The Effect of Mesenchymal Stem Cell-Derived Extracellular Vesicles on Hematopoietic Stem Cells Fate

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Abstract
Hematopoietic stem cells (HSCs) are multipotent stem cells, with self-renewal ability as well as ability to generate all blood cells. Mesenchymal stem cells (MSCs) are multipotent stem cells, with self-renewal ability, and capable of differentiating into a variety of cell types. MSCs have supporting effects on hematopoiesis; through direct intercellular communications as well as secreting cytokines, chemokines, and extracellular vesicles (EVs). Recent investigations demonstrated that some biological functions and effects of MSCs are mediated by their EVs. MSC-EVs are the cell membrane and endosomal membrane compartments, which are important mediators in the intercellular communications. MSC-EVs contain some of the molecules such as proteins, mRNA, siRNA, and miRNA from their parental cells. MSC-EVs are able to inhibit tumor, repair damaged tissue, and modulate immune system responses. MSC-EVs compared to their parental cells, may have the specific safety advantages such as the lower potential to trigger immune system responses and limited side effects. Recently some studies demonstrated the effect of MSC-EVs on the expansion, differentiation, and clinical applications of HSCs such as improvement of hematopoietic stem cell transplantation (HSCT) and inhibition of graft versus host disease (GVHD). HSCT may be the only therapeutic choice for patients who suffer from malignant and non-malignant hematological disorders. However, there are several severe side effects such GVHD that restricts the successfullness of HSCT. In this review, we will discuss the most important effects of MSCs and MSC-EVs on the improvement of HSCT, inhibition and treatment of GVHD, as well as, on the expansion of HSCs.

Introduction
Bone marrow (BM) microenvironment or BM niche plays a significant role in the control of hematopoietic stem cells (HSCs) fate through mesenchymal stem cells (MSCs) and other stromal cells.1 HSCs include a very small portion of the BM cells which are multipotent cells, with the self-renewal ability and capable of generating all blood cells.2,3 MSCs are multipotent and non-hematopoietic stem cells with self-renewal ability and capability of proliferating and differentiating into several cell types such as adipocytes, osteocytes, chondrocytes, fibroblasts, cartilage, bone, cardiomyocytes, skeletal myocytes, and stromal cells.4,6 MSCs are particularly low immunogenic, and due to their immunomodulatory features that affect a broad range of innate and adaptive immune system responses, act as a therapeutic agent in the regenerative medicine to repair injured tissues, tumor inhibition, and immunosuppression.7,8 These effects of MSCs in the result of the MSCs differentiation into various cell types, including both mesenchymal and non-mesenchymal cell types and MSCs paracrine molecules, including cytokines, chemokines, growth factors, and extracellular vesicles (EVs).9,10 MSCs have supporting function in hematopoiesis in the BM microenvironment through expression of multiple adhesion molecules that are necessary for cell-cell, cell-matrix interactions, homing and mobilization of HSCs, as well as, production of cytokines, chemokines, growth factors, and EVs that affect the HSCs expansion, differentiation, and transplantation.11-14 Nowadays, gathering data from investigations show that the most therapeutic effects of MSCs as a result of their paracrine activities.15,16 On the other hand, recent studies demonstrated that mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) used as a possible therapeutic agent in many disorders and their biological functions and effects is almost like MSCs.5,17,18 EVs from different cell sources including MSCs have been involved in many pathological and physiological processes that also most of them performed by their parental cells such as cell proliferation, cell differentiation, cleaning undesired proteins, genetic exchanges, antigen presentation, angiogenesis, immune system responses, tumor metastasis and inhibition, inflammation, and distributing of oncogenes and
pathogens.\textsuperscript{19,20} MSC-EVs are safer than their parental cells due to their specific safety advantages such as the lack of risk for aneuploidy due to their not self-renewal ability, the lower potential to trigger immune system responses and subsequently lower immune rejection due to their very small size (nm) and lower expression of cell surface molecules such as MHC molecules, their preserved function during storage and their preserved cargo versus \textit{in vivo} degradation due to their encapsulated cargo, and limited side effects or toxicity.\textsuperscript{21-23} Moreover, recent \textit{ex vivo} and \textit{in vivo} investigations showed that MSC-EVs therapy can use in the scope of improving hematopoietic stem cells transplantation (HSCT), and HSCs expansion, as well as, treatment of graft versus host disease (GVHD).\textsuperscript{12,24,25}

The goals of this article, are to review the most important effects of MSCs and MSC-EVs on the improvement of clinical applications in the scope of HSCT, treatment and inhibition of GVHD following HSCT, as well as, improvement of \textit{ex vivo} expansion of HSCs.

