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Optimization of MSC therapeutic strategies for improved GVHD treatment

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Abstract: Mesenchymal stem cells (MSCs) have a powerful immunosuppressive capacity, and they have been used to treat numerous immune diseases, such as refractory graft-versus-host disease. Nevertheless, there are conflicting clinical data. To our knowledge, MSCs from different donors do not share the same qualities and have different immunosuppressive capacities. Infused MSCs are cleared by the recipient's immune cells or macrophages. Therefore, the MSC therapeutic strategy might be the most important factor that determines treatment success. Repeated infusions would lead to a relatively stable MSC concentration, which would benefit a sustained therapeutic effect. In this review, we focus on the quality of MSCs and the associated therapeutic strategy, as well as other potential variables affecting their utility as a cellular pharmaceutical.

Keywords: mesenchymal stem cell, graft-versus-host disease, systematic review

1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a well-established form of therapy for malignant hematological disorders [1,2]. The treatment is complicated by treatment-related mortality because of infections, regimen-related toxicity, and engraftment failure [3-5]. Graft-versus-host disease (GVHD) is a pivotal factor that contributes to engraftment failure [6,7], despite an intensive preparative regimen, an adequate cell dose, and complete donor chimerism. Patients with grade III and grade IV acute GVHD have estimated 5-year survival rates of 25% and 5%, respectively [8].

Because of their powerful immunosuppressive ability, mesenchymal stem cells (MSCs) have been used in the treatment of immune diseases. Animal studies have shown that MSCs improve the engraftment of HSCs and ameliorate GVHD [9-12], and subsequent clinical data have further confirmed their efficacy in treating refractory GVHD [13-17]. For example, patients receiving co-transplantation of MSCs at the time of cord blood transplantation (CBT) had a significantly faster hematopoietic recovery of neutrophils and platelets than those receiving CBT alone ($P=0.02$ and 0.01 , respectively) [17].

However, negative results from a phase III trial on the use of MSCs to treat steroid-refractory acute GVHD almost ended this avenue of study [18]. A review analyzed the possible reasons that contribute to four distinct MSC product variables: donor variance, epigenetic reprogramming, immunogenicity, and cryopreservation [19]. Furthermore, another comprehensive study focused on discrepancies in clinical trials that used autologous MSCs to treat heart disease, finding that only trials containing flaws, such as in study design or reporting errors, showed positive outcomes [20,21]. Hence, investigators might need to identify the critical impacts of MSCs on therapeutic efficacy and optimize MSC therapeutic strategies according to different clinical indications. In this review, we focus on two pivotal parameters of MSC-based cytotherapy: quality of MSCs and therapeutic regimen (dose and frequency).

2 Quality of MSCs

Before addressing questions regarding the definition of, as well as the characteristics that are closely related to, the quality of MSCs, several characteristics that have been used to reflect the MSC quality, such as proliferative potential, the scale of cell senescence, and immunosuppression, will be discussed.

Aging, as well as long-term culture and diseases, exert a very important influence on the quality of MSCs. Several early investigations have provided evidence that the loss of differentiation potential and loss of proliferative potential are the most common age-related changes [22–26]. Age-related changes also include a decreased viable number of cells according to fibroblast colony-forming unit assay results and an increased number of senescent cells [26,27]. Furthermore, MSCs from aged individuals lose the ability to replenish progenitor cells [27,28].

After allogeneic HSCT, the frequency of bone marrow (BM)-MSCs within the BM-mononuclear cell (MNC) fraction was shown to be significantly reduced compared to healthy donors, which suggested that HSC transplantation may be the main reason for MSC damage [29,30]. BM-MSCs from hematological disease patients receiving high-dose chemotherapy display low levels of clonogenic potential and a defective proliferative capacity, indicating potential disadvantages of the use of autologous MSCs in chemotherapeutically pretreated patients [31]. Furthermore, the number of MSCs is estimated to be small in an average BM harvest (2–5 MSCs per 1×10^6 MNCs, suggesting that a BM graft composed of 2×10^8 MNCs/kg contains 400–1000 MSCs/kg) [32]. We have discussed and summarized the relation between abnormalities of self-MSCs and diseases [33]. Better clinical results can be obtained using healthy allogeneic MSCs (off-self).

