

Third-party Wharton's jelly mesenchymal stem cells for treatment of steroid-resistant acute and chronic graft-versus-host disease: a report of 10 cases

Dariusz BORUCZKOWSKI¹, Dominika GŁADYSZ¹, Sławomir RUMIŃSKI²,
Iwona CZAPLIKA-SZMAUS¹, Magdalena MURZYN¹, Artur OLKOWICZ¹, Krzysztof KAŁWAK³,
Monika MIELCAREK³, Katarzyna DRABKO⁴, Jan STYCZYŃSKI⁵, Mirosław MARKIEWICZ⁶,
Katarzyna PAWELEC^{1,7,*}, Maciej BORUCZKOWSKI⁸, Tomasz OŁDAK¹

¹Polish Stem Cell Bank, Warsaw, Poland

²Warsaw Medical University, Poland

³Department of Bone Marrow Transplantation, Pediatric Hematology and Oncology, Wrocław Medical University, Wrocław, Poland

⁴Department of Pediatric Hematology and Oncology and Transplantology, Medical University of Lublin, Lublin, Poland

⁵Department of Pediatric Hematology and Oncology, Collegium Medicum, UMK, Bydgoszcz, Poland

⁶Medical University of Silesia, School of Medicine in Katowice, Department of Hematology and Bone Marrow Transplantation, Katowice, Poland

⁷Department of Pediatric Hematology and Oncology, Medical University of Warsaw, Warsaw, Poland

⁸Poznan University of Medical Sciences, Poznan, Poland

Received: 13.08.2015 • Accepted/Published Online: 19.10.2015 • Final Version: 23.02.2016

Abstract: Severe graft-versus-host disease (GvHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT). Bone marrow mesenchymal stem cells (BM-MSCs) display immunosuppressive activity and have been previously shown to improve the outcome of GvHD therapy. We describe the use of umbilical cord mesenchymal stem cells (WJ-MSCs) expanded in serum-free medium for the treatment of acute GvHD (aGvHD) and chronic GvHD (cGvHD) in 10 patients. After 1 to 3 WJ-MSC infusions at a median dose of 1.5×10^6 cells/kg recipient body weight, a complete or partial response was observed in 4/7 patients with aGvHD and in 2/3 patients with cGvHD. Five out of 6 patients (83.3%) from the responders group and only 1 out of 4 nonresponders (25%) survived the follow-up. The deaths occurred in patients with acute GvHD. In the responders group, 2 patients showed complete remission of GvHD while symptoms of 4 patients were alleviated and the intensity of immunosuppressive therapy could be reduced. No serious adverse effects were observed. In conclusion, WJ-MSCs appear to be safe and can be successfully applied in the treatment of acute and chronic GvHD, but the therapeutic conditions should be optimized in prospective studies to maximize their efficacy.

Key words: Umbilical cord, mesenchymal stem cells, hematopoietic stem cell transplantation, graft-versus-host disease, Wharton's jelly

1. Introduction

Hematopoietic stem cell transplantations (HSCTs) can be required as a consequence of marrow-ablative high-dose chemotherapy in cases of several hematological and neoplastic disorders. Hematopoietic stem cells for those therapies are obtained usually from mobilized peripheral blood, bone marrow, or umbilical cord blood (Tabbara et al., 2002). Approximately 50% of patients receiving HSCTs develop acute or chronic graft-versus-host disease (aGvHD and cGvHD, respectively) (Pavletic et al., 2012). The disease is caused by donor immunological cells that recognize and attack host cells. Systemic inflammation evoked by donor cells has a destructive effect on many

organs of the recipient, including the skin, gastrointestinal tract, and liver (Blazar et al., 2012).

Mesenchymal stem cells (MSCs) are multipotent stromal cells with unusual immunoregulatory features (Le Blanc and Mougiakakos, 2012). They can be isolated from various adult and neonatal tissues, with bone marrow being the best-recognized source of MSCs, since their first characterization by Pitenger et al. (1999). MSCs are plastic adherent, have at least trilineage (osteoblasts, chondroblasts, and adipocytes) differentiation capacity in vitro, and show a characteristic surface antigen profile (Horwitz et al., 2005; Dominici et al., 2006). Additionally, MSCs are well recognized for their immunomodulatory

* Correspondence: katarzyna.pawelec@litewska.edu.pl

plasticity, due to their ability to inhibit as well as increase immune response (Wang et al., 2014).