\textbf{Characteristics and therapeutic applications of MSC-EVs}

EVs are cell-derived vesicles which secreted by a variety of cell types such as MSCs, cytotoxic T cells, mast cells, neurons and other cells into the extracellular milieu.\textsuperscript{17,26} EVs include exosomes, microvesicles (also called microparticles or ectosomes), and apoptotic bodies, which are different in size and mechanism of formation.\textsuperscript{5,26} Exosomes are derived from the internal budding of the late endosomes that led to the formation of multivesicular bodies (MVBs) and are released from cells when MVBs fuse with the cell membrane, with the size range from 40 to 100 nm in diameter.\textsuperscript{5,17} Microvesicles (MVs) are derived from the direct outward budding of the cell membrane, with the size range from 50 to 1000 nm in diameter.\textsuperscript{5} Apoptotic bodies are cell fragmentations that released from cells that undergoing apoptosis and are identified via expression of phosphatidylserine on their surface, with the size range from 50 to 5000 nm in diameter.\textsuperscript{26} MSC-EVs express cell surface molecules from their parental cells such as CD29, CD44, CD73, and CD105, as well as, express endosome-associated surface molecules such as CD81, CD82, CD63, CD53, CD9, and CD37. They contain endosome-associated proteins such as TSG101 (tumor susceptibility gene 101), Alix, Flotillin, Annexins, SNAREs, and Rab GTPase, and lipids such as cholesterol, ceramides, and phospholipids, as well as, several types of RNA such as siRNA, mRNA, RNA and tRNA fragments.\textsuperscript{26-28}

EVs have been separated from various biological body fluids such as serum, milk, urine, amniotic fluid, saliva, synovial fluid, and as well as from the supernatant of many cell cultures such as MSCs, dendritic cells, platelets, T cells, B cells, and other cells.\textsuperscript{5,17} EVs due to their very small size (nm) could easily be transported through interstitial space, blood and other biological body fluids, even the blood-brain barrier.\textsuperscript{29} Therefore, they exert their effects in the intercellular communications on the target cells via an endocrine effect on distant cells and paracrine effect on adjacent cells.\textsuperscript{29} EVs could be uptake by target cells through direct fusion with the cell membrane and the variety of molecular endocytic pathways such as clathrin-dependent endocytosis, caveolin-dependent endocytosis, phagocytosis, macropinocytosis, and lipid raft-dependent endocytosis. EVs uptake mechanisms depend on types of proteins, glycoproteins, and proteoglycans that located on the membrane of EVs and target cells.\textsuperscript{29,30}

MSC-EVs are important mediators in the intercellular communications that change the wide spectrum of pathological and physiological processes of the target cells by transferring of biological molecules from MSCs.\textsuperscript{31} Factors such as inflammatory stimuli, hypoxic conditions, stress, acidic PH, and high levels of intracellular calcium influence the secretion of EVs from MSCs both in pathological and physiological conditions.\textsuperscript{32,34} Recent research activities on the MSC-EVs have shown supporting therapeutic effects in the field of cardiovascular disease, neurological diseases, liver disease, kidney disease, lung disease, immune system disease, cutaneous wound healing, and tumor inhibition.\textsuperscript{5,35} The effect of MSC-EVs in the recent studies on various conditions is summarized in Table 1.

Nowadays, attention has been focused on the production of EVs by genetic engineering from their parental cells for therapeutic goals in order to by engineering EVs that contain therapeutic cargo for targeting particular tissues.\textsuperscript{36,37} For example, exosomes derived from MSCs could decrease renal fibrosis and transforming growth factor β1 (TGFB1) stimulated damage via delivering exogenous miRNA let-7c to damaged kidney cells in the mouse model of unilateral ureteral obstruction.\textsuperscript{38} Also, use of MSC-EVs for active drug delivery that first time reported by Pascucci et al. for inhibiting of pancreatic tumor.\textsuperscript{39} In this regard, MSC-EVs that express surface TRAIL (TNF-related apoptosis-inducing ligand) that is an anti-cancer soluble recombiant protein, could induce apoptosis in 11 TRAIL-resistant cancer cell lines in a dose-dependent way but could not induce apoptosis in primary human bronchial epithelial cells because of TRAIL neutralization or caspase activity inhibition.\textsuperscript{40}