After expansion in vitro, MSCs inevitably undergo replicative senescence [34–37]. In later passages, the number of enlarged MSCs increases [26], and telomeres are shortened [38]. Later-passage MSCs initiate significantly more instant blood-mediated inflammatory reactions [39] and activate the complement pathway [39,40], limiting their survival and function in vivo. It has been reported based on in vitro experiments that the immunosuppressive effects of MSCs did not differ from passage 2 to passage 7 [41]. However, in therapy-resistant GVHD patients, the 1-year survival rate was 75% in patients who received healthy donors' early-passage MSCs (from passages 1–2) in contrast to a rate of 21% when using later-passage MSCs (from passages 3–4) [42].

3 Therapeutic Strategy (Dose and Frequency)

When using MSCs to treat diseases, the dose may be the first important factor to consider. More evidence is needed to define the optimal dose. Patient conditions are more complicated than those of animal models. MSCs are not specific enough to exert an exclusive therapeutic effect on one condition, such as an immune reaction or a tissue injury. When an immune reaction and a tissue injury coexist, MSCs not only suppress the immune reaction but also repair the injured tissue. Therefore, the same dose might not be appropriate for different patients. The doses of MSCs commonly used in most clinical studies are listed in Table 1. However, questions such as what would happen when a higher dose of MSCs is used in a single infusion and whether there is a human maximum tolerated dose of MSCs remain unanswered.

Although one infusion of MSCs has been used in most clinical studies, increasing amounts of data suggest that multiple infusions could improve effectiveness. Studies have shown that <1% of transplanted cells survive longer than 1 week after injection for treatment of cardiac diseases [43,44], which means that multiple infusions are needed to maintain a therapeutic effect. This result is supported by animal experiments that showed that only multiple infusions of MSCs could prevent GVHD [45]. There was no difference with respect to efficacy between the low and high Prochymal® (industrial MSC product) doses if a single treatment was used [46]. Moreover, some GVHD patients needed repeated MSC infusions to obtain a better response [42,47,48]. Repeated infusions of MSCs were also shown to lead to better clinical outcomes in liver failure diseases [49,50], whereas a single treatment of MSCs failed to result in marked long-term (48 weeks) outcomes [51].

Table 1. Intervention characteristics of clinical studies on MSC-treated GVHD

Published years	Study design	Age of patients	Status of GVHD	MSC source	MSC passage	Frequency	Dose/infusion	Response
2004	1 patient: case report	9 years	Severe, treatment-resistant, grade IV, acute	Allogeneic BM	N	2 doses, separated by 77 days	1st: 2.0×10^6 /kg; 2nd: 1.0×10^6 /kg	100%
2008	Multicenter, phase II study	0.5–64 years	Severe, steroid-resistant, acute, grades II–IV	Allogeneic BM	1–4	27 patients: 1 dose; 22 patients: 2 doses; 6 patients: 3–5 doses	$0.4\text{--}9.0 \times 10^6$ /kg	Complete: 53%; others: 16%
2009	Randomized, multicenter, phase II trial	34–67 years	Acute, grades II–IV	Allogeneic BM	5	1 dose	Two groups: 2.0×10^6 /kg; 8.0×10^6 /kg	Complete: 77%; partial: 16%
2011	Multicenter	0.4–15 years	Severe, steroid-refractory, acute, grades III–IV	Allogeneic BM	N	Twice weekly for 4 weeks	2.0×10^6 /kg; 8.0×10^6 /kg	Complete: 58%; partial: 17%; mix: 25%
2012	Single center	1–67 years	Steroid-refractory, acute	Allogeneic BM	1–4	10 patients: 2–5 doses	$0.65\text{--}3.0 \times 10^6$ /kg	Complete: children: 83%; adults: 44%; MSCs 1–2P: 86%; MSCs 3–4P: < 36%
2013	Two centers	0.7–18 years	Steroid-refractory, acute, grades III–IV	Allogeneic BM	2–3	22 patients: 2 doses; 12 patients: 3–5 doses; 3 patients: > 6 doses	$0.9\text{--}3.0 \times 10^6$ /kg	Complete: 65%; partial: 22%
2014	Open-label, multicenter	0.2–17.5 years	Severe, steroid-refractory, acute, grades II–IV	Allogeneic BM	N	1 stage: twice weekly for 4 weeks; 2 stage: once weekly for 4 weeks	2.0×10^6 /kg	1 stage: complete: 61.3%; 2 stage: complete: 40%*
2016	Randomized, multicenter, double-blind controlled, phase II	N	Chronic	Allogeneic UC	N	1 dose per month for 2–4 months	3.0×10^7 per patient	Treatment: 72.6%; control: 51%