Bone marrow mesenchymal stem cells (BM-MSCs) have been shown to inhibit proliferation of both CD4+ and CD8+ T lymphocytes upon activation through cellular or mitogenic stimuli (Di Nicola et al., 2002). Furthermore, BM-MSCs inhibited proliferation of lymphocytes stimulated by their cognate antigen and decreased the number of IFN- γ expressing CD8+ T cells (Krampera et al., 2013). The immunomodulatory effect of BM-MSCs on lymphocytes is manifested also by induction of differentiation of regulatory T cells, defined as CD4+CD25+ FoxP3+ or CTLA4+ from CD4+ T helper cells upon co-culture with BM-MSCs (Maccario et al., 2005; English et al., 2009). However, what is important from the clinical point of view is that BM-MSCs do not suppress virus-induced cytotoxic T cell proliferation or IFN- γ production, and therefore upon infusion will not compromise T cell mediated virus immunity (Karlsson et al., 2008). Those immunomodulatory properties are exerted through soluble factors such as transforming growth factor β (TGF- β), hepatocyte growth factor (HGF) (Di Nicola et al., 2012), and prostaglandin E2 as well as through direct cell-to-cell contact (English et al., 2009).

Due to their immunosuppressive activity, BM-MSCs have already been applied in experimental therapies and clinical studies for the treatment of GvHD. The first successful application of BM-MSCs was described by Le Blanc et al. (2004) in a boy with steroid-resistant grade IV aGvHD and resulted in complete remission. Another study showed successful treatment of aGvHD with $0.7\text{--}9 \times 10^6$ BM-MSCs per kg body weight in six out of eight patients (Ringdén et al., 2006). A large phase II study on the use of BM-MSCs in steroid-resistant aGvHD in both children and adults was conducted by Le Blanc et al. (2008). In total, 55 patients were treated with a median dose of 1.4×10^6 BM-MSCs per kg body weight. Approximately half of the patients received two or more cell infusions. Most of the cells used in the study were derived from HLA-mismatched third-party donors. Complete or partial treatment response was observed in 70% of the patients regardless of HLA identity. No toxic effects after cell infusions were noted. Another study (Kebriaei et al., 2009) used BM-MSCs in combination with steroids and confirmed high response rates: complete in 77% and partial in 16% of patients. The study administered two different MSC doses (2 or 8×10^6 per kg body weight), but the investigators did not notice any influence of the cell dose on the efficacy of stem cell therapy.

In contrast to aGvHD, data about the use of BM-MSCs for cGvHD therapy are limited. Intrabone marrow infusion of BM-MSCs was used to treat cGvHD after nonmyeloablative HSCT in 4 patients (Zhou et al., 2010).

BM-MSC infusions ameliorated clinical symptoms and all patients were alive after a follow-up period of 4–23 months. In another study (Weng et al., 2010), conducted in 19 patients, 14 achieved complete or partial treatment response within the median follow-up of 697 days. Fourteen patients survived the therapy and ten of them were able to taper or discontinue immunosuppressive therapy.

MSCs can be also isolated from Wharton's jelly, which is located within the umbilical cord. These umbilical cord mesenchymal stem cells (WJ-MSCs) are plastic adherent and are capable of multilineage differentiation. Similar to BM-MSCs, WJ-MSCs express characteristic mesenchymal surface antigens such as CD29, CD90, and CD105 although lacking the hematopoietic antigen CD34 (Secco et al., 2008; Troyer and Weiss et al., 2008). However, WJ-MSCs are more easily isolated as the umbilical cord can be collected without any additional medical procedures. The quantity of mesenchymal cells in the umbilical cord is higher than that in the bone marrow. In addition, WJ-MSCs have shorter doubling times, proliferate more efficiently, and can be cultured in vitro longer than BM-MSCs (Lu et al., 2006). Furthermore, WJ-MSCs display immunomodulatory properties greater than BM-MSCs. WJ-MSCs have been shown to inhibit proliferation of stimulated splenocytes and lymphocytes as well as expressing IL-6, which is associated with MSC-induced immunosuppression. What is important is that WJ-MSCs did not elicit lymphocyte proliferation and expressed low levels of co-stimulatory molecules (Weiss et al., 2008). In addition, WJ-MSCs inhibited production of IFN- γ and TNF- α by stimulated T cells (Yoo et al., 2009). Finally, WJ-MSCs decreased maturation of monocyte-derived dendritic cells (DCs) and inhibited the endocytic activity of DCs (Saeidi et al., 2013). Wu et al. (2011) described the use of WJ-MSCs for the treatment of aGvHD in 2 children. After 4 infusions of WJ-MSCs both patients showed complete treatment response and discontinued immunosuppressive therapy. This was probably the first clinical WJ-MSC application.

We would like to describe the results of a clinical study on the use of WJ-MSCs for the treatment of 10 patients with steroid-resistant GvHD including 7 patients diagnosed with aGvHD and 3 patients with cGvHD.