\textbf{The effect of MSCs on HSCs expansion}

The main sources for isolation of HSPCs, including umbilical cord blood (UCB), mobilized peripheral blood with granulocyte colony-stimulating factor (G-CSF), and BM.\textsuperscript{48,49} UCB has important advantages such as easy to acquire, easy availability, less stringent for HLA matching, lower incidence of GVHD, and lower risk for transmission of infectious diseases than peripheral blood and BM. The major disadvantage of UCB is the low number of HSPCs in a cord blood unit, leading to delays in platelets and neutrophils recovery in the peripheral blood after HSCT.\textsuperscript{50,51} To overcome this disadvantage, there are two main ways for increasing the number of HSCs: co-infusion of two cord blood units and \textit{ex vivo} expansion of HSCs.\textsuperscript{52} Co-infusion of two cord blood units has shown some possible improvement than the infusion of single cord blood unit, but hematopoietic recovery still remains sub-optimal with moderate improvement in platelets and neutrophils recovery after HSCT\textsuperscript{52} and may be increased the incidence of GVHD.\textsuperscript{54} Therefore recent works that have been done, further focusing on the improvement of \textit{ex vivo} expansion...
methods for increasing the number of HSCs. In numerous protocols, cytokines such as stem cell factor (SCF), thrombopoietin (TPO), and FMS-like tyrosine kinase 3 ligand (FLT3-L) that important for maintaining of HSCs in a more primitive fraction (CD34+/CD38-) have been used to promote ex vivo expansion of HSCs, and the addition of other cytokines such as interleukin-3 (IL-3), IL-6, IL-11, and G-CSF had optimized HSCs ex vivo expansion rate. Nevertheless, most of these protocols confirmed that cytokine-mediated ex vivo expansion, shown moderate increases in the number of HSCs with lesser improvement in platelets and neutrophils recovery after HSCT, as well as, promoting differentiation of HSCs to the mature cell lines. For this reasons, the use of MSCs has been suggested for improving ex vivo expansion of HSCs in the recent years.

Table 1. Effects of MSCs-EVs on the various conditions

| Source of EVs | Type of EVs and their size | Isolation method | Identify method | Administration way of EVs | Outcome | Ref |
|--------------|---------------------------|------------------|----------------|--------------------------|---------|-----|
| Human UCB-MSCs | Exosome 40-100 nm | Ultracentrifugation (100000 g 1 h) | TEM and western blotting | Direct injection into lobes of mouse liver | Reduce mouse liver fibrosis and hepatic inflammation | 41 |
| Human fetal tissue MSCs | Exosome Size not shown | HPLC | Not shown | Intraspelvic injection | Improve mouse liver regeneration through increasing of hepatocyte proliferation and survival | 42 |
| Mouse BM-MSCs | MVs 80-1000 nm | Ultracentrifugation (100000 g 1 + 1 h) | Flowcytometry, TEM, and SEM | Intravenous injection (tail) | MVs with or without IFN-γ stimulation suppress T-cell proliferation through increasing the percentage of CD4+ CD25+ FoxP3+ Treg cells ex vivo. Only MVs without IFN-γ stimulation preserved rats kidney from AKI in vivo | 43 |
| Human UCB-MSCs | MVs 20-1000 nm | Ultracentrifugation (100000 g 1 + 1 h) | NTA, TEM, and SEM | Co-culture (ex vivo) Injection into left carotid artery of rat | Ex vivo suppress the expression of CX3CL1 (chemotactic factor for macrophages) in HUVECs under hypoxia-induced damage. Improve ischemia/reperfusion AKI in rats by increased proliferation, and decreased inflammation and apoptosis of renal cells in vivo | 44 |
| Human ESC-MSCs | Exosome 50-100 nm | Ultrafiltration and HPLC | Electron microscopy | Intravenous injection (tail) | Decrease myocardial ischemia/reperfusion damage in a mouse model through reduction of infarct size | 22 |
| Mouse BM-MSCs and Human WJ-MSCs | Exosome 30-100 nm | Ultrafiltration and size-exclusion chromatography | Electron microscopy | Intravenous injection (left jugular and tail vein) | Suppress hypoxic pulmonary hypertension in a murine model by inhibition of hypoxia-activated signaling pathway that causes lung inflammation | 24 |
| Rat BM-MSCs | Exosome Peak 116 t 49 nm by qNano system | ExoQuick-TCM™ kit | qNano system, TEM, western blotting, and confocal fluorescence microscopy | Intravenous injection (tail) | Promote functional recovery through increased endogenous brain angiogenesis and neurogenesis, as well as, decreased neuroinflammation in a rat model of TBI | 45 |
| Human MSCs | EVs Mean 100 nm | Anion exchange chromatography | Not shown | Intravenous injection (tail) | Efficiently suppress autoimmunity in murine models of IDDM and EAU. Suppress activation of APCs and proliferation of Th1 and Th17 Cells as well as increase the expression of IL-10 ex vivo | 46 |
| Human BM-MSCs | Exosome 30-100 nm | Ultracentrifugation (100000 g 70 + 70 + 70 min) | TEM and western blotting | Ex vivo coculture | Could induce the proliferation and migration of dermal fibroblasts derived from healthy donors and chronic wound patients, as well as induce angiogenesis of HUVECs ex vivo | 47 |