*40% patients achieved complete resolution of acute GVHD with additional 4 times MSC infusions when these patients failed to achieve complete response in the first stage treatment.

BM = bone marrow; GVHD = graft-versus-host disease; MSC = mesenchymal stem cell; UC = umbilical cord.

It is important to note that good clinical outcomes are obtained when MSCs are combined with drugs and/or other treatments, instead of therapy with MSCs alone. For example, MSC therapy for the treatment of acute GVHD reached 100% response with the addition of corticosteroid therapy [46].

4 Other Factors

The cell delivery route may have a critical effect on therapeutic efficacy for clinical translation. Most MSCs are trapped in the lungs after intravenous injection (>70%) [52]. Compared to intravenous injection, the intraperitoneal injection of MSCs resulted in better colitis recovery in the treatment of mice with dextran sulfate sodium (DSS)-induced colitis [53]. MSCs have the characteristic of chemotaxis, which enables their migration to sites of injured/inflammatory tissue. Preparing MSCs with optimal homing capacities might improve the efficacy of therapeutic applications [54].

It has been found that repeated infusions of MHC-mismatched MSCs from the same source leads to MSC alloimmunization and the subsequent rejection of MSCs by the host immune system [55–57]. These animal data suggest that using different sources is a better option for repeated infusions of MSCs.

Culturing MSCs in fetal calf/bovine serum containing xenoantigens may also enhance the intrinsic immunogenicity of MSCs [58,59]. The use of platelet lysate may also circumvent this possible problem [60]. Additionally, freshly thawed cryopreserved MSCs have been shown to upregulate heat shock proteins, be refractory to interferon- γ , and cooperate in suppressing CD3/CD28-driven T-cell proliferation [61]. Animal experiments have provided evidence that interferon- γ enhances the suppression function of MSCs [62–64]. However, following IFN- γ stimulation, MSCs induce the surface expression of *de novo* major histocompatibility complex (MHC) II [65] and present antigens as professional antigen-presenting cells [66,67]. Therefore, more clinical studies are needed to clarify whether MSCs require licensing by IFN- γ .

5 Discussion and Conclusion

Steroid-refractory GVHD is a devastating disease, and about half of the patients have a very poor overall survival rate of <10% [8,68]. Numerous animal data and results from small-scale clinical studies have demonstrated that promising outcomes can result from the powerful immunosuppressive capacity of MSCs. However, multiple transfusions of Prochymal failed to meet its primary clinical end point of achieving an increased GVHD overall complete response rate, compared with a placebo control, in a large-scale clinical trial [18]. Intrinsic heterogeneity, coupled with large-scale clinical manufacturing, may explain, in part, why data from MSC-based clinical trials are largely incongruent [69]. A meta-analysis review found that MSC treatment had a beneficial effect on the 6-month survival rate of patients with steroid-refractory acute GVHD and that survival did not differ with respect to age, MSC culture medium, or dose of MSCs delivered [70]. However, another clinical study showed that the response rate was better among children than among adults treated for GVHD (83% complete or partial response, compared with 44% for adults), and this result was reflected in a significantly better survival rate [42].

GVHD is an immune disease caused by host T-cells attacking the tissues of the transplant recipient. MSCs have a powerful immunosuppressive capacity to suppress T-cell activation and proliferation, which should contribute to GVHD treatment. To the best of our knowledge, conflicting clinical outcomes may be the result of differences in experimental design. Therefore, careful attention should be paid to the quality of MSCs (intrinsic heterogeneity) and to the therapeutic strategy.

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Authors' Contributions: HY Wang made the literature analysis and wrote, discussed, and revised the manuscript of this review. WH Kuang critically analyzed and corrected the manuscript. All authors read and approved the final manuscript.