2. Materials and methods

2.1. Sampling and cell culture

All umbilical cord (UC) samples were obtained after patients provided informed consent. Ethical approval was given by the Bioethical Committee. UC samples were collected after natural delivery as well as cesarean sections. Transport conditions were monitored and tissue was processed within 48 h of delivery. UC fragments were

washed in a sterile saline with Antibiotic Antimycotic solution (Gibco, USA). They were dissected and blood vessels were removed. Wharton's jelly was minced into 2-cm³ scraps with a sterile lancet and placed into 6-well plates covered with MSC Attachment solution (Biological Industries, Israel) according to the manufacturer's recommendations and grown in serum-free medium for human mesenchymal stem cells NutriStem XF (Biological Industries, Israel) with NutriStem XF Supplement Mix (Biological Industries, Israel) with the addition of Antibiotic-Antimycotic solution (Gibco, Poland). The culture was incubated at 37 °C in 5% CO₂ in the air. The nonadherent cells were washed off and the attached cells were expanded further. Tissue explants were removed after 2–3 weeks of culture. Adherent cells were passaged upon reaching 90% confluence and reseeded at 1.2×10^4 cells/cm² in 75-cm² tissue culture flasks (BD, USA) for further culture expansion. After trypsinization with trypsin solution (Biological Industries, Israel) the number of cells was evaluated in a hemocytometer. When the required number of cells was obtained, they were transferred to a freezing bag and resuspended in human albumin (CSL Behring, USA) in the presence of 10% DMSO (WAK-Chemie, Germany), cooled with controlled rate freezer (Sy-Lab IceCube 14S, Austria), and then placed in the vapor phase of liquid nitrogen. Viability was determined on the basis of the thawed reference sample and counted by trypan blue exclusion in the hemocytometer. The viability of cells used for transplantation was between 95% and 97%.

2.2. Immunophenotyping of human umbilical cord MSCs

Characterization of human umbilical cord derived MSCs was carried out in accordance with the minimal criteria of MSCs described elsewhere (Dominici et al., 2006) by immunophenotyping using both MSC-positive and MSC-negative surface markers. Briefly, 60% to 80% confluent flasks of expanded MSCs were trypsinized and then incubated with following antibodies in darkness for 30 min. Cells were stained with antibodies against CD34 FITC, CD14 FITC, CD19 FITC, CD45 FITC, and HLA-DR FITC as a negative surface markers; CD73 PE, CD90 PE, CD105 PE, and HLA-ABC FITC as positive surface markers; and mouse IgG1 FITC with mouse IgG1 PE as controls. Then cells were washed with a cell wash solution (BD, USA), resuspended in Cell Fix solution (BD, USA), and finally analyzed using a FACS CALIBUR (BD, USA) flow cytometer. Flow cytometry analysis confirmed the mesenchymal phenotype of the cells.

3. Results

The study group varied in terms of age, number of infusions, previous treatment applied, the dose of cells,

and type of GvHD. Furthermore, the study was performed in 10 patients only; therefore, general analysis of clinical outcomes and statistical assessment were not feasible. Instead, clinical results will be presented as a case study.

3.1. Case series

3.1.1. aGvHD patients

Case 2. A 20-year-old woman, diagnosed with ALL, was subjected to bone marrow transplantation obtained from an HLA-fully matched unrelated donor. HSCT resulted in complete remission with positive minimal residual disease. However, the woman developed severe, grade IV aGvHD. The symptoms affected the gastrointestinal tract, liver, and skin. The patient received extended immunosuppressive treatment including etanercept (ETN), antithymocyte globulin, methotrexate, and monoclonal antibody treatment. Despite multiple treatment regimes, her bilirubin level reached critical levels of over 20 mg/dL. Therefore, 51 days after the GvHD diagnosis, the patient received WJ-MSCs at a dose of 1.1×10^6 cells per kg body weight. Cell infusion was repeated 7 days later at the same dose. Unfortunately, patient died 25 days after the second infusion due to hepatic encephalopathy.

Case 3. A 7-year-old girl was admitted to hospital due to ALL in second remission. She was subjected to BM-HSCT from an HLA-matched unrelated donor and developed grade IV aGvHD 19 days after the HSCT. The symptoms were restricted to the gastrointestinal tract, with stool volume over 4000 mL per day and presence of blood. Additional pharmacological treatment, which consisted of tacrolimus (FK506), intravenous immunoglobulin (IVIG), monoclonal antibodies, and ETN, was applied. Furthermore, the patient received a third party WJ-MSC infusion at a dose of 1.7×10^6 cells per kg body weight 22 days after the diagnosis of aGvHD. After cell infusion the girl was subjected to multiple extracorporeal photopheresis (ECP) procedures. The combined treatment resulted in remission of GvHD. Approximately 4 months after the diagnosis, the patient was discharged from hospital in good general condition, without symptoms of GvHD.

