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Review Article

Update on the Use of Mesenchymal Stem Cells and their Products in Hematopoietic Stem Cell Transplantation

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Abstract

Graft Versus Host Disease (GVHD) is a major limitation to the success of allogeneic Hematopoietic Stem Cell Transplantation (HSCT) as Steroid-Refractory (SR) acute GVHD carries poor prognosis due to the absence of an efficacious second-line therapy. Mesenchymal Stem Cells (MSCs) which have immunosuppressive, immunomodulatory, and regenerative properties may become a highly effective therapeutic modality for SR-GVHD in the near future.

MSCs have already been approved to treat childhood SR-GVHD in Japan, and they have been conditionally licensed in New Zealand and Canada. It is expected that MSCs will be approved for the treatment of SR-GVHD in adults in Europe, North America, and other parts of the world within a few years. Utilization of the recently introduced techniques including the use of MSC products such as exosomes and Extracellular Vesicles (ECVs) instead of the parent MSCs, robotic manufacturing technology, and genetic engineering of MSCs will ultimately overcome the remaining obstacles facing the widespread utilization of MSCs and their products as therapeutics not only in HSCT but also in other medical fields. The aim of this review is to provide an update on the remarkable progress achieved in the use of MSCs and their products in the field of HSCT.

More Information

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Introduction

Over the last 50 years, Hematopoietic Stem Cell Transplantation (HSCT) has been successfully used to treat various benign and malignant hematologic diseases [1-3]. Despite the recent advances in stem cell therapies, acute and chronic Graft Versus Host Disease (GVHD) remain major limitations to the success of allogeneic HSCT as they constitute leading causes of morbidity and mortality in recipients of allogeneic stem cell transplantation [3,4]. GVHD is a severe inflammatory condition that results from an immune-mediated attack on the recipient tissues by donor T-cells contained in the allogeneic graft [1]. Acute and chronic GVHD have several risk factors related to the: primary disease, donor, recipient, conditioning therapy prior to HSCT, stem cell source and dose, and GVHD prophylaxis in addition to other factors as shown in Table 1 [5-9]. As per the MAGIC criteria, acute GVHD is classified into 5 clinical stages and 5 overall grades as demonstrated in Tables 2 and 3 respectively [10,11]. The current and future therapeutic modalities for acute GVHD are shown in Table 4 [10,12-14]. However, systemic steroids remain the standard first-line treatment of acute GVHD, but there is no standard second-line treatment available for SR or steroid-dependent acute GVHD [10,12-14].

Poor Graft Function (PGF) after HSCT is a rare but life-threatening complication that occurs after allogeneic transplantation and has a poor prognosis [15-18]. PGF is defined as follows: severe cytopenia affecting at least 2 cell lines; persistent thrombocytopenia with platelet count ≤ 20 x 10^9 / Liter (L); hemoglobin ≤ 70 grams / L; neutrophils ≤ 0.5 x 10^9 / L for at least 3 days to 2 consecutive weeks taking place beyond day +28 post-HSCT with hypocellular Bone Marrow (BM) and full donor chimerism without severe GVHD or disease relapse [16-19]. Primary PGF refers to incomplete engraftment or no hematopoietic recovery post-HSCT while secondary PGF refers to loss of donor cells after initial engraftment [17,18]. Primary PGF carries a very poor prognosis with a one-year Overall Survival (OS) of 25% and a 2-year OS of only 6% [18,19]. Patients with primary PGF have a lower

Table 1: Risk Factors for Acute and Chronic Graft Versus Host Disease.
1. Primary disease: chronic myeloid leukemia rather than acute leukemia.
2. Disparity or mismatching and use of unrelated donors or HLA A-26.
3. Older age of the recipient and donor.
4. Cytomegalovirus positivity in both recipient and donor.
5. Race: white/black more than Asian or Hispanic.
6. Previous donor alloreactivity, history of blood transfusion, or pregnancy in female donors.
7. Karnofsky performance score < 90.
8. Receipt of more than one hematopoietic stem cell transplantation.
9. High-intensity conditioning therapy such as total body irradiation and cyclophosphamide.
10. Graft versus host disease prophylaxis other than methotrexate and cyclosporine-A.
11. Peripheral blood as a source of stem cells.
12. Total number of CD 19 + stem cells in allograft ≥ 82 X 10^6 cells/kg body weight.
13. Total number of CD 3 + stem cells in allograft ≥ 325 X 10^6 cells/kg body weight.

Table 2: Staging of Acute Graft Versus Host Disease (GVHD) according to MAGIC Criteria.

Clinical Stage	Lower Gastrointestinal Tract	Upper Gastrointestinal Tract	Liver profile (Bilirubin in mg/dL)	Skin [% rash of body surface area (BSA)]
0	Diarrhea < 500 ml/day OR < 3 episodes/day	None OR Intermittent anorexia, nauseas or vomiting	< 2	No active erythematous (GVHD) rash
1	Diarrhea 500 - 999 ml/day OR 3 - 4 episodes/day	Persistent anorexia, nausea, vomiting	2 - 3	< 25% of BSA Maculopapular rash
2	Diarrhea 1000 - 1500 ml/day OR 5 - 7 episodes/day	-------	3.1 - 6	25% - 50% of BSA Maculopapular rash
3	Diarrhea > 1500 ml/day	-------	6.1 - 15	> 25 % of BSA Maculopapular rash
4	Severe abdominal pain with/without ileus OR grossly bloody diarrhea regardless of the stool volume	-------	> 15	Bullae / desquamation > 5% Generalized erythema > 50% of BSA

Table 3: Overall Grading of Acute Graft Versus Host Disease According to MAGIC Criteria.

Grade	Stage Lower Gastrointestinal Tract (Stool output/day)	Stage Upper Gastrointestinal Tract	Stage Liver profile (Bilirubin in mg/dL)	Stage Skin (Active erythema only)
0	0	0	0	0
I	0	0	0	1 - 2
II	1	1	1	3
III	2 - 3	***	2 - 3	***
IV	4	***	4	4

*** These manifestations are not required for grading.

response rate to treatment and poorer prognosis compared to secondary PGF [17]. Incidence of PGF ranges between 5% and 27% (16-18,20). The pathogenetic mechanisms responsible for the evolution of PGF are complex, poorly understood, and may be related to: BM microenvironment components particularly MSCs and endothelial cells, and damage caused by the pre-HSCT conditioning therapy, BM suppression during HSCT, and drugs as well as specific post-HSCT complications such as GVHD, Veno-Occlusive Disease (VOD) of the liver and infections [18-20]. The risk factors for PGF and the available therapeutic modalities for graft failure and PGF after HSCT are shown in Table 5 [16-20] and Table 6 [15,17] respectively.