References

- [1] Socie, G., Stone, J.V., Wingard, J.R., Weisdorf, D., Henslee-Downey, P.J., Bredeson, C., et al., Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry, *N. Engl. J. Med.*, 1999, 341(1), 14-21.
- [2] Hansen, J.A., Gooley, T.A., Martin, P.J., Appelbaum, F., Chauncey, T.R., Clift, R.A., et al., Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia, *N. Engl. J. Med.*, 1998, 338(14), 962-968.
- [3] Tabbara, I.A., Zimmerman, K., Morgan, C., Nahleh, Z., Allogeneic hematopoietic stem cell transplantation: complications and results, *Arch. Intern. Med.*, 2002, 162(14), 1558-1566.
- [4] Wingard, J.R., Majhail, N.S., Brazauskas, R., Wang, Z., Sobocinski, K.A., Jacobsohn, D., et al., Long-term survival and late deaths after allogeneic hematopoietic cell transplantation, *J. Clin. Oncol.*, 2011, 29(16), 2230-2229.
- [5] Gratwohl, A., Brand, R., Frassoni, F., Rocha, V., Niederwieser, D., Reusser, P., et al., Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. *Bone Marrow Transplant.*, 2005, 36(9), 757-769.
- [6] Deeg, H.J., How I treat refractory acute GVHD, *Blood*, 2007, 109(10), 4119-4126.
- [7] Lee, S.J., Vogelsang, G., Gilman, A., Weisdorf, D.J., Pavletic, S., Antin, J.H., et al., A survey of diagnosis, management, and grading of chronic GVHD, *Biol. Blood Marrow Transplant.*, 2002, 8(1), 32-39.
- [8] Cahn, J.Y., Klein, J.P., Lee, S.J., Milpied, N., Blaise, D., Antin, J.H., et al., Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Societe Francaise de Greffe de Moelle et Therapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMT) prospective study, *Blood*, 2005, 106(4), 1495-1500.
- [9] Polchert, D., Sobinsky, J., Douglas, G., Kidd, M., Moadsiri, A., Reina, E., et al., IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease, *Eur. J. Immunol.*, 2008, 38(6), 1745-1755.
- [10] Maitra, B., Szekely, E., Gjini, K., Laughlin, M.J., Dennis, J., Haynesworth, S.E., et al., Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation, *Bone Marrow Transplant.*, 2004, 33(6), 597-604.
- [11] Angelopoulou, M., Novelli, E., Grove, J.E., Rinder, H.M., Civin, C., Cheng, L., et al., Cotransplantation of human mesenchymal stem cells enhances human myelopoiesis and megakaryocytopoiesis in NOD/SCID mice, *Exp. Hematol.*, 2003, 31(5), 413-420.
- [12] Noort, W.A., Kruisselbrink, A.B., in't Anker, P.S., Kruger, M., van Bezooijen, R.L., de Paus, R.A., et al., Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice, *Exp. Hematol.*, 2002, 30(8), 870-878.
- [13] Le Blanc, K., Frassoni, F., Ball, L., Locatelli, F., Roelofs, H., Lewis, I., et al., Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study, *Lancet*, 2008, 371(9624), 1579-1586.
- [14] Le Blanc, K., Rasmusson, I., Sundberg, B., Gotherstrom, C., Hassan, M., Uzunel, M., et al., Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells, *Lancet*, 2004, 363(9419), 1439-1441.
- [15] Baron, F., Lechanteur, C., Willems, E., Bruck, F., Baudoux, E., Seidel, L., et al., Cotransplantation of mesenchymal stem cells might prevent death from graft-versus-host disease (GVHD) without abrogating graft-versus-tumor effects after HLA-mismatched allogeneic transplantation following nonmyeloablative conditioning, *Biol. Blood Marrow Transplant.*, 2010, 16(6), 838-847.
- [16] Lee, S.T., Jang, J.H., Cheong, J.W., Kim, J.S., Maeng, H.Y., Hahn, J.S., et al., Treatment of high-risk acute myelogenous leukaemia by myeloablative chemoradiotherapy followed by co-infusion of T cell-depleted haematopoietic stem cells and culture-expanded marrow mesenchymal stem cells from a related donor with one fully mismatched human leucocyte antigen haplotype, *Br. J. Haematol.*, 2002, 118(4), 1128-1131.
- [17] Wu, K.H., Sheu, J.N., Wu, H.P., Tsai, C., Sieber, M., Peng, C.T., et al., Cotransplantation of umbilical cord-derived mesenchymal stem cells promote hematopoietic engraftment in cord blood transplantation: a pilot study, *Transplantation*, 2013, 95(5), 773-777.
- [18] Allison, M., Genzyme backs Osiris, despite Prochymal flop, *Nat. Biotechnol.*, 2009, 27(11), 966-967.
- [19] Galipeau, J., The mesenchymal stromal cells dilemma – does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? *Cytotherapy*, 2013, 15(1), 2-8.
- [20] Nowbar, A.N., Mielewicz, M., Karavassilis, M., Dehbi, H.M., Shun-Shin, M.J., Jones, S., et al., Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis, *BMJ*, 2014, 348, g2688.