Case 4. The fourth case of WJ-MSC application was in a 5-year-old boy, who suffered from AML. The patient received two subsequent HSCTs from BM and mobilized peripheral blood. He developed grade IV aGvHD that affected his skin, gastrointestinal tract, and liver and resulted in microangiopathy. In addition to the standard therapy, he received the second-line pharmaceutical treatment with ETN. Furthermore, the boy was subjected to 6 ECP procedures. No clinical improvement was achieved; therefore, WJ-MSCs from a third-party donor were applied 36 days after the GvHD diagnosis. Two subsequent cell infusions were performed, with a total cell dose of 2.6×10^6 cells per kg body weight. Partial clinical improvement was obtained in terms of reduced stool volume and

disappearance of the obstruction. Unfortunately, the boy died 3 months after stem cell treatment due to bleeding to the central nervous system and sudden cardiac arrest.

Case 5. An 18-year-old man with ALCL in second complete remission was treated with HSCT from a 9/10 HLA-matched related donor. The HSCT caused development of grade IV aGvHD. The symptoms affected the gastrointestinal tract, skin, and liver. Despite extended treatment with ETN, ECP, anti-thymocyte globulin (ATG), and monoclonal antibodies, the clinical state of the patient did not improve. Therefore, 26 days after the diagnosis of GvHD, WJ-MSc infusion was performed. The cells were infused at a dose of 2.4×10^6 cells per kg body weight. He received two MSC doses; however, the GvHD symptoms were not alleviated after cell infusions. The patient died 4 months after cell application due to sepsis.

Case 6. A 3-year-old girl suffering from AML underwent a bone marrow transplantation from an unrelated, HLA-matched donor. After HSCT the patient was diagnosed with grade III acute GvHD that affected the gastrointestinal tract. Application of steroids did not result in clinical improvement. Additional treatment with ATG and monoclonal antibodies was applied. Despite therapy, GvHD symptoms persisted. Therefore, WJ-MSCs were applied in 3 subsequent infusions at a total dose of 4.9×10^6 cells per kg body weight. The first infusion was conducted 28 days after the diagnosis. As a result of cell therapy, a reduction in stool volume was noted and the patient could be fed orally.

Case 7. A 3½-year-old boy underwent HSCT from mobilized peripheral blood, from an unrelated, HLA-matched donor, due to ALL in second remission. After transplantation the patient was diagnosed with grade IV aGvHD. The symptoms were widespread, affecting the gastrointestinal tract, skin, liver, and bone marrow. In

addition to steroids, the patient received ETN, ATG, IVIG, and monoclonal antibodies but the pharmacological treatment did not lead to any improvement. MSCs were applied 38 days after the diagnosis in the form of one WJ-MSc infusion, using a dose of 1.6×10^6 cells per kg body weight. The cell infusion did not result in any clinical improvement. The patient died 17 days after the infusion due to GvHD progression and sepsis.

Case 8. A 3-year-old boy underwent transplantation due to AML transformed from MDS. The patient received HSCT from an HLA-matched, unrelated donor, which caused development of grade IV aGvHD. The disease was manifested in the gastrointestinal tract and skin. In addition to standard immunosuppressive treatment, ETN, ATG, and MoAB were applied. Furthermore, the patient was subjected to ECP. Due to lack of clinical improvement, he received one WJ-MSc infusion at a dose of 1.5×10^6 cells per kg body weight. After cell infusion, remission of the gastrointestinal tract symptoms occurred, but the skin symptoms were not significantly affected. Eventually, the symptoms were completely alleviated and the patient was discharged from hospital without GvHD manifestations.

For a summary of the aGvHD patients, please see Table 1.

3.1.2. cGvHD patients

Case 1. A 16-year-old patient suffering from MDS was given an HSCT from an unrelated donor. Despite preventive immunosuppressive treatment the patient showed an exacerbation of extensive cGvHD. The disease symptoms affected the skin, gastrointestinal tract, liver, mucosa, and bone marrow. Furthermore, the patient suffered from microangiopathy and dry eye syndrome. In addition to general immunosuppressive treatment she also received ETN and IVIG and was subjected to ECP. However, the treatment did not bring about a significant improvement. Therefore, 186 days after the diagnosis the patient received

Table 1. Treatment of acute GvHD with WJ-MSCs.