General outline of mesenchymal stem cells

Mesenchymal Stem Cells (MSCs); which were first described by Alexander Friedenstein in the 1960s; are heterogeneous, non-hematopoietic, adult multipotent stromal progenitor cells that are capable of self-renewal and differentiation into multiple lineages and various cell types [21-26]. They can be isolated from several sources including BM, peripheral blood, umbilical cord blood, placenta, amniotic fluid, adipose tissue, dental pulp, synovial fluid, salivary glands, as well as skin, lung, liver, and skeletal muscle tissues [21-26]. MSCs have the following distinguishing features: (1) being plastic adherent; (2) ability to differentiate into osteoblasts, adipocytes, and chondrocytes; and (3) having characteristic surface markers on flow cytometry as they are characteristically positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD11b, CD14, CD19, CD79a, and HLA-DR [21-27]. MSCs have immunomodulatory, immunosuppressive properties, as well as antimicrobial actions that enable them to have several therapeutic and clinical applications including: (1) enhancement of engraftment as well as prevention and treatment of GVHD in recipients of allogeneic HSCT; (2) treatment of several autoimmune disorders such as systemic lupus erythematosus, systemic sclerosis, and type 1 diabetes mellitus; (3) role in regenerative medicine and tissue repair including treatment of myocardial ischemia and infarction, as well as chronic non-healing wounds, and spinal cord injuries; (4) neurological disorders such as multiple sclerosis; (5) bone and cartilage diseases such as osteoarthritis and osteogenesis imperfecta; and (6) treatment of various infectious

Table 4: Current and Future Therapies of Acute Graft Versus Host Disease.
1. Corticosteroids: intravenous methylprednisolone and oral prednisolone
2. Anti-thymocyte globulin: a. Induction of B-cell lineage apoptosis. b. Induction of T-regulatory cells and natural killer (NK) T-cells.
3. Infliximab: anti-tumor necrosis factor-α (TNF-α) monoclonal antibody.
4. Etanecept: anti-TNF-α monoclonal antibody.
5. Ruxolitinib: a. JAK1 / JAK2 inhibito.r b. Inhibition of proinflammatory cytokines. c. Suppression of T-cell expansion. d. Promotion of T-regulatory cell proliferation.
6. Alemtuzumab: a. Anti-CD52 monoclonal antibody. b. Targets T-cells, B-cells, natural killer cells, monocytes, and macrophages.
7. Daclizumab: a. Humanized monoclonal antibody against interleukin (IL)-2Rx. b. Inhibits activated T-cells.
8. Basiliximab: a. Chimeric monoclonal antibody against IL-2Rx. b. Inhibits activated T-cells.
9. Inolimomab: a. Murine monoclonal antibody against interleukin (IL)-2Rx. b. Inhibits activated T-cells.
10. Extracorporal photopheresis
11. Mesenchymal and decidual stem cells: immunomodulatory activity (of IL-10).
12. Methotrexate: a. Inhibition of dihydrofolate reductase. b. production of thymidine and purines. c. Suppression of T-cell response, proliferation, and expression of adhesion molecules.
13. Other agents: mycophenolate mofetil, pentostatin, sirolimus.
14. Other novel therapies in clinical trials: a. Fecal microbiota transplantation. b. α1 anti-trypsin. c. Anti-CD3/CD7 immunotoxin. d. Vedolizumab: a monoclonal antibody that recognizes integrin α4β7 present on circulating lymphocytes and inhibits their relocation to the gastrointestinal tract.

Table 5: The risk factors for poor graft function after hematopoietic stem cell transplantation (HSCT).
1. Low CD34+stem cell dose infused.
2. Non-sibling donor for allogeneic HSCT.
3. Donor-recipient blood group mismatching.
4. Donor-specific anti-human leukocyte antigen antibodies.
5. Factors related to the primary disease such as splenomegaly and bone marrow fibrosis.
6. Iron overload with high serum ferritin level.
7. Graft versus host disease.
8. Veno-occlusive disease of the liver.
9. Viral infections particularly cytomegalovirus.
10. Post-HSCT infections with positive blood cultures .
11. Damage to the bone marrow microenvironment .
12. Early admission to the intensive care unit in the post-HSCT period.

Table 6: The available therapeutic modalities for poor graft function following hematopoietic stem cell transplantation (HSCT).
1. Treatment of the cause if identified.
2. Provision of full supportive measures including granulocyte-colony stimulating factor, platelet and packed red blood cell transfusions, and use of appropriate antimicrobial agents.
3. Administration of CD34+ - selected stem cell boost.
4. Administration of mesenchymal stem cells or their products .
5. Thrombopoietin receptor agonists such as eltrombopag.
6. N-acetyl-cysteine.
7. Second allogeneic HSCT.

complications such as sepsis and acute respiratory distress syndrome [21,22,25,28].

The role of mesenchymal stem cells in hematopoietic stem cell transplantation

The BM niche is composed of several cell types including MSCs, endothelial cells, Treg cells, macrophages, osteoblasts, osteoclasts, neuronal tissue, and adipocytes as well as secretory products such as cytokines which play vital roles in maintaining homeostasis and regulating the functions of hematopoietic stem cells (17-19). BM-derived MSCs play a crucial role in the regulation of hematopoiesis [29]. In

addition to supporting hematopoiesis, MSCs are capable of modulating immune and inflammatory responses and participating in tissue repair [30,31]. MSCs promote an immunosuppressive and immunoregulatory environment via multifactorial mechanisms that include the transfer of mitochondria, secretion of proteins/peptides/hormones, and transfer of exosomes or microvesicles containing RNA and other molecules [32]. The clinical applications of MSCs in HSCT include prevention and treatment of GVHD, enhancement of hematopoietic engraftment and prevention of engraftment failure, reduction in aplasia post-chemotherapy, acceleration of lymphocyte recovery, and repair of tissue damage [30,31,33-35].

Use of mesenchymal stem cells in the treatment of poor graft function following hematopoietic stem cell transplantation

In a study that included 8 patients with graft failure after allogeneic HSCT, a second allogeneic HSCT was performed and intra-osseous injection of donor MSCs was given, donor hematopoiesis was restored in 75% of patients indicating the contribution of MSCs to the success of the second allograft and patient survival [36]. In three studies that included 55 patients with PGF following HSCT, MSCs obtained from BM of third-party donors were infused. The Overall Response (OR) rate was approximately 73% and this was translated into improvement in white blood cell count, hemoglobin level, and platelet count, and consequently, transfusion independence for platelets and packed Red Blood Cells (RBCs) was achieved. However, significant numbers of treated patients developed viremia due to cytomegalovirus and Epstein-Barr virus and at least 5 patients developed post-transplant lymphoproliferative diseases [15,37,38]. In a phase 1 clinical trial, 21 patients with incomplete hematopoietic recovery after HSCT were treated with escalating doses of placenta-derived MSCs (ranging from $1x10^6$ cells/kg to $4x10^6$ cells/kg). Blood counts improved in almost all patients, peaking between 6 and 9 months after MSC therapy and these elevated blood counts were maintained for 12 months post-MSC therapy. The increases in blood counts were reflected by transfusion independence for platelets and packed RBCs in a significant number of patients [39].