- [21] Abbott, A., Doubts over heart stem-cell therapy, *Nature*, 2014, 509(7498), 15-16.
- [22] Baxter, M.A., Wynn, R.F., Jowitt, S.N., Wraith, J.E., Fairbairn, L.J., Bellantuono, I., Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion, *Stem Cells*, 2004, 22(5), 675-682.
- [23] Majors, A.K., Boehm, C.A., Nitto, H., Midura, R.J., Muschler, G.F., Characterization of human bone marrow stromal cells with respect to osteoblastic differentiation, *J. Orthop. Res.*, 1997, 15(4), 546-557.
- [24] D'Ippolito, G., Schiller, P.C., Ricordi, C., Roos, B.A., Howard, G.A., Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow, *J. Bone Miner. Res.*, 1999, 14(7), 1115-1122.
- [25] Stolzing, A., Scutt, A., Age-related impairment of mesenchymal progenitor cell function, *Aging Cell*, 2006, 5(3), 213-224.
- [26] Stolzing, A., Jones, E., McGonagle, D., Scutt, A., Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies, *Mech. Ageing Dev.*, 2008, 129(3), 163-173.
- [27] Sethe, S., Scutt, A., Stolzing, A., Aging of mesenchymal stem cells, *Ageing Res. Rev.*, 2006, 5(1), 91-116.
- [28] Banfi, A., Muraglia, A., Dozin, B., Mastrogiacomo, M., Cancedda, R., Quarto, R., Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy, *Exp. Hematol.*, 2000, 28(6), 707-715.
- [29] Prata Kde, L., Orellana, M.D., De Santis, G.C., Kashima, S., Fontes, A.M., Carrara Rde, C., et al., Effects of high-dose chemotherapy on bone marrow multipotent mesenchymal stromal cells isolated from lymphoma patients, *Exp. Hematol.*, 2010, 38(4), 292 e4-300 e4.
- [30] Wang, B., Hu, Y., Liu, L., Hu, K., Tie, R., He, Y., et al., Phenotypical and functional characterization of bone marrow mesenchymal stem cells in patients with chronic graft-versus-host disease, *Biol. Blood Marrow Transplant.*, 2015, 21(6), 1020-1028.
- [31] Kemp, K., Morse, R., Wexler, S., Cox, C., Mallam, E., Hows, J., et al., Chemotherapy-induced mesenchymal stem cell damage in patients with hematological malignancy, *Ann. Hematol.*, 2010, 89(7), 701-713.
- [32] Bacigalupo, A., Mesenchymal stem cells and haematopoietic stem cell transplantation, *Best Pract. Res. Clin. Haematol.*, 2004, 17(3), 387-399.
- [33] Wang, H., Wu, M., Liu, Y., Are mesenchymal stem cells major sources of safe signals in immune system? *Cell. Immunol.*, 2012, 272(2), 112-116.
- [34] Shibata, K.R., Aoyama, T., Shima, Y., Fukiage, K., Otsuka, S., Furu, M., et al., Expression of the p16INK4A gene is associated closely with senescence of human mesenchymal stem cells and is potentially silenced by DNA methylation during in vitro expansion, *Stem Cells*, 2007, 25(9), 2371-2382.