EVs: extracellular vesicles; MVs: microvesicles; UCB-MSCs: umbilical cord blood-derived mesenchymal stem cells; BM-MSCs: bone marrow-derived mesenchymal stem cells; WJ-MSCs: Wharton’s jelly-derived mesenchymal stem cells; ESC-MSCs: embryonic stem cell-derived mesenchymal stem cells; HPLC: high performance liquid chromatography; TEM: transmission electron microscopy; SEM: scanning electron microscopy; NTA: nanoparticle tracking analysis; NOD/SCID mice: nonobese diabetic/severe combined immunodeficiency mice; Treg: regulatory T cells; Th: helper T cells; CXCL12: C-X-C motif chemokine ligand 1; HUVECs: human umbilical vein endothelial cells; AKI: acute kidney injury; TBI: traumatic brain injury; IDDM: insulin dependent diabetes mellitus; APCs: antigen presenting cells; EAU: experimental autoimmune uveoretinitis.
MSCs have a supporting effect on ex vivo expansion of CD34+ HSCs. Early passages of MSCs, early stage of the co-culture, irradiated MSCs, and children’s BM-MSCs, further increase ex vivo expansion of HSCs in a more primitive fraction (CD34+/CD38-) adding exogenous growth factors of HSCs in the co-culture medium with MSCs and forced expression of HSC-supportive factors by engineering in MSCs, further enhanced ex vivo expansion of HSCs. In this regard, CD34+ HSCs in the co-culture medium with MSCs as a feeder layer in the presence of exogenous cytokines such as SCF, TPO, and FLT3L compared with cytokines and MSCs conditions alone, further enhanced their ex vivo expansion.

MSCs in addition to the BM, also have been isolated from various tissues, including UCB, lung, dental pulp, fetal blood and liver, adipose tissue, skeletal muscle, cardiac tissues, placenta, synovial membrane, Wharton’s jelly, and amniotic fluid. MSCs from different sources induce the expansion of CD34+ HSCs in the co-culture system. However, BM-MSCs are significantly more efficient than other sources of MSCs such as Wharton’s jelly, amnion, and chorion for the ex vivo expansion of CD34+ HSCs. While in the another study it has been shown, Wharton’s jelly-derived mesenchymal stem cells (WJ-MSCs) have better potential to support ex vivo expansion of CD34+ HSCs in a more primitive condition than BM-MSCs. Moreover, no important difference observed with BM-MSCs compared to UCB-MSCs, and amniotic fluid. One group in 2015 reported that placenta-derived mesenchymal stem cells (P-MSCs) are better feeder layer for ex vivo expansion of HSCs than UCB-MSCs. They also reported, there is similar potential between BM-MSCs and P-MSCs for ex vivo expansion of HSCs. Additionally, it has been shown that adipose tissue-derived mesenchymal stem cells (AD-MSCs) with faster expansion rate than BM-MSCs, could support ex vivo expansion of CD34+ peripheral blood HSCs to the higher rate than BM-MSCs.

Da Silva et al. showed that direct cellular interactions between MSCs and CD34+ HSPCs, enhance ex vivo expansion of CD34+ and CD34+/CD38+ HSPCs. Similar results from other studies also showed the importance of this result. Moreover, it has been shown that in the co-culture system of MSCs and HSCs, MSCs surface is suitable for the ex vivo expansion of HSCs. In contrast to these studies, a study demonstrated indirect contact of MSCs with HSCs compared to direct contact, further enhance proliferation and ex vivo expansion rate of HSCs.

In the recent years, methods employed for the ex vivo expansion of HSCs, use natural or synthetic biomaterials in 2-dimensional (2D) or 3-dimensional (3D) culture systems. These biomaterials closely mimic the characteristics of in vivo HSC niche and so control the expansion and differentiation potential of HSCs. MSCs led to further expansion of CD34+ HSPCs in the 3D co-culture system compared to the 2D co-culture system. In the 3D collagen-based co-culture with MSCs, HSCs in contact with the collagenous matrix had higher expansion potential for a more primitive phenotype (CD34+, CD38+). MSCs in the 3D fibrin scaffold enhance further ex vivo expansion of CD34+ HSCs than collagen and poly-epsilon-caprolactone (PCL) scaffolds. It was also reported that 2D fibrin-based cultures without MSCs enhance further expansion of CD34+ HSCs than 2D PCL based cultures. Differentiated Osteoblasts from BM-MSCs have a supporting effect on ex vivo expansion of HSCs in the 2D co-culture system. In this regard, a group in 2016 reported that 3D co-culture system of BM-MSCs and differentiated osteoblasts from BM-MSCs with HSCs (3D mix) on human bio-derived bone scaffolds promote ex vivo expansion of CD34+ HSCs. On the other hand, ex vivo expansion of CD34+ HSCs in the 3D mix co-culture system significantly higher than 3D co-culture system of differentiated osteoblasts with CD34+ HSCs. Additionally, ex vivo expansion of CD34+ HSCs in the 3D MSCs with CD34+ HSCs co-culture system is close to 3D mix co-culture system. Huang et al. in 2016, also used human bio-derived bone scaffolds to establish a 3D co-culture system for BM-MSCs and human umbilical vein endothelial cells (HUVECs) with HSCs, that led to long-term ex vivo expansion of HSCs. Meanwhile, ex vivo expansion of HSCs in the 3D mix co-culture system (BM-MSCs and HUVECs with HSCs) significantly higher than 3D BM-MSCs and 3D HUVECs. Regarding the above-mentioned studies developing the new and improved biomaterials are needed for 3D co-culture systems for improving HSCs ex vivo expansion. However, these efforts must answer the demands in the clinical setting, especially improve the HSCs engraftment.