Use of mesenchymal stem cells in the treatment and prevention of graft versus host disease following hematopoietic stem cell transplantation

MSCs have the following characteristic properties which make them suitable for treatment of acute GVHD: (1) low immunogenicity as they express low levels of HLA class I and II and they lack expression of co-stimulatory molecules; (2) under pro-inflammatory conditions MSCs have immunomodulatory effects such as induction of M2 macrophages to reduce inflammation, promotion of T-cell regulation, and promotion of helper T-cell suppression; (3) migration into areas of inflammation and thus they do not induce systemic immunosuppression; and (4) ability to produce interleukin (IL)-6, IL-8, and granulocyte-monocyte colonystimulating factor which may recruit and activate neutrophils to enhance the antimicrobial effect and promote the local protective immune system [40-44]. Amelioration of acute GVHD by the therapeutic infusion of BM-MSC-derived ECVs has been shown to be associated with the preservation of circulating naive T cells, possibly due to the unique microRNA profiles of BM-MSC-derived ECVs [45]. Also, once ECVs of MSCs are given in combination with HSCs, they can modulate the immune system and inhibit the development of GVHD following HSCT [29,30].

In the year 2004, Le Blanc, et al. published the first case report of a 9-year-old boy with grade IV acute GVHD which resolved dramatically after infusion of MSCs obtained from the BM [40,43,44]. In September 2015, an MSC product; TEMCELL; was approved by the Japanese government as one of the first cellular and tissue products for the treatment of SR acute GVHD in children following the promising results of 2 clinical trials (phase 1/2 and phase 2/3) [40,46,47]. As the safety and therapeutic potential of the clinical application of MSCs in HSCT has been well established by numerous clinical trials, commercial MSC products for pediatric SR-GVHD have already been licensed in Japan, conditionally licensed in Canada and New Zealand, and may obtain approval by the Food and Drug Administration in United States of America (USA) soon [34].

Systematic reviews and meta-analyses on the use of mesenchymal stem cell in graft versus host disease

Six systematic reviews and meta-analyses, that included 181 studies comprising 8103 patients, on the use of MSCs in prevention of GVHD, treatment of SR-acute and SR-chronic GVHD showed the following results: (1) in patients with acute GVHD, 39% - 67% of patients achieved complete response (CR: resolution of all signs of acute GVHD]; and one-third of patients achieved partial response (PR: decrease in staging and severity but no resolution of all signs of acute GVHD); (2) in patients with chronic GVHD, 23% of patients achieved CR while 66% of patients achieved PR; (3) acute GVHD of the skin responded to MSC therapy better than acute GVHD of the liver or gastrointestinal tract; (4) acute GVHD grade II responded to MSCs much better than grades III and IV acute GVHD; (5) children, younger than 10 years of age, with acute GVHD showed better responses than adults with acute GVHD; (6) not only response to MSC therapy but also OS correlated well with the dose of MSCs administered; (7) patients who made more benefit from MSC therapy were: patients with mild degrees of tissue damage, those with lower levels of pro-inflammatory chemokines, and patients with higher proportions of naïve T and B-cells and immature dendritic cells; and (8) once used prophylactically, MSC treatment was effective in reducing the incidence of chronic GVHD, the OS was increased by 17%, and improvement of engraftment was achieved [48-53]. Additionally, one major review that included 9 studies on

the use of MSCs in the treatment of SR acute GVHD showed: (1) CR of SR acute GVHD was achieved in up to 50% - 83% of patients; (2) CR, but not PR, was associated with prolonged OS; and (3) no serious adverse effects of MSC therapy were reported [54]. On the contrary, one systematic review and meta-analysis that included 9 clinical trials (CTs) comprising 309 patients showed no statistically significant effect of co-transplantation of multipotent MSC in the setting of allogeneic HSCT [55]. Also, prophylactic cotransplantation of MSCs in addition to HSCs in patients with severe aplastic anemia undergoing haploidentical HSCT failed to show efficacy in preventing GVHD after HSCT [56].

Phase 3 randomized controlled trials on use of mesenchymal stem cells in treatment of steroid refractory-acute graft versus host disease

In the first phase 3 CT (study 275; NCT00759018), 241 children with grades II to IV SR acute GVHD at 50 sites in 8 countries were included. The mean age was 9.6 years, 39% of patients were females and 60% of patients were white. Patients with grades III and IV acute GVHD constituted 30% and 50% of the entire study group respectively. Patients were given 8 biweekly intravenous (IV) infusions of human MSCs at doses of 2 x 10^6/kg for 4 weeks. Patients who achieved partial or mixed responses were given the option of 4 more weekly MSC infusions after day +28 of treatment. The primary end-point was OR [the sum of partial response (PR) and CR] at day +28 of therapy. The secondary end-point was survival through day +100 of therapy [57]. Response rates to MSC therapy were: 65.1% OR, 14.1% CR, and 51.3% PR. OR rates at day +28 of therapy were: 72.9% for patients with grade II, 67.1% for grade III, and 60.8% for grade IV SR acute GVHD. OR at day +100 of MSC therapy was 66.9%. Day +100 survival for responders and non-responders at day +28 of treatment were 82.1% and 38.6% respectively (with *p* value less than 0.001). Additionally, remstemcel-L was generally well tolerated without infusion toxicities or specific safety concerns. This study, which was an update on the remestemcel-L expanded access program, confirmed the reported clinical and survival benefits of remestemcel-L therapy in children with acute GVHD who have exhausted all conventional therapeutic options [57].

The second phase 3 CT (NCT00366145) is a randomized multicenter study that was performed between August 2006 and May 2009 to assess the efficacy of *ex vivo* cultured adult human MSCs; remestemcel-L; in addition to the second line treatment for SR-acute GVHD according to institutional standards. Two hundred and sixty patients with ages ranging between 6 months and 70 years were included. The patients were randomized 2:1 to have either remestemcel-L or placebo. In the study group, a total of 8 injections of remestemcel-L were administered IV weekly over 4 weeks. In patients achieving incomplete response at day +28 of therapy, 4 additional doses of remestemcel-L were given over the following 4 weeks. Durable CR was defined as the complete resolution of acute GVHD symptoms for any period of at least 28 days after starting MSC therapy [58]. Unfortunately, remestemcel-L did not meet the primary endpoint of greater durable CR in the intent-to-treat population [35% (study group) versus 30% (control group) with a *p* - value of 0.42]. In post-hoc analysis, the following results were highlighted: (1) patients with liver involvement by GVHD who received at least 1 infusion of remestemcel-L had higher durable CR and higher overall CR and PR than those who received placebo [29% versus 5% with a *p* - value of 0.047]; (2) among high-risk patients, with grades III and IV acute GVHD, remestemcelL demonstrated significantly higher OR at day+28 than placebo [58% versus 37 with a *p* - value of 0.03]; and (3) pediatric patients had higher OR with MSCs compared to placebo [64% versus 23% with a *p* - value of 0.05]. Additionally, the safety and tolerability of remestemcel-L were well illustrated. So, the results of the study did not demonstrate superior durable CR compared to placebo when remestemcel-L was added to the standard of care [58].