Case no.	GvHD	Treatment before MSCs	Time from diagnosis	Clinical outcome	Follow-up
2	Grade IV: S, GI, L	GCs, CsA, MMF, ETN, MoAB	51 days	NR	Death
3	Grade IV: GI	GCs, CsA, MMF, FK506, ETN, MoAB, IVIG, ECP	22 days	CR	Alive
4	Grade IV: S, GI, L, microangiopathy	GCs, CsA, MMF, ETN, IVIG, ECP	36 days	PR	Death
5	Grade IV: S, GI, L	GCs, CsA, MMF, ETN, ATG, MoAB, IVIG, ECP	26 days	NR	Death
6	Grade III: GI	GCs, CsA, MMF, ATG, MoAB	28 days	PR	Alive
7	Grade IV: S, GI, L, bone marrow	GCs, MMF, ETN, ATG, MoAB, IVIG	38 days	NR	Death
8	Grade IV: S, GI	GCs, CsA, MMF, ETN, ATG, MoAB, IVIG, ECP	24 days	CR	Alive

Abbreviations: GvHD – Graft-versus-host disease, S – skin, GI – gastrointestinal tract, L – liver, MSCs – mesenchymal stem cells, GCs – glucocorticoids, CsA – cyclosporine A, MMF – mycophenolate mofetil, ETN – entanercept, MoAB – monoclonal antibodies, FK506 – tacrolimus, IVIG – intravenous immunoglobulins, ECP – extracorporeal photopheresis, NR – no response, CR – complete response, PR – partial response.

2 WJ-MSC infusions with a total dose of 1.8×10^6 cells per kg body weight. The infusion was repeated 1 week after the first procedure. The cell injections resulted in complete remission of gastrointestinal tract symptoms and alleviation of symptoms affecting other organs. At the end of the follow-up period, the condition of the patient was classified as limited cGvHD. Immunosuppressive treatment could be reduced and the patient was discharged from hospital.

Case 9. The patient was an 18-year-old male who underwent HSCT due to SAA. The transplantation was repeated because of primary graft failure. After the second HSCT the patient was diagnosed with extensive cGvHD with symptoms manifested mainly in the skin. He was treated with standard immunosuppressive agents, GCs, CsA, and MMF. However, during long-term treatment his condition did not improve. Therefore, the patient was qualified for WJ-MSC administration. Nearly 2 years after the onset of cGvHD, he received 1 infusion with a dose of 1.1×10^6 cells per kg body weight. Three months from the infusion, the intensity of symptoms decreased significantly and the dose of immunosuppressants could be reduced. The patient was alive at the end of the follow-up period and suffered from limited cGvHD.

Case 10. A 17-year-old male was given HSCT due to AML in second remission. The transplantation caused the development of extensive cGvHD. The symptoms were restricted to the skin, which was affected by diffuse scleroderma. Prolonged immunosuppressive treatment with GCs, CsA, and MMF did not lead to clinical improvement. Therefore, 454 days after the diagnosis of cGvHD, the patient received a single injection of WJ-MSCs at a dose of 1.5×10^6 cells per kg of body weight. The cell injection did not lead to alleviation of cGvHD symptoms. Immunosuppressive treatment had to be continued. At the end of the follow-up period, the patient was alive and still suffering from extensive cGvHD.

For a summary of the cGvHD patients, please see Table 2.

3.2. Conclusions

MSC therapy was applied to 10 patients who were first diagnosed with myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), acute myeloid leukemia

(AML), severe aplastic anemia (SAA), or anaplastic large-cell lymphoma (ALCL). All of them underwent HSCT as a treatment for the primary disorder. After HSCT, despite prophylactic immunosuppressive treatment (CsA, MMF), patients developed GvHD. The group consisted of 7 aGvHD and 3 cGvHD cases. The age of the patients ranged from 3 to 20 years (median age of 11.5 years). GvHD symptoms were not alleviated despite GCs, CsA, and MMF treatment. A number of patients were given additional treatment, such as anti-TNF therapy (e.g., etanercept), monoclonal antibody treatment, and extracorporeal photopheresis (ECP). The only enrollment criterion was the lack of clinical improvement after standard therapy. MSC infusion was considered as a salvage therapy. Each patient received 1–3 infusions of third party WJ-MSCs. In the aGvHD group, the patients received the first dose 22–51 days (median of 28 days) after the diagnosis of GvHD. Patients suffering from cGvHD were treated with cells 186–625 days (median of 454 days) after the diagnosis. Cells were injected intravenously at a median dose of 1.5×10^6 cells per kg body weight (from 1.1 to 4.9×10^6 cells/kg).