The effect of MSCs on HSCT and GVHD management following HSCT

HSCT achieved clinical therapeutic progress in the recent decades and is a useful and practical solution for cell therapy of many malignant and non-malignant hematological disorders, and immune system disorders. The first transplantation of HSCs are successfully performed in HLA-matched siblings from a UCB of a sister in 1988, was used to treat her younger brother with severe Fanconi anemia. Several factors affect the HSCs successful engraftment after transplantation, including dose of transplanted stem cells, the intensity of the primary treatment regimen, the HLA compatibility level between donor and recipient, the T cells amount in the graft, and the immunosuppression intensity after transplantation. MSCs possess various properties that by using them could promote HSCT and help to prevent graft rejection and GVHD following HSCT. These properties including secreted various cytokines and growth factors, extracellular adhesion molecules, the ability to differentiate into different stromal cells, tendency to homing into damaged and inflamed tissues after infusion, the immunoregulatory and anti-inflammatory effects on different subsets of immune system cells, easy isolation, and ex vivo expansion. MSCs overexpressing C-X-C motif chemokine receptor 4 (CXCR4) that generated by transduction in co-
transplantation with HSCs, promote HSCT and hematopoiesis in lethally irradiated mice. Another study showed that overexpression of CXCR4 on MSCs with a cocktail of cytokines, such as SCF, FLT3-L, IL-6, HGF (hepatocyte growth factor), and IL-3, accelerate the hematopoietic recovery after transplantation in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Moreover, hematopoietic engrafment process enhanced by culture-expanded UCB-MSCs transfected with cytokine genes required for growth of HSCs such as G-CSF and SCF, that co-transplanted with UCB-HSCs into NOD/SCID mice. Genetically modified MSCs that express stromal cell-derived factor-1/homeobox B4 (SDF-1/HOXB4) fusion protein in co-engraftment with human UCB-HSCs enhance survival and hematopoietic recovery in irradiated mice after transplantation. MSCs from sources such as adipose tissue and UCB have a potential as BM-MSCs for enhancing the engrafment of HSCs into NOD/SCID mice. UCB-MSCs from different donors have different effects in enhancing the engrafment of HSCs. On the contrary, it has been shown that AD-MSCs compared to BM-MSCs through producing a higher level of C-X-C motif chemokine ligand 12 (CXCL12), further promote HSCs homing and engrafment in a mouse model. Van der Garde et al in 2015 showed that WJ-MSCs or BM-MSCs in co-engraftment with UCB-HSCs have similarly enhanced effects on hematopoietic recovery into NOD/SCID mice. Moreover, WJ-MSCs have better potential to support CD34+ HSCs in a more primitive condition than BM-MSCs. Since WJ of UCB is a waste product and WJ-MSCs have some advantages such as higher rate of expansion, obtained at low-cost, minimal ethical concerns, lower immunogenicity, painless isolation method with any risk or harm to the donor compared to BM-MSCs. Therefore, WJ-MSCs can be used as a practical alternative source for HSCT. HSPCs derived from umbilical cord blood, expanded on WJ-MSCs in HP01 serum-free medium without adding exogenous cytokines, improve the hematopoietic engrafment in NOD/SCID mice. Without adding exogenous cytokines, ex vivo expanded HSCs on MSCs improve long-term hematopoietic engrafment in NOD/SCID mice. While in some studies, it has been shown that ex vivo expanded HSCs on MSCs in the presence of cocktails of cytokines such as TPO, SCF, G-CSF, and FLT3-L, enhance short-term hematopoietic engrafment in NOD/SCID mice. A group showed that combination of ex vivo expanded CD34+ umbilical cord blood cells and unmanipulated CD34+ umbilical cord blood cells on MSCs improve engrafment and decrease the time of platelet and neutrophil recovery after HSCT in patients with hematological malignancies compared with unmanipulated CD34+ umbilical cord blood cells only. Co-transplantation of MSCs with CD34+ cord blood cells into NOD/SCID mice, improved hematopoietic engrafment and platelet recovery after transplantation, and ex vivo expanded CD34+ cord blood cells with TPO improved early platelet recovery after transplantation, while co-transplantation of TPO-expanded CD34+ cord blood cells and MSCs into NOD/SCID mice not only had no synergistic effect on these results but also caused the risk of very low engrafment or rejection. Co-transplantation of adipose tissue-derived insulin-secreting MSCs (AD-IS-MSCs) that generated ex vivo with BM-HSCs into insulin-dependent diabetes Mellitus (IDDM) patients is a safe and efficient treatment choice for IDDM, and autologous co-infusion show a supported effect of the decreased need to exogenous insulin and therefore better long-term control of hyperglycemia compared with allogeneic co-infusion. Researchers in 2015 reported that co-transplantation of mouse AD-MSCs with HSCs, improve HSCs engrafment in an autologous mouse model. As respects, MSCs enhance the HSCT in allogeneic or xenogeneic transplantation model and this is probably due to the immunomodulatory features of MSCs, and this study performed in an autologous transplantation model, so no immune system rejection expected in this model. Therefore, strongly suggested that supportive hematopoietic engrafment effect of autologous AD-MSCs not due to the paracrine effects, but is due to direct contact between the HSCs and the AD-MSCs. Co-transplantation of autologous MSCs with HSCs, not only promote the recovery of platelets and neutrophils but also promote early recovery of T cell subsets and T cell reconstitution (especially reconstitution of naïve CD4+ T cells) in patients with malignant lymphomas. Also, it has been shown that co-engraftment of autologous HSCs and allogeneic MSCs enhance CD4+ CD25+ FoxP3+ regulatory T cells (Treg cells) after transplantation in a case report of a patient who suffered from refractory systemic lupus erythematosus (SLE). HSCs engrafment effect of AD-MSCs is dose-dependent, and high doses of AD-MSCs significantly increase HSCs engrafment after transplantation, in line with the studies in NOD/SCID mice that co-transplanted with human HSCs and human MSCs. On the other hand, higher doses of MSCs might decrease engrafment of HSCs. Moreover, co-engraftment of MSCs in a dose-dependent manner not only increase HSCs engrafment but also decrease GVHD. There are some complications such as GVHD, relapse, tissue damage due to the intensity of the treatment regimen, and infection that are dangerous for life in HSCT patients and they can restrict the widespread application and successfulness of HSCT therapy. GVHD is the most common complication that causes death after allogeneic HSCT and complicated inflammatory response that is known by the increased release of pro-inflammatory cytokines, activation of many types of donor T cells, and subsequently led to tissue damage in healthy tissues of the recipient. There are some prophylactic procedures that used for reducing the severity and incidence of GVHD after allogeneic HSCT such as ex vivo depletion of donor T cells by active depletion or enrichment of CD34+ HSCs and immunosuppressive agents. Nevertheless, ex vivo depletion of donor T cells causes an increased rate of graft versus leukemia (GVL) effect, infection because of the removing of donor T cells, and relapse. Moreover, immunosuppressive agents that are first-line factors for
treatment of GVHD associated with low response rate, decreased immune system reconstitution in patients, and the increased risk of GVL effect and opportunistic infections. For these reasons, new strategies are needed to prevent and treat the GVHD.