In the third phase 3 multicenter, prospective, single-arm CT (NCT02336230), 54 children with SR acute GVHD were included. No treatment other than corticosteroids was given prior to MSCs. The MSC product; remestemcel-L; was administered at doses of 2 x 10^6/kg twice weekly for 4 weeks [59]. ORs on days +28 of treatment were 70.4% in the treatment arm and 45% in the control arm with a *p* - value of 0.0003, while ORs in the treatment arm on days +100 and +180 were 74.1% and 68.5% respectively. The CR rates on days +28 and +100 of treatment were 29.6% and 44.45 respectively. Survival rates on days +100 and +180 of MSC therapy in responders were 86.8% and 78.9% and in nonresponders 47.1% and 43.8% respectively. Additionally, OR on day +28 was highly predictive of improved survival through 180 days of MSC therapy. Remestemcel-L therapy was well tolerated without identified infusion-related reactions or other safety concerns. The study provided robust prospective evidence of the safety, tolerability, and efficacy of remestemcel-L as first-line salvage therapy after steroid failure in pediatric SR acute GVHD [59]. However, extended follow-up showed evidence of long-term benefit in treated children as OS was reported to be 63% at 1 year, 51% at 2 years, and 49% at 4 years [60]. In August 2023, it was announced that the Food and Drug Administration in the USA has provided a complete response to its Biologics License Application resubmission for remestemcel-L for the treatment of SR acute GVHD in children [61].

The fourth phase 3 CT, was performed at 9 centers in China between September 2014 and March 2019 and included 203 patients with SR-acute GVHD. Patients were randomized 1:1 to second-line treatment for GVHD or MSCs plus second-line treatment. The primary end-point was OR at day 28 of treatment, while the secondary and safety end-points were:

durable OR at day +56 of treatment, failure-free survival, OS, chronic GVHD, infection, hematological toxicity, and disease relapse. Finally, 198 patients completed the study. The mean age was 30.1 years and 40.4% of patients were females [44]. The final results of the CT were as follows: (1) ORs at day +28 were 82.8% and 70% for the study group and the control group respectively with a *p* - value of 0.043; (2) durable ORs at day +56 were 78.8% and 64.6% for the study group and the control group respectively with a *p* - value of 0.027; (3) median failure-free survivals were 11.3 months and 6 months for the study group and the control group respectively with a *p* - value of 0.024; (4) the 2-year cumulative incidences of chronic GVHD were 39.5% and 62.7% for the study group and the control group respectively with a *p* - value of 0.005; (5) the 3-year cumulative incidences of disease relapse were 10.1% and 13.5% respectively with a *p* - value of 0.610; and (6) within 180 days of treatment, the most common adverse events were: infections in 65.7% of the study group and 78.8% of the control group, and hematological toxicity in 37.4% of the study group and 53.5% of the control group [44]. So, the CT showed that compared to the second-line therapy alone, the combination of MSCs and second-line therapy for SR acute GVHD not only superior efficacy but also higher safety and tolerability, in addition to lower rates of infections, chronic GVHD, and hematological toxicity [44].

Allogeneic versus autologous mesenchymal stem cells in hematopoietic stem cell transplantation

Off-the-shelf allogeneic human MSC products are clinically available to treat acute GVHD, but real-world data have revealed the limitations of these products as well as their feasibility, safety, and efficacy. However, there is emerging evidence that the immunomodulatory and regenerative effects of MSCs can be recapitulated by ECVs released from MSCs. MSC-ECVs may have advantages over parental MSCs as drugs because of their distinguished biodistribution and importantly dose-dependent therapeutic effects [62]. Also, autologous and culture-recovered MSCs have been shown to be safe in the setting of refractory GVHD following HCT for hematologic malignancy, with the most notable clinical responses in patients with acute GVHD [63].

Use of mesenchymal stem cells in chronic graft versus host disease

Studies have shown that MSCs do not contribute to the pathogenesis of chronic GVHD. Hence, it is feasible to use autologous cell therapy in patients who are not completely responding to standard immunosuppressive therapy for chronic GVHD [64]. Also, third-party MSCs can be prepared and stored frozen to be used for the treatment of SR-extensive chronic GVHD therapy [65]. The use of MSCs in the treatment of chronic GVHD has yielded responses in approximately two-thirds of patients [66,67]. Additionally, MSCs may prevent chronic GVHD after allogeneic HSCT and increase the survival rate by increasing the ratio of CD4/CD8 and the proportion of regulatory T cells *in vivo* [65].

Due to their immunomodulatory and pro-angiogenic characteristics, MSCs have been extensively explored as new cell-based therapies in the treatment of different eye diseases [68]. In patients with ocular GVHD, MSCs differentiate in corneal epithelial cells, suppress eye inflammation, and restore the function of lacrimal glands [69]. MSC-derived exosomes, which address all the safety issues related to the transplantation of their parental cells including the risk of unwanted differentiation and aggravation of intraocular inflammation, have been shown to limit the extent of eye injury and inflammation [68,69]. Also, MSC-derived exosomes and ECVs contain MSC-derived growth factors and immunoregulatory proteins that can easily by-pass all biological barriers in the eyes and deliver their cargo directly in injured corneal epithelial cells and eye-infiltrated leukocytes, modulating their viability and function [69]. MSCs appear to exert their effects by triggering the generation of CD8(+) CD28 (-) T cells, which may regulate the balance between Th1 and Th2. Hence, transfusion of MSCs has improved the clinical symptoms in about 55% of patients with refractory dry eye secondary to chronic GVHD [70].

The challenges facing the clinical application of mesenchymal stem cells therapies and methods of improving the outcomes of these therapies

Factors that still limit the wide use of MSCs in the treatment of GVHD include variability in MSC donor types, production methodology, dose regimens, and variations in study design. Additionally, extensive culture expansion of primary donor-derived MSCs leads to marked changes in functionality, and hence there is a high level of inter-donor variability in MSC properties [32]. The following maneuvers have been used to improve the outcome of MSC therapies: (1) genetic engineering/manipulation to enhance expression of genes regulating survival, proliferation, and immunomodulation leading ultimately to enhancement of therapeutic efficacy of MSCs as the use of gene therapy and gene-editing technologies to produce next-generation MSCs has been shown to improve functionality, specificity, and responsiveness to MSC therapies; (2) preconditioning of MSCs with pro-inflammatory cytokines such as tumor necrosis factor-α, IL-17, and IL-1β and treatment with immune receptor agonists including toll-like receptor agonists, prior to transplantation, in order to stimulate the expression of immunomodulatory factors by MSCs and to prepare them for the inflammatory environment of the target tissues; (3) the recent manufacturing innovations and use of automated, robotic and closed production systems so as to provide the most efficient manufacturing strategy; and (4) the use of MSC products, mainly ECVs and exosomes, that have the following advantages over the parent MSCs: safety with less adverse effects particularly predisposition to infections and malignancy compared to the parent MSCs, more stability and

reversibility, and lower possibility of immune rejection due to having small size and lower expression of membrane-bound molecules such as histocompatibility molecules [29,30,32, 71-74].

Conclusion and future perspectives

Over the last 50 years, HSCT has been successfully utilized to treat a variety of benign and malignant disorders, but the widespread use of allogeneic HSCT is limited by the development of GVHD. Acute and chronic SR-GVHD are associated with poor outcomes as there are no efficacious or curative therapies yet.