A positive treatment response was observed in 6 out of 10 patients. In the group of positive responders, 2 patients suffering from aGvHD showed complete remission of symptoms with no signs of GvHD during follow-up. Four patients displayed amelioration of GvHD symptoms. This group consisted of 2 patients with cGvHD and 2 patients with aGvHD. One of the patients with aGvHD, who showed initial improvement, died after 3 months from cell infusion due to central nervous system bleeding. Four patients out of 10 enrolled in the study did not respond to cell infusions. One patient died within 4 months of the cell infusion due to progression of aGvHD symptoms. The condition of 1 patient suffering from cGvHD did not improve, but he stayed alive throughout the follow-up period. The survival rate at 1 year follow-up was 83.3% (5/6) for patients who responded to the therapy (complete remission or partial response) and only 25% (1/4) for nonresponders.

None of the patients showed any immediate or long-term toxic effects directly related to cell therapy. Only one patient had mild tachycardia, which did not need pharma-

Table 2. Treatment of chronic GvHD with WJ-MSCs.

Case no.	GvHD	Treatment before MSCs	Time from diagnosis	Clinical outcome	Follow-up
1	Extensive: S, GI, L, mucosa, bone marrow, microangiopathy, dry eye syndrome	GCs, CsA, MMF, ETN, IVIG, ECP	186 days	PR	Alive, currently treated with ECP
9	Extensive: S	GCs, CsA, MMF	625 days	PR	Alive
10	Extensive: S, with diffuse scleroderma	GCs, CsA, MMF	454 days	NR	Alive

Abbreviations: GvHD – Graft-versus-host disease, S – skin, GI – gastrointestinal tract, L – liver, MSCs – mesenchymal stem cells, GCs – glucocorticoids, CsA – cyclosporine A, MMF – mycophenolate mofetil, ETN – etanercept, IVIG – intravenous immunoglobulins, ECP – extracorporeal photopheresis, PR – partial response, NR – no response.

colytic treatment. As a result of the multiple therapeutic approaches applied, one of the patients showed an adverse side effect, which was stable, mild tachycardia.

4. Discussion

A number of studies describing the use of BM-MSCs for the treatment of aGvHD have been performed; see an excellent review by Amorin et al. (2014). However, very limited data on the use of WJ-MSCs exist. WJ-MSCs were successfully expanded in a chemically defined, serum-free medium, which makes the cell preparation protocol suitable for routine clinical application. We have observed positive effects of cell application in both aGvHD and cGvHD patients including 2 patients with complete remission of disease symptoms. Moreover, MSC therapy appears safe as none of the patients developed adverse effects. The study was performed in 10 patients only; therefore, detailed analysis of therapy outcome with relation to number of infusions, time from diagnosis to application, cell dose, type of GvHD, and age of patient could not be performed. WJ-MSC infusions were performed in severe GvHD cases where pharmacological treatment did not lead to clinical improvements.

Application of WJ-MSCs was also described by Wu et al. (2011) in 2 children with aGvHD. The patients received 2 or 3 infusions of cells before the symptoms disappeared. In this paper, the authors provide further data of seven aGvHD cases treated with WJ-MSCs. The patients received from 1 to 3 infusions, yet the therapy outcome did not correlate with the number of infusions. However, it is worth noting that 2 cases of complete disease remission were observed in patients who received only 1 infusion. Furthermore, those patients received cell infusions 22 and 24 days after the GvHD diagnosis, which were the earliest cell applications in the aGvHD group. This result suggests that timing rather than number of infusions is crucial for therapy outcomes. Early infusion of BM-MSCs has been shown to increase the frequency of complete remission cases in children, which reached 78% compared to 52% in late infusion cases (Ball et al., 2013). The same relationship probably exists for WJ-MSCs, but the hypothesis has to be verified experimentally.

We also provide data on the treatment of severe cGvHD with WJ-MSCs, which has not been described previously. Two out of 3 patients partially responded to cell infusion and alleviation of symptoms was observed. A different study described the results of BM-MSC infusion for treatment of cGvHD, where 4 patients showed improvement, but some symptoms persisted (Zhou et al., 2010). The outcomes of a study performed on a larger group of cGvHD patients were similar. Out of 19 patients treated, 10 showed partial response and complete remission was observed in 4 cases (Weng et al., 2010). Therefore, the first cases of cGvHD