MSCs due to their immunomodulatory, anti-inflammatory, and tissue repair features could be effective in the field of GVHD treatment and prevention. BM-MSCs reduce the incidence of GVHD in mice that received allogeneic UCB transplantation. While some works suggested that MSCs cannot prevent GVHD or MSCs can decrease the incidence of GVHD but cannot promote hematopoietic recovery after transplantation. Also, Han et al. reported that co-infusion of MSCs with haploidentical HSCs compared to infusion of HSCs alone can reduce the time of neutrophil recovery after engraftment but cannot reduce the incidence of GVHD and has no impact on the infection and relapse in HSCT patients. 

MSCs through an increased in the Treg cells content, decreased donor T cells infiltration into target organs, increased expression of cytotoxic T lymphocyte antigen 4 (CTLA-4) molecule that expresses on Treg cells, and decreased of CD80/86 expression on recipient splenic dendritic cells (DCs) could suppress or reduce the incidence and severity of GVHD. In this setting, a recent study showed that third-party BM-MSCs administration decrease cutaneous sclerodermaetous GVHD by blocking immune effector cells infiltration into skin and this effect as a result of reduced expression of chemokines in skin such as C-C motif chemokine ligand 1 (CCL1), CCL3, CCL8, CCL17, and CCL22 and chemokine receptors such as C-C motif chemokine receptor 8 (CCR8) and CCR4 on CD4+ T cells and CCR1 on CD11b+ monocytes and macrophages. Another study showed that repeated infusions of UCB-MSCs can decrease the incidence of chronic GVHD after HLA-Haploidentical HSCT through an increased number of memory B cells and Treg cells and a decreased number of natural killer cells (NKs), as well as the increased ratio of Th1 cells to Th2 cells. BM-MSCs combined with conventional immunosuppressive agents are effective to refractory steroid-resistant acute GVHD, and decrease the incidence and severity of chronic GVHD by repairing damaged thymus and inducing the production of CD4+ CD25+ Foxp3+ Treg cells, although no differences observed in the incidence of infection and tumor relapse between patients with or without MSCs treatment. Third party MSCs through the increased numbers of IL-10+ FoxP3+ Treg cells, decreased numbers of pro-inflammatory Th17 cells, shifted immune system responses toward Th2 cells, increased the ratio of CD4+ T cells to CD8+ T cells, and increased secretion of IL-2, improve the treatment of steroid-refractory acute GVHD. Whereas above-mentioned studies demonstrated that the number of Treg cells was lower in the onset of GVHD and MSCs through an increased number of Treg cells could decrease the incidence of GVHD, some studies demonstrated that the number of Treg cells in the onset of GVHD was higher or MSCs infusion cannot increase the number of Treg cells and these issues complicate the role of Treg cells in the reduction of GVHD. The relevant information about the recent clinical applications of MSCs infusion to treat GVHD is summarized in Table 2.