Hopefully, the recently published as well as the ongoing clinical studies particularly the RCTs on the use of MSCs and their products in the treatment of SR-GVHD will pave the way for not only the approval but also the widespread use of MSCs and their products in the treatment of acute and chronic SR-GVHD at all ages. It is expected that the use of MSC-derived ECVs and exosomes, the recently adopted robotic manufacturing techniques, and genetic engineering of MSCs will bypass the remaining obstacles and safety issues related to the use of MSCs not only in HSCT but also the treatment of other difficult to treat medical conditions. Not only allogeneic MSC products which have become commercially available recently but also autologous MSCs have been shown to be safe and effective in the treatment of specific allogeneic HSCT complications such as PGF as well as acute and chronic GVHD.

References

1. Kim EJ, Kim N, Cho SG. The potential use of mesenchymal stem cells in hematopoietic stem cell transplantation. Exp Mol Med. 2013 Jan 10;45(1):e2. doi: 10.1038/emm.2013.2. PMID: 23306700; PMCID: PMC3584660.
2. Zhou X, Jin N, Wang F, Chen B. Mesenchymal stem cells: a promising way in therapies of graft-versus-host disease. Cancer Cell Int. 2020 Apr 7; 20:114. doi: 10.1186/s12935-020-01193-z. PMID: 32280306; PMCID: PMC7137413.
3. Wolff D, Fatobene G, Rocha V, Kröger N, Flowers ME. Steroid-refractory chronic graft-versus-host disease: treatment options and patient management. Bone Marrow Transplant. 2021 Sep;56(9):2079-2087. doi: 10.1038/s41409-021-01389-5. Epub 2021 Jul 3. PMID: 34218265; PMCID: PMC8410585.
4. Nassereddine S, Rafei H, Elbahesh E, Tabbara I. Acute Graft Versus Host Disease: A Comprehensive Review. Anticancer Res. 2017 Apr;37(4):1547-1555. doi: 10.21873/anticanres.11483. PMID: 28373413.
5. Hahn T, McCarthy PL Jr, Zhang MJ, Wang D, Arora M, Frangoul H, Gale RP, Hale GA, Horan J, Isola L, Maziarz RT, van Rood JJ, Gupta V, Halter J, Reddy V, Tiberghien P, Litzow M, Anasetti C, Pavletic S, Ringdén O. Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. J Clin Oncol. 2008 Dec 10;26(35):5728-34. doi: 10.1200/JCO.2008.17.6545. Epub 2008 Nov 3. PMID: 18981462; PMCID: PMC2645611.
6. Flowers ME, Inamoto Y, Carpenter PA, Lee SJ, Kiem HP, Petersdorf EW, Pereira SE, Nash RA, Mielcarek M, Fero ML, Warren EH, Sanders JE, Storb RF, Appelbaum FR, Storer BE, Martin PJ. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood. 2011 Mar 17;117(11):3214-9. doi: 10.1182/blood-2010-08-302109. Epub 2011 Jan 24. PMID: 21263156; PMCID: PMC3062319.
7. Weisdorf D, Hakke R, Blazar B, Miller W, McGlave P, Ramsay N, Kersey J, Filipovich A. Risk factors for acute graft-versus-host disease in histocompatible donor bone marrow transplantation. Transplantation. 1991 Jun;51(6):1197-203. doi: 10.1097/00007890-199106000-00010. PMID: 2048196.
8. Vargas-Díez E, Fernández-Herrera J, Marin A, Cámara R, García-Díez A. Analysis of risk factors for acute cutaneous graft-versus-host disease after allogeneic stem cell transplantation. Br J Dermatol. 2003 Jun;148(6):1129-34. doi: 10.1046/j.1365-2133.2003.05336.x. PMID: 12828739.
9. Gale RP, Bortin MM, van Bekkum DW, Biggs JC, Dicke KA, Gluckman E, Good RA, Hoffmann RG, Kay HE, Kersey JH, et al. Risk factors for acute graft-versus-host disease. Br J Haematol. 1987 Dec;67(4):397-406. doi: 10.1111/j.1365-2141.1987.tb06160.x. PMID: 3322360.
10. Malard F, Holler E, Sandmaier BM, Huang H, Mohty M. Acute graft-versus-host disease. Nat Rev Dis Primers. 2023 Jun 8;9(1):27. doi: 10.1038/s41572-023-00438-1. PMID: 37291149.
11. Akahoshi Y, Spyrou N, Hogan WJ, Ayuk F, DeFilipp Z, Weber D, Choe HK, Hexner EO, Rösler W, Etra AM, Sandhu K, Yanik GA, Chanswangphuwana C, Kitko CL, Reshef R, Kraus S, Wölfl M, Eder M, Bertrand H, Qayed M, Merli P, Grupp SA, Aguayo-Hiraldo P, Schechter T, Ullrich E, Baez J, Beheshti R, Gleich S, Kowalyk S, Morales G, Young R, Kwon D, Nakamura R, Levine JE, Ferrara JLM, Chen YB. Incidence, clinical presentation, risk factors, outcomes, and biomarkers in de novo late acute GVHD. Blood Adv. 2023 Aug 22;7(16):4479-4491. doi: 10.1182/bloodadvances.2023009885. PMID: 37315175; PMCID: PMC10440469.
12. Kasikis S, Etra A, Levine JE. Current and Emerging Targeted Therapies for Acute Graft-Versus-Host Disease. BioDrugs. 2021 Jan;35(1):19-33. doi: 10.1007/s40259-020-00454-7. PMID: 33201499; PMCID: PMC7855093.
13. Choe H, Ferrara JLM. New therapeutic targets and biomarkers for acute graft-versus-host disease (GVHD). Expert Opin Ther Targets. 2021 Sep;25(9):761-771. doi: 10.1080/14728222.2021.1992383. Epub 2021 Nov 1. PMID: 34669521; PMCID: PMC8602762.
14. Malard F, Huang XJ, Sim JPY. Treatment and unmet needs in steroid-refractory acute graft-versus-host disease. Leukemia. 2020 May;34(5):1229-1240. doi: 10.1038/s41375-020-0804-2. Epub 2020 Apr 3. PMID: 32242050; PMCID: PMC7192843.
15. Servais S, Baron F, Lechanteur C, Seidel L, Baudoux E, Briquet A, Selleslag D, Maertens J, Poire X, Schroyens W, Graux C, De Becker A, Zachee P, Ory A, Herman J, Kerre T, Beguin Y. Multipotent mesenchymal stromal cells as treatment for poor graft function after allogeneic hematopoietic cell transplantation: A multicenter prospective analysis. Front Immunol. 2023 Feb 1; 14:1106464. doi: 10.3389/fimmu.2023.1106464. PMID: 36817464; PMCID: PMC9929549.
16. Zhao Y, Gao F, Shi J, Luo Y, Tan Y, Lai X, Yu J, Huang H. Incidence, Risk Factors, and Outcomes of Primary Poor Graft Function after Allogeneic Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2019 Sep;25(9):1898-1907. doi: 10.1016/j.bbmt.2019.05.036. Epub 2019 Jun 6. PMID: 31176790.
17. Chen J, Wang H, Zhou J, Feng S. Advances in the understanding of poor graft function following allogeneic hematopoietic stem-cell transplantation. Ther Adv Hematol. 2020 Aug 17; 11:2040620720948743. doi: 10.1177/2040620720948743. PMID: 32874483; PMCID: PMC7436797.
18. Man Y, Lu Z, Yao X, Gong Y, Yang T, Wang Y. Recent Advancements in Poor Graft Function Following Hematopoietic Stem Cell Transplantation. Front Immunol. 2022 Jun 2; 13:911174. doi: 10.3389/fimmu.2022.911174. PMID: 35720412; PMCID: PMC9202575.