treatment with WJ-MSCs are in agreement with previous studies utilizing BM-MSCs and indicate that WJ-MSCs can successfully treat cGvHD. Partial response of patients with cGvHD to WJ-MSC infusion might originate from the mechanism of MSCs' immunosuppressive activity. It has been demonstrated that BM-MSCs inhibit T cells and B cells upon exposure to IFN- γ (Krampera et al., 2003). The immunosuppressive functions of WJ-MSCs are also markedly more efficient in the presence of IFN- γ (Prasanna et al., 2010). As aGvHD is accompanied by high levels of IFN- γ , MSCs are primed towards immunosuppression after infusion. However, in cGvHD IFN- γ levels are lower (Blazar et al., 2012) and, as a consequence, suppression of T cell proliferation and activation by MSCs might be less efficient. Therefore, in future applications of MSCs for cGvHD, a transient exposure of cells to IFN- γ before infusion might provide additional stimulation of cells' immunosuppressive activity and thus improve therapy outcome. In an alternative approach, MSCs have been co-infused with hematopoietic stem cells to enhance engraftment and prevent GvHD. SAA has been treated with both BM-MSCs and WJ-MSCs co-infused during allogeneic HSCT. The co-infusions resulted in low risk of severe GvHD occurrence, efficient engraftment, and allowed a decrease in the intensity of immunosuppressive conditioning. However, the studies did not include a control group of patients who received HSCT without MSCs; therefore, lower incidence of GvHD cannot be unambiguously linked with WJ-MSC co-infusion (Li XH et al., 2014; Li ZY et al., 2014; Wu et al., 2014). Data gathered in preclinical studies on mice suggest that umbilical cord blood (UCB) and BM-MSC co-transplantation after bone marrow ablation resulted in reduced severity of GvHD compared to the control group that received only UCB (Li ZY et al., 2014). However, co-infusion of adipose tissue-derived MSCs and UCB in a model of relapsed chronic myeloid leukemia did not result in decreased frequency of GvHD occurrence (Oviedo et al., 2013). Therefore, administration of MSCs together with hematopoietic transplantation might not prevent GvHD if leukemic cells are present in circulation.

In conclusion, WJ-MSCs can be effectively isolated and expanded in a serum-free medium. Infusion of WJ-MSCs was safe and provided clinical improvement in several patients when applied as a salvage therapy for acute and chronic GvHD. WJ-MSC therapy for GvHD has to be further optimized in prospective studies. Based on our own experience and previous studies, in order to maximize therapeutic efficacy, we suggest that cells should be administered early after GvHD diagnosis and multiple infusions could be conducted if initial improvement is not observed.

References

- Amorin B, Alegretti AP, Valim V, Pezzi A, Laureano AM, da Silva MA, Wiecek A, Silla L (2014). Mesenchymal stem cell therapy and acute graft-versus-host disease: a review. *Hum Cell* 27: 137–150.
- Ball LM, Bernardo ME, Roelofs H, van Tol MJ, Contoli B, Zwaginga JJ, Avanzini MA, Conforti A, Bertaina A, Giorgiani G et al. (2013). 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* 163: 501–509.
- Blazar BR, Murphy WJ, Abedi M (2012). Advances in graft-versus-host disease biology and therapy. *Nat Rev Immunol* 12: 443–458.
- Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, Grisanti S, Gianni AM (2002). Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 99: 3838–3843.
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315–317.
- English K, Ryan JM, Tobin L, Murphy MJ, Barry FP, Mahon BP (2009). 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* 156: 149–160.
- Fu Y, Wang Q, Zhou J, Liu S, Fang B, Wei X, Song Y (2013). Reduced intensity conditioning and co-transplantation of unrelated peripheral stem cells combined with umbilical cord mesenchymal stem/stroma cells for young patients with refractory severe aplastic anemia. *Int J Hematol* 98: 658–663.
- Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A (2005). Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 7: 393–395.
- Karlsson H, Samarasinghe S, Ball LM, Sundberg B, Lankester AC, Dazzi F, Uzunel M, Rao K, Veys P, Le Blanc K et al. (2008). Mesenchymal stem cells exert differential effects on alloantigen and virus-specific T-cell responses. *Blood* 112: 532–541.
- Kebriaei P, Isola L, Bahceci E, Holland K, Rowley S, McGuirk J, Devetten M, Jansen J, Herzig R, Schuster M et al. (2009). Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol Blood Marrow Transplant* 15: 804–811.
- Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F (2003). Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 101: 3722–3729.
- Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M et al. (2008). Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 371: 1579–1586.
- Le Blanc K, Mougiakakos D (2012). Multipotent mesenchymal stromal cells and the innate immune system. *Nat Rev Immunol* 12: 383–396.
- Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363: 1439–1441.
- Li XH, Gao CJ, Da WM, Cao YB, Wang ZH, Xu LX, Wu YM, Liu B, Liu ZY, Yan B et al. (2014). Reduced intensity conditioning, combined transplantation of haploidentical hematopoietic stem cells and mesenchymal stem cells in patients with severe aplastic anemia. *PLoS One* 9:e89666.
- Li ZY, Wang CQ, Lu G, Pan XY, Xu KL (2014). Effects of bone marrow mesenchymal stem cells on hematopoietic recovery and acute graft-versus-host disease in murine allogeneic umbilical cord blood transplantation model. *Cell Biochem Biophys* 70: 115–122.
- Lim JY, Park MJ, Im KI, Kim N, Jeon EJ, Kim EJ, Cho ML, Cho SG (2014). Combination cell therapy using mesenchymal stem cells and regulatory T-cells provides a synergistic immunomodulatory effect associated with reciprocal regulation of TH1/TH2 and th17/treg cells in a murine acute graft-versus-host disease model. *Cell Transplant* 23: 703–714.
- Lu LL, Liu YJ, Yang SG, Zhao QJ, Wang X, Gong W, Han ZB, Xu ZS, Lu YX, Liu D et al. (2006). Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Haematologica* 91: 1017–1026.
- Maccario R, Podestà M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S et al. (2005). 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.
- Oviedo A, Yañez R, Colmenero I, Aldea M, Rubio A, Bueren JA, Lamana ML (2013). Reduced efficacy of mesenchymal stromal cells in preventing graft-versus-host disease in an in vivo model of haploidentical bone marrow transplant with leukemia. *Cell Transplant* 22: 1381–1394.
- Pavletic SZ, Fowler DH (2012). Are we making progress in GVHD prophylaxis and treatment? *Hematology Am Soc Hematol Educ Program* 2012: 251–264.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143–147.