**The effect of MSC-EVs on HSCT and GVHD management following HSCT**

EVs are the significant regulators in the determination of HSCs fate. In the investigation that performed by Ratajczak et al. it has been shown that mouse embryonic stem cell-derived microvesicles (ESC-MVs) considerably improve the survival and *ex vivo* expansion of murine HSPCs. As well as, co-culture of MVs derived from megakaryocytes (Mks) with HSPCs induce differentiation of HSPCs toward mature Mks lineages without adding exogenous TPO. Mesenchymal stem cell-derived microvesicles (MSC-MVs) enhance the *ex vivo* expansion of cord blood CD34+ HSCs and cord blood-derived mononuclear cells (CB-MNCs). But this enhancement was lower compared with the enhancement by MSCs alone. This is because of, various of growth factors secreted by MSCs in addition to MVs. Moreover, MSC-MVs enhanced further expansion of HSCs, when added to the co-culture system of MSCs and HSCs. The MSC-MVs through Wnt/β-catenin signaling pathway increase *ex vivo* expansion, self-renewal, and block differentiation of HSCs. MSC-MVs contain miRNAs that have regulatory effects on HSCs and miRNAs that target genes that have inhibitory effects on Wnt/β-catenin signaling pathway. De Luca et al. demonstrated that MSC-EVs miRNAs and Piwi-interacting RNAs (piRNAs) influence HSCs gene expression pattern such as inducing cell survival, inhibiting apoptosis, and decreasing cellular differentiation to the all hematopoietic lineages. As well as, MSC-EVs miRNAs through an increased expression of CXCR4 in the CD34+ HSCs that transplanted with MSC-EVs miRNAs or treated with MSC-EVs, cause increased migration of CD34+ HSCs from peripheral blood (PB) to BM niche, significantly increase HSCs engraftment in NOD/SCID/IL-2Rγnull (NSG) mice. On the contrary, another study showed that MSC-EVs treatment induces the decreased quiescence and expansion of HSCs and increased differentiation of HSCs to myeloid progenitors. MSC-EVs promoted HSCs differentiation via cell surface interaction with Toll-like receptor 4 (TLR4) instead of their cargo. This interaction led to activation of myeloid differentiation primary response (MyD88) adaptor protein and NFκB transcription factor. MSC-EVs or whole BM cells derived EVs can decrease *ex vivo* radiation injury to murine HSCs cell line by stimulating of HSC proliferation, suppression of DNA destruction and apoptosis. But *in vivo* effect of MSC-EVs to decrease radiation injury to murine marrow HSCs was partial. Also, overexpression of miRNAs that are abundant in MVs in the murine HSCs cell line could partially decrease the radiation injury.
MSC-EVs have immunomodulatory effects on the immune system response and therefore they can prevent GVHD, adjust active immune system responses or inflammation-associated disorders. In a study for the first time, it has been shown that MSC-derived exosomes infusion significantly improve symptoms of the steroid-resistant acute GVHD patients shortly after administration of exosomes without reported side effects as well as in vivo and ex vivo decrease of pro-inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ has been observed.146 Additionally, Amarnath et al. identified that CD73 expressing exosomes derived from human BM-MSCs modulate GVHD in a mouse model through converting of ATP to adenosine, and this exosome-associated mechanism inhibits Th1 cells function in the mouse and therefore modulate GVHD.131 Finally, a group showed that MSC-EVs could decrease acute GVHD by modulating immune system responses in mice that undergo allogeneic HSCT, decrease the in vivo manifestations of acute GVHD, and therefore significantly increased the survival of recipient mice. They also suggested because of the safety concerns of MSCs clinical applications, MSC-EVs can use as an ideal alternative for preventing of acute GVHD after allogeneic HSCT.25 Recipient mice showed the important decrease of tolerated frequency and the absolute number of alloreactive T cells; HSCT. alternative for preventing of acute GVHD after allogeneic HSCT.