19. Prabahran A, Koldej R, Chee L, Ritchie D. Clinical features, pathophysiology, and therapy of poor graft function post-allogeneic stem cell transplantation. Blood Adv. 2022 Mar 22;6(6):1947-1959. doi: 10.1182/bloodadvances.2021004537. PMID: 34492685; PMCID: PMC8941468.

20. Xiao Y, Song J, Jiang Z, Li Y, Gao Y, Xu W, Lu Z, Wang Y, Xiao H. Risk-factor analysis of poor graft function after allogeneic hematopoietic stem cell transplantation. Int J Med Sci. 2014 Apr 30;11(6):652-7. doi: 10.7150/ijms.6337. PMID: 24834012; PMCID: PMC4021098.

21. Auletta JJ, Deans RJ, Bartholomew AM. Emerging roles for multipotent, bone marrow-derived stromal cells in host defense. Blood. 2012 Feb 23;119(8):1801-9. doi: 10.1182/blood-2011-10-384354. Epub 2012 Jan 6. PMID: 22228625; PMCID: PMC3293637.

22. Al-Anazi KA, Al-Jasser AM. Mesenchymal stem cells-their antimicrobial effects and their promising future role as novel therapies of infectious complications in high-risk patients. In: Progress in stem cell transplantation. Edited by: Demirer T. Intech Open. 2015. doi: 10.5772/60640

23. Abdal Dayem A, Lee SB, Kim K, Lim KM, Jeon TI, Seok J, Cho AS. Production of Mesenchymal Stem Cells Through Stem Cell Reprogramming. Int J Mol Sci. 2019 Apr 18;20(8):1922. doi: 10.3390/ijms20081922. PMID: 31003536; PMCID: PMC6514654.

24. Bobis S, Jarocha D, Majka M. Mesenchymal stem cells: characteristics and clinical applications. Folia Histochem Cytobiol. 2006;44(4):215-30. PMID: 17219716.

25. Kim N, Cho SG. Clinical applications of mesenchymal stem cells. Korean J Intern Med. 2013 Jul;28(4):387-402. doi: 10.3904/kjim.2013.28.4.387. Epub 2013 Jul 1. PMID: 23864795; PMCID: PMC3712145.

26. Squillaro T, Peluso G, Galderisi U. Clinical Trials with Mesenchymal Stem Cells: An Update. Cell Transplant. 2016;25(5):829-48. doi: 10.3727/096368915X689622. Epub 2015 Sep 29. PMID: 26423725.

27. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7. doi: 10.1080/14653240600855905. PMID: 16923606.

28. Leyendecker A Jr, Pinheiro CCG, Amano MT, Bueno DF. The Use of Human Mesenchymal Stem Cells as Therapeutic Agents for the in vivo Treatment of Immune-Related Diseases: A Systematic Review. Front Immunol. 2018 Sep 11; 9:2056. doi: 10.3389/fimmu.2018.02056. PMID: 30254638; PMCID: PMC6141714.

29. Sarvar DP, Effatpanah H, Akbarzadehlaleh P, Shamsasenjan K. Mesenchymal stromal cell-derived extracellular vesicles: novel approach in hematopoietic stem cell transplantation. Stem Cell Res Ther. 2022 May 16;13(1):202. doi: 10.1186/s13287-022-02875-3. PMID: 35578300; PMCID: PMC9109321.

30. De Luca L, Trino S, Laurenzana I, Lamorte D, Caivano A, Del Vecchio L, Musto P. Mesenchymal Stem Cell Derived Extracellular Vesicles: A Role in Hematopoietic Transplantation? Int J Mol Sci. 2017 May 9;18(5):1022. doi: 10.3390/ijms18051022. PMID: 28486431; PMCID: PMC5454935.

31. Battiwalla M, Hematti P. Mesenchymal stem cells in hematopoietic stem cell transplantation. Cytotherapy. 2009;11(5):503-15. doi: 10.1080/14653240903193806. PMID: 19728189; PMCID: PMC2766085.

32. Kelly K, Rasko JEJ. Mesenchymal Stromal Cells for the Treatment of Graft Versus Host Disease. Front Immunol. 2021 Oct 26; 12:761616. doi: 10.3389/fimmu.2021.761616. PMID: 34764962; PMCID: PMC8577186.

33. Kim EJ, Kim N, Cho SG. The potential use of mesenchymal stem cells in hematopoietic stem cell transplantation. Exp Mol Med. 2013 Jan 10;45(1):e2. doi: 10.1038/emm.2013.2. PMID: 23306700; PMCID: PMC3584660.

34. Burnham AJ, Daley-Bauer LP, Horwitz EM. Mesenchymal stromal cells in hematopoietic cell transplantation. Blood Adv. 2020 Nov 24;4(22):5877-5887. doi: 10.1182/bloodadvances.2020002646. PMID: 33232479; PMCID: PMC7686890.

35. Wu KH, Wu HP, Chan CK, Hwang SM, Peng CT, Chao YH. The role of mesenchymal stem cells in hematopoietic stem cell transplantation: from bench to bedsides. Cell Transplant. 2013;22(4):723-9. doi: 10.3727/096368912X655217. Epub 2012 Oct 12. PMID: 23068433.

36. Petinati N, Drize N, Sats N, Risinskaya N, Sudarikov A, Drokov M, Dubniak D, Kraizman A, Nareyko M, Popova N, Firsova M, Kuzmina L, Parovichnikova E, Savchenko V. Recovery of Donor Hematopoiesis after Graft Failure and Second Hematopoietic Stem Cell Transplantation with Intraosseous Administration of Mesenchymal Stromal Cells. Stem Cells Int. 2018 Apr 10;2018: 6495018. doi: 10.1155/2018/6495018. PMID: 29760731; PMCID: PMC5914104.

37. Liu XD, Fan ZP, Peng YW, Huang F, Jiang QL, Zhang X, Yu GP, Zhao J, Sun J, Xiang P, Liu QF. [The outcome and safety of mesenchymal stem cells from bone marrow of a third party donor in treatment of secondary poor graft function following allogeneic hematopoietic stem cell transplantation]. Zhonghua Xue Ye Xue Za Zhi. 2012 Feb;33(2):98-102. Chinese. PMID: 22730656.

38. Liu X, Wu M, Peng Y, Chen X, Sun J, Huang F, Fan Z, Zhou H, Wu X, Yu G, Zhang X, Li Y, Xiao Y, Song C, Xiang AP, Liu Q. Improvement in poor graft function after allogeneic hematopoietic stem cell transplantation upon administration of mesenchymal stem cells from third-party donors: a pilot prospective study. Cell Transplant. 2014;23(9):1087-98. doi: 10.3727/096368912X661319. PMID: 23294601.