- [35] Wagner, W., Horn, P., Castoldi, M., Diehlmann, A., Bork, S., Saffrich, R., et al., Replicative senescence of mesenchymal stem cells: a continuous and organized process, *PLoS One*, 2008, 3(5), e2213.
- [36] Wagner, W., Ho, A.D., Zenke, M., Different facets of aging in human mesenchymal stem cells, *Tissue Eng. Part B Rev.*, 2010, 16(4), 445-453.
- [37] Wagner, W., Senescence is heterogeneous in mesenchymal stromal cells: kaleidoscopes for cellular aging, *Cell Cycle*, 2010, 9(15):2923-4.
- [38] Bernardo, M.E., Zaffaroni, N., Novara, F., Cometa, A.M., Avanzini, M.A., Moretta, A., et al., Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms, *Cancer Res.*, 2007, 67(19), 9142-9149.
- [39] Moll, G., Rasmusson-Duprez, I., von Bahr, L., Connolly-Andersen, A.M., Elgue, G., Funke, L., et al., Are therapeutic human mesenchymal stromal cells compatible with human blood? *Stem Cells*, 2012, 30(7), 1565-1574.
- [40] Li, Y., Lin, F., Mesenchymal stem cells are injured by complement after their contact with serum, *Blood*, 2012, 120(17), 3436-3443.
- [41] Samuelsson, H., Ringden, O., Lonnies, H., Le Blanc, K., Optimizing in vitro conditions for immunomodulation and expansion of mesenchymal stromal cells, *Cytotherapy*, 2009, 11(2), 129-136.
- [42] von Bahr, L., Sundberg, B., Lonnies, L., Sander, B., Karbach, H., Hagglund, H., et al., Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy, *Biol. Blood Marrow Transplant.*, 2012, 18(4), 557-564.
- [43] Toma, C., Pittenger, M.F., Cahill, K.S., Byrne, B.J., Kessler, P.D., Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart, *Circulation*, 2002, 105(1), 93-98.
- [44] Song, H., Song, B.W., Cha, M.J., Choi, I.G., Hwang, K.C., Modification of mesenchymal stem cells for cardiac regeneration, *Expert Opin. Biol. Ther.*, 2010, 10(3), 309-319.
- [45] Tisato, V., Naresh, K., Girdlestone, J., Navarrete, C., Dazzi, F., Mesenchymal stem cells of cord blood origin are effective at preventing but not treating graft-versus-host disease, *Leukemia*, 2007, 21(9), 1992-1999.
- [46] Kebriaei, P., Isola, L., Bahceci, E., Holland, K., Rowley, S., McGuirk, J., et al., Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease, *Biol. Blood Marrow Transplant.*, 2009, 15(7), 804-811.
- [47] Gao, L., Zhang, Y., Hu, B., Liu, J., Kong, P., Lou, S., et al., Phase II multicenter, randomized, double-blind controlled study of efficacy and safety of umbilical cord-derived mesenchymal stromal cells in the prophylaxis of chronic graft-versus-host disease after HLA-haploidentical stem-cell transplantation, *J. Clin. Oncol.*, 2016, 34(24), 2843-2850.