### Table 2. Clinical use of MSCs infusion for treatment of GVHD

| Clinical Context | Number of patients | MSCs source | The dose of MSCs | Source of transplanted HSCs | Outcome | Ref |
|------------------|--------------------|-------------|------------------|-----------------------------|---------|-----|
| steroid-resistant grade II-IV acute and chronic GVHD after allogeneic HSCT | 40 adults and children patients | Third party PL-expanded BM-MSCs | Median 1.5 × 10^6 cells/kg Median 3 dose | BM, UCB, and PB, HLA-matched, HLA-mismatched, and Haploidentical | OR: 67.5%; CR: 27.5%; PR: 40.0%. No severe toxicity, better in grade II and children. | 132 |
| steroid-resistant grade II-IV acute GVHD after allogeneic HSCT | 46 adults and children patients | Third party FBS-expanded BM-MSCs | Median 6.81 × 10^6 cells/kg Median 3 dose | BM, UCB, and PB, HLA-matched and HLA-mismatched | OR: 50%; CR: 6.5%; PR: 30.5%; TPR: 13%; severe transient side effects during cell injection: 4.3%; No acute or late side effects. | 133 |
| steroid-resistant grade I-IV acute or chronic GVHD after allogeneic HSCT | 11 children patients | Third party PL-expanded BM-MSCs | Median 1.2 × 10^6 cells/kg Median 3 dose | BM, UCB, and PB, HLA-matched and HLA-mismatched | OR: 71.4%; CR: 23.8%; PR: 47.6%. No acute or late side effects, better in acute GVHD. | 134 |
| steroid-resistant grade III-IV acute GVHD after allogeneic HSCT | 13 patients | Third party PL-expanded BM-MSCs | Median 0.9 × 10^6 cells/kg Median 3 dose | PB, HLA-matched and HLA-mismatched | OR: 54%; CR: 7.5%; PR: 7.5%; MR:39%. No toxicity during or quickly after the injection. | 135 |
| steroid-resistant grade III-IV acute GVHD after allogeneic HSCT | 37 children patients | Third party FBS-expanded BM-MSCs | 1−2 × 10^6 cells/kg Median 2 dose | BM, UCB, and PB, HLA-matched, HLA-mismatched, and Haploidentical | OR: 86%; CR: 65%; PR: 21%. Better OS, if MSC treatment quickly after the beginning of acute GVHD. | 136 |
| steroid-resistant grade I-IV acute GVHD after allogeneic HSCT | 58 patients | Third party PL-expanded BM-MSCs | Median 0.99 × 10^6 cells/kg Median 3 dose | BM and PB, HLA-matched and HLA-mismatched | OR: 47%; CR: 9%; VGPR: 9; PR: 29%. No better OS compared to patients that have no MSCs infusion. | 137 |
| steroid-resistant chronic GVHD after allogeneic HSCT | 23 patients | Third party BM-MSCs | 1 × 10^6 cells/kg Median 2 dose | BM and PB, HLA-matched and HLA-mismatched | OR: 87%. Increased number of CD5+ regulatory B cells. | 138 |
| steroid-resistant grade III-IV acute GVHD after allogeneic HSCT | 25 adults and children patients | Third party BM-MSCs | 2 × 10^6 cells/kg Median 2 dose | BM, UCB, and PB, HLA-identical and HLA-mismatch | OR: 60%; CR: 24%; PR: 36%. No side effects. | 139 |
| Sclerodermatous chronic GVHD after allogeneic HSCT | 4 patients | Third party BM-MSCs | 1−2 × 10^7 cells/kg Median 4-8 dose | BM, HLA-identical sibling | gradually improvement of the symptoms of chronic GVHD. Intra bone marrow injection. No side effects. Increased ratio of Th1/Th2 cells. | 140 |
| steroid-resistant grade II-IV acute GVHD after allogeneic HSCT | 25 patients | Third party PL-expanded BM-MSCs | Median 1.1 × 10^6 cells/kg Median 2−4 dose | Not shown | OR: 71%; CR: 46%; PR: 25%. Lower toxicity. | 141 |

GVHD: graft versus host disease; HSCT: hematopoietic stem cell transplantation; PL-expanded BM-MSCs: platelet lysate expanded bone marrow-derived mesenchymal stem cells; FBS-expanded BM-MSCs: fetal bovine serum expanded bone marrow-derived mesenchymal stem cells; BM: bone marrow; UCB: umbilical cord blood; PB: peripheral blood; OR: overall response; CR: complete response; VGPR: very good partial response; PR: partial response; TPR: transient partial response; MR: mixed response; OS: overall survival.

Advanced Pharmaceutical Bulletin, 2017, 7(4), 531-546 | 537
such as TNF-α, IL-2, and IFN-γ; and increased serum levels of anti-inflammatory cytokines such as IL-10.23
Compatible with data from previous investigations,44,147 The therapeutic effect of MSC-EVs in the recent studies on HSCs expansion, HSCT, and GVHD is summarized in Table 3.

| Source of EVs | Type of EVs and size | Isolation method | Identify method | Source of HSCs | Outcome | Ref |
|---------------|----------------------|------------------|----------------|---------------|---------|-----|
| Human BM-MSCs | MVs 100-1000 nm Centrifugation (16000 g 1 + 1 h) Flowcytometry and TEM | Human cord blood CD34+ cells | Enhance the ex vivo expansion of cord blood-derived CD34+ cells | | 12 |
| Human BM-MSCs | EVs 100-2000 nm Ultracentrifugation (100000 g 70 + 70 min) Flowcytometry and TEM | Human cord blood CD34+ cells | Induce the ex vivo cell survival and decrease differentiation of cord blood CD34+ cells. Significantly increase in vivo HSCs engraftment in NSG mice | | 24 |
| Mouse BM-MSCs and mouse AD-MSCs | