stem cells to heart allografts during chronic rejection. *Transplantation* 75:679-685, 2003

56. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA: Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449:557-563, 2007

57. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, Noel D, Jorgensen C: Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood* 102:3837-3844, 2003

58. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis MA, McElmurry RT, Bell S, Xia L, Zhou N, Riddle M, Schroeder TM, Westendorf JJ, McIvor RS, Hogendoorn PC, Szuhai K, Oseth L, Hirsch B, Yant SR, Kay MA, Peister A, Prockop DJ, Fibbe WE, Blazar BR: Sarcoma derived from cultured mesenchymal stem cells. *Stem Cells* 25:371-379, 2007

59. Centeno CJ, Schultz JR, Cheever M, Robinson B, Freeman M, Marasco W: Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. *Curr Stem Cell Res Ther* 5:81-93, 2010