- [48] Ball, L.M., Bernardo, M.E., Roelofs, H., van Tol, M.J., Contoli, B., Zwaginga, J.J., et al., Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease, *Br. J. Haematol.*, 2013, 163(4), 501-509.
- [49] Shi, M., Zhang, Z., Xu, R., Lin, H., Fu, J., Zou, Z., et al., Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients, *Stem Cells Transl. Med.*, 2012, 1(10), 725-731.
- [50] Li, Y.H., Xu, Y., Wu, H.M., Yang, J., Yang, L.H., Yue-Meng, W., Umbilical cord-derived mesenchymal stem cell transplantation in hepatitis B virus related acute-on-chronic liver failure treated with plasma exchange and Entecavir: a 24-month prospective study, *Stem Cell Rev.*, 2016, 12(6), 645-653.
- [51] Peng, L., Xie, D.Y., Lin, B.L., Liu, J., Zhu, H.P., Xie, C., et al., Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes, *Hepatology*, 2011, 54(3), 820-828.
- [52] Yukawa, H., Watanabe, M., Kaji, N., Okamoto, Y., Tokeshi, M., Miyamoto, Y., et al., Monitoring transplanted adipose tissue-derived stem cells combined with heparin in the liver by fluorescence imaging using quantum dots, *Biomaterials*, 2012, 33(7), 2177-2186.
- [53] Wang, M., Liang, C., Hu, H., Zhou, L., Xu, B., Wang, X., et al., Intraperitoneal injection (IP), Intravenous injection (IV) or anal injection (AI)? Best way for mesenchymal stem cells transplantation for colitis, *Sci. Rep.*, 2016, 6, 30696.
- [54] De Becker, A., Riet, I.V., Homing and migration of mesenchymal stromal cells: how to improve the efficacy of cell therapy? *World J Stem Cells*, 2016, 8(3), 73-87.
- [55] 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(13), 4057-4065.
- [56] Nauta, A.J., Westerhuis, G., Kruisselbrink, A.B., Lurvink, E.G., Willemze, R., Fibbe, W.E., Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting, *Blood*, 2006, 108(6), 2114-2120.
- [57] Zangi, L., Margalit, R., Reich-Zeliger, S., Bachar-Lustig, E., Beilhack, A., Negrin, R., et al., Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells, *Stem Cells*, 2009, 27(11), 2865-2874.
- [58] Sundin, M., Ringden, O., Sundberg, B., Nava, S., Gotherstrom, C., Le Blanc, K., No alloantibodies against mesenchymal stromal cells, but presence of anti-fetal calf serum antibodies, after transplantation in allogeneic hematopoietic stem cell recipients, *Haematologica*, 2007, 92(9), 1208-1215.
- [59] Horwitz, E.M., Gordon, P.L., Koo, W.K., Marx, J.C., Neel, M.D., McNall, R.Y., et al., Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone, *Proc. Natl. Acad. Sci. U.S.A.*, 2002, 99(13), 8932-8937.
- [60] Fekete, N., Gadelorge, M., Furst, D., Maurer, C., Dausend, J., Fleury-Cappellesso, S., et al., Platelet lysate from whole blood-derived pooled platelet concentrates and apheresis-derived platelet concentrates for the isolation and expansion of human bone marrow mesenchymal stromal cells: production process, content and identification of active components, *Cytotherapy*, 2012, 14(5), 540-554.
- [61] Francois, M., Copland, I.B., Yuan, S., Romieu-Mourez, R., Waller, E.K., Galipeau, J., Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing, *Cytotherapy*, 2012, 14(2), 147-152.
- [62] Ren, G., Zhang, L., Zhao, X., Xu, G., Zhang, Y., Roberts, A.I., et al., Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide, *Cell Stem Cell*, 2008, 2(2), 141-150.
- [63] Duijvestein, M., Wildenberg, M.E., Welling, M.M., Hennink, S., Molendijk, I., van Zuylen, V.L., et al., Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis, *Stem Cells*, 2011, 29(10), 1549-1558.
- [64] Krampera, M., Mesenchymal stromal cell 'licensing': a multistep process, *Leukemia*, 2011, 25(9), 1408-1414.
- [65] Romieu-Mourez, R., Francois, M., Boivin, M.N., 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(3), 1549-1558.
- [66] 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(6), 2570-2577.
- [67] Li, W., Ren, G., Huang, Y., Su, J., Han, Y., Li, J., et al., Mesenchymal stem cells: a double-edged sword in regulating immune responses, *Cell Death Differ.*, 2012, 19(9), 1505-1513.
- [68] Arai, S., Margolis, J., Zahurak, M., Anders, V., Vogelsang, G.B., Poor outcome in steroid-refractory graft-versus-host disease with antithymocyte globulin treatment, *Biol. Blood Marrow Transplant.*, 2002, 8(3), 155-160.
- [69] Phinney, D.G., Functional heterogeneity of mesenchymal stem cells: implications for cell therapy, *J. Cell. Biochem.*, 2012, 113(9), 2806-2812.
- [70] Hashmi, S., Ahmed, M., Murad, M.H., Litzow, M.R., Adams, R.H., Ball, L.M., et al., Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis, *Lancet Haematol.*, 2016, 3(1), e45-e52.