- Prasanna SJ, Gopalakrishnan D, Shankar SR, Vasandan AB (2010). Pro-inflammatory cytokines, IFN γ and TNF α , influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. *PLoS One* 5: e9016.
- Ringdén O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, Lönnies H, Marschall HU, Dlugosz A, Szakos A, Hassan Z et al. (2006). Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation* 81: 1390–1397.
- Saeidi M, Masoud A, Shakiba Y, Hadjati J, Mohyeddin Bonab M, Nicknam MH, Latifpour M, Nikbin B (2013). Immunomodulatory effects of human umbilical cord Wharton's jelly-derived mesenchymal stem cells on differentiation, maturation and endocytosis of monocyte-derived dendritic cells. *Iran J Allergy Asthma Immunol* 12: 37–49.
- Secco M, Zucconi E, Vieira NM, Fogaça LL, Cerqueira A, Carvalho MD, Jazedje T, Okamoto OK, Muotri AR, Zatz M (2008). Multipotent stem cells from umbilical cord: cord is richer than blood! *Stem Cells* 26: 146–150.
- Tabbara IA, Zimmerman K, Morgan C, Nahleh Z (2002). Allogeneic hematopoietic stem cell transplantation: complications and results. *Arch Intern Med* 162: 1558–1566.
- Troyer DL, Weiss ML (2008). Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells* 26: 591–599.
- Wang Y, Chen X, Cao W, Shi Y (2014). Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* 15: 1009–1016.
- Weiss ML, Anderson C, Medicetty S, Seshareddy KB, Weiss RJ, VanderWerff I, Troyer D, McIntosh KR (2008). Immune properties of human umbilical cord Wharton's jelly-derived cells. *Stem Cells* 26: 2865–2874.
- Weng JY, Du X, Geng SX, Peng YW, Wang Z, Lu ZS, Wu SJ, Luo CW, Guo R, Ling W et al. (2010). Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. *Bone Marrow Transplant* 45: 1732–1740.
- Wu KH, Chan CK, Tsai C, Chang YH, Sieber M, Chiu TH, Ho M, Peng CT, Wu HP, Huang JL (2011). Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. *Transplantation* 91: 1412–1416.
- Wu Y, Cao Y, Li X, Xu L, Wang Z, Liu P, Yan P, Liu Z, Wang J, Jiang S et al. (2014). Cotransplantation of haploidentical hematopoietic and umbilical cord mesenchymal stem cells for severe aplastic anemia: successful engraftment and mild GVHD. *Stem Cell Res* 12: 132–138.
- Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, Lee JE, Kim YJ, Yang SK, Jung HL et al. (2009). Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cell Immunol* 259: 150–156.
- Zhou H, Guo M, Bian C, Sun Z, Yang Z, Zeng Y, Ai H, Zhao RC (2010). Efficacy of bone marrow-derived mesenchymal stem cells in the treatment of sclerodermatous chronic graft-versus-host disease: clinical report. *Biol Blood Marrow Transplant* 2010 16: 403–412.