39. McGuirk JP, Metheny L 3rd, Pineiro L, Litzow M, Rowley SD, Avni B, Tamari R, Lazarus HM, Rowe JM, Sheleg M, Rothenstein D, Halevy N, Zuckerman T. Placental expanded mesenchymal-like cells (PLX-R18) for poor graft function after hematopoietic cell transplantation: A phase I study. Bone Marrow Transplant. 2023 Nov;58(11):1189-1196. doi: 10.1038/s41409-023-02068-3. Epub 2023 Aug 8. PMID: 37553467; PMCID: PMC10622312.

40. Najima Y, Ohashi K. Mesenchymal stem cells. The first approved stem cell drug in Japan. J Hemat Cell Transplant. 2017; 6 (3): 125-132. doi: 10.7889/hct16-031

41. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell. 2013; 13(4):392-402. doi: 10.1016/j.stem.2013.09.006. PMID: 24094322.

42. English K. Mechanisms of mesenchymal stromal cell immunomodulation. Immunol Cell Biol. 2013 Jan;91(1):19-26. doi: 10.1038/icb.2012.56. Epub 2012 Oct 23. PMID: 23090487.

43. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol. 2012 Apr 25;12(5):383-96. doi: 10.1038/nri3209. PMID: 22531326.

44. Zhao K, Lin R, Fan Z, Chen X, Wang Y, Huang F, Xu N, Zhang X, Zhang X, Xuan L, Wang S, Lin D, Deng L, Nie D, Weng J, Li Y, Zhang X, Li Y, Xiang AP, Liu Q. Mesenchymal stromal cells plus basiliximab, calcineurin inhibitor as treatment of steroid-resistant acute graft-versus-host disease: a multicenter, randomized, phase 3, open-label trial. J Hematol Oncol. 2022 Mar 7;15(1):22. doi: 10.1186/s13045-022-01240-4. PMID: 35255929; PMCID: PMC8900437.

45. Fujii S, Miura Y, Fujishiro A, Shindo T, Shimazu Y, Hirai H, Tahara H, Takaori-Kondo A, Ichinohe T, Maekawa T. Graft-Versus-Host Disease Amelioration by Human Bone Marrow Mesenchymal Stromal/Stem Cell-Derived Extracellular Vesicles Is Associated with Peripheral Preservation of Naive T Cell Populations. Stem Cells. 2018 Mar;36(3):434-445. doi: 10.1002/stem.2759. Epub 2017 Dec 27. PMID: 29239062.

46. Muroi K, Miyamura K, Ohashi K, Murata M, Eto T, Kobayashi N, Taniguchi S, Imamura M, Ando K, Kato S, Mori T, Teshima T, Mori M, Ozawa K. Unrelated allogeneic bone marrow-derived mesenchymal stem cells for steroid-refractory acute graft-versus-host disease: a phase I/II study. Int J Hematol. 2013 Aug;98(2):206-13. doi: 10.1007/s12185-013-1399-4. Epub 2013 Jul 17. PMID: 23860964.

47. Muroi K, Miyamura K, Okada M, Yamashita T, Murata M, Ishikawa T, Uike N, Hidaka M, Kobayashi R, Imamura M, Tanaka J, Ohashi K, Taniguchi S, Ikeda T, Eto T, Mori M, Yamaoka M, Ozawa K. Bone marrow-derived mesenchymal stem cells (JR-031) for steroid-refractory grade III or IV acute graft-versus-host disease: a phase II/III study. Int J Hematol. 2016 Feb;103(2):243-50. doi: 10.1007/s12185-015-1915-9. Epub 2015 Nov 25. PMID: 26608364.

48. Chen X, Wang C, Yin J, Xu J, Wei J, Zhang Y. Efficacy of Mesenchymal Stem Cell Therapy for Steroid-Refractory Acute Graft-Versus-Host Disease following Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. PLoS One. 2015 Aug 31; 10(8):e0136991. doi: 10.1371/journal.pone.0136991. PMID: 26323092; PMCID: PMC4554731.

49. Morata-Tarifa C, Macías-Sánchez MDM, Gutiérrez-Pizarraya A, Sanchez-Pernaute R. Mesenchymal stromal cells for the prophylaxis and treatment of graft-versus-host disease-a meta-analysis. Stem Cell Res Ther. 2020 Feb 18;11(1):64. doi: 10.1186/s13287-020-01592-z. PMID: 32070420; PMCID: PMC7027118.

50. Hashmi S, Ahmed M, Murad MH, Litzow MR, Adams RH, Ball LM, Prasad VK, Kebriaei P, Ringden O. Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. Lancet Haematol. 2016 Jan; 3(1):e45-52. doi: 10.1016/S2352-3026(15)00224-0. Epub 2015 Nov 27. PMID: 26765648.

51. Li Y, Hao J, Hu Z, Yang YG, Zhou Q, Sun L, Wu J. Current status of clinical trials assessing mesenchymal stem cell therapy for graft versus host disease: a systematic review. Stem Cell Res Ther. 2022 Mar 4;13(1):93. doi: 10.1186/s13287-022-02751-0. PMID: 35246235; PMCID: PMC8895864.

52. Li T, Luo C, Zhang J, Wei L, Sun W, Xie Q, Liu Y, Zhao Y, Xu S, Wang L. Efficacy and safety of mesenchymal stem cells co-infusion in allogeneic hematopoietic stem cell transplantation: a systematic review and meta-analysis. Stem Cell Res Ther. 2021 Apr 20;12(1):246. doi: 10.1186/s13287-021-02304-x. PMID: 33879242; PMCID: PMC8056684.

53. Wang L, Zhu CY, Ma DX, Gu ZY, Xu CC, Wang FY, Chen JG, Liu CJ, Guan LX, Gao R, Gao Z, Fang S, Zhuo DJ, Liu SF, Gao CJ. Efficacy and safety of mesenchymal stromal cells for the prophylaxis of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation: a meta-analysis of randomized controlled trials. Ann Hematol. 2018 Oct;97(10):1941-1950. doi: 10.1007/s00277-018-3384-8. Epub 2018 Jun 8. PMID: 29947972.

54. Munneke JM, Spruit MJ, Cornelissen AS, van Hoeven V, Voermans C, Hazenberg MD. The Potential of Mesenchymal Stromal Cells as Treatment for Severe Steroid-Refractory Acute Graft-Versus-Host Disease: A Critical Review of the Literature. Transplantation. 2016 Nov;100(11):2309-2314. doi: 10.1097/TP.0000000000001029. PMID: 26714122.

55. Kallekleiv M, Larun L, Bruserud Ø, Hatfield KJ. Co-transplantation of multipotent mesenchymal stromal cells in allogeneic hematopoietic stem cell transplantation: A systematic review and meta-analysis. Cytotherapy. 2016 Feb;18(2):172-85. doi: 10.1016/j.jcyt.2015.11.010. PMID: 26794711.

56. Li R, Tu J, Zhao J, Pan H, Fang L, Shi J. Mesenchymal stromal cells as prophylaxis for graft-versus-host disease in haplo-identical hematopoietic stem cell transplantation recipients with severe aplastic anemia? -a systematic review and meta-analysis. Stem Cell Res Ther. 2021 Feb 4;12(1):106. doi: 10.1186/s13287-021-02170-7. PMID: 33541414; PMCID: PMC7860635.

57. Kurtzberg J, Prockop S, Chaudhury S, Horn B, Nemecek E, Prasad V, Satwani P, Teira P, Hayes J, Burke E; MSB-275 Study Group. Study 275: Updated Expanded Access Program for Remestemcel-L in Steroid-Refractory Acute Graft-versus-Host Disease in Children. Biol Blood Marrow Transplant. 2020 May;26(5):855-864. doi: 10.1016/j.bbmt.2020.01.026. Epub 2020 Feb 7. PMID: 32044400; PMCID: PMC8292970.

58. Kebriaei P, Hayes J, Daly A, Uberti J, Marks DI, Soiffer R, Waller EK, Burke E, Skerrett D, Shpall E, Martin PJ. A Phase 3 Randomized Study of Remestemcel-L versus Placebo Added to Second-Line Therapy in Patients with Steroid-Refractory Acute Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2020 May;26(5):835-844. doi: 10.1016/j.bbmt.2019.08.029. Epub 2019 Sep 7. PMID: 31505228; PMCID: PMC7060124.

59. Kurtzberg J, Abdel-Azim H, Carpenter P, Chaudhury S, Horn B, Mahadeo K, Nemecek E, Neudorf S, Prasad V, Prockop S, Quigg T, Satwani P, Cheng A, Burke E, Hayes J, Skerrett D; MSB-GVHD001/002 Study Group. A Phase 3, Single-Arm, Prospective Study of Remestemcel-L, Ex Vivo Culture-Expanded Adult Human Mesenchymal Stromal Cells for the Treatment of Pediatric Patients Who Failed to Respond to Steroid Treatment for Acute Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2020 May;26(5):845-854. doi: 10.1016/j.bbmt.2020.01.018. Epub 2020 Feb 1. PMID: 32018062; PMCID: PMC8322819.

60. Kurtzburg J. LBA17 (Abstract): Long-term survival in children treated with Remestemcel-L for steroid-refractory acute GVHD. Tandem Meetings: Transplantation & Cellular Therapy Meetings of ASTCT and CIBMTR. 2023. Orlando, Florida, USA.

61. Fitch J. Mesoblast receives complete response from US Food and Drug Administration for Biologics License Application for steroid-refractory acute graft versus host disease in children. Contemp Pediatr.2023. https://investorsmedia.mesoblast.com/static-files/422cd6da-a0b9-49cf-a177- 7fd106f111f2

62. Fujii S, Miura Y. Immunomodulatory and Regenerative Effects of MSC-Derived Extracellular Vesicles to Treat Acute GVHD. Stem Cells. 2022 Nov 29;40(11):977-990. doi: 10.1093/stmcls/sxac057. PMID: 35930478.

63. Stenger E, Giver CR, Langston A, Kota D, Das PK, Chinnadurai R, Galipeau J, Waller EK, Qayed M. Safety of autologous freshly expanded mesenchymal stromal cells for the treatment of graft-versus-host disease. Front Immunol. 2022 Sep 14; 13:959658. doi: 10.3389/fimmu.2022.959658. PMID: 36189324; PMCID: PMC9515357.

64. Wang B, Hu Y, Liu L, Hu K, Tie R, He Y, Fu S, Zhu N, Luo Y, Yu X, Huang H. Phenotypical and functional characterization of bone marrow mesenchymal stem cells in patients with chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2015 Jun;21(6):1020-8. doi: 10.1016/j.bbmt.2015.02.013. Epub 2015 Feb 20. PMID: 25708216.

65. Zhang LS, Liu QF, Huang K, Zhang Y, Fan ZP, Huang SL. [Mesenchymal stem cells for treatment of steroid-resistant chronic graft-versus-host disease]. Zhonghua Nei Ke Za Zhi. 2009 Jul;48(7):542-6. Chinese. PMID: 19957792.

66. Erkers T, Kaipe H, Nava S, Molldén P, Gustafsson B, Axelsson R, Ringdén O. Treatment of severe chronic graft-versus-host disease with decidual stromal cells and tracing with (111) indium radiolabeling. Stem Cells Dev. 2015 Jan 15;24(2):253-63. doi: 10.1089/scd.2014.0265. PMID: 25162829; PMCID: PMC4291217.

67. Ringden O. Mesenchymal stem cells for treatment and prevention of graft-versus-host disease and graft failure after hematopoietic stem cell transplantation and future challenges. In: Mesenchymal Stem Cell Therapy. Chase IG, Vemuri MC, eds. Springer Verlag, Humana Press, New York. 2013; 173-206.

68. Harrell CR, Simovic Markovic B, Fellabaum C, Arsenijevic A, Djonov V, Arsenijevic N, Volarevic V. Therapeutic Potential of Mesenchymal Stem Cell-Derived Exosomes in the Treatment of Eye Diseases. Adv Exp Med Biol. 2018; 1089:47-57. doi: 10.1007/5584_2018_219. PMID: 29774506.

69. Harrell CR, Djonov V, Volarevic V. Therapeutic Potential of Mesenchymal Stem Cells in the Treatment of Ocular Graft-Versus-Host Disease. Int J Mol Sci. 2022 Oct 31;23(21):13254. doi: 10.3390/ijms232113254. PMID: 36362040; PMCID: PMC9656879.

70. Weng J, He C, Lai P, Luo C, Guo R, Wu S, Geng S, Xiangpeng A, Liu X, Du X. Mesenchymal stromal cells treatment attenuates dry eye

in patients with chronic graft-versus-host disease. Mol Ther. 2012 Dec;20(12):2347-54. doi: 10.1038/mt.2012.208. Epub 2012 Oct 16. PMID: 23070118; PMCID: PMC3519994.

71. Keshavarz Shahbaz S, Mansourabadi AH, Jafari D. Genetically engineered mesenchymal stromal cells as a new trend for treatment of severe acute graft-versus-host disease. Clin Exp Immunol. 2022 May 13;208(1):12-24. doi: 10.1093/cei/uxac016. PMID: 35274673; PMCID: PMC9113247.

72. Ochs J, Barry F, Schmitt R, Murphy M. Advances in automation for the production of clinical-grade mesenchymal stromal cells: the AUTOSTEM robotic platform. Cell Gene Ther Insights. 2017; 3(8): 739-748. doi: 10.18609/ cgti.2017.073

73. Kimbrel EA, Lanza R. Next-generation stem cells - ushering in a new era of cell-based therapies. Nat Rev Drug Discov. 2020 Jul;19(7):463-479. doi: 10.1038/s41573-020-0064-x. Epub 2020 Apr 6. PMID: 32612263.

74. Sarsenova M, Kim Y, Raziyeva K, Kazybay B, Ogay V, Saparov A. Recent advances to enhance the immunomodulatory potential of mesenchymal stem cells. Front Immunol. 2022 Sep 23; 13:1010399. doi: 10.3389/fimmu.2022.1010399. PMID: 36211399; PMCID: PMC 9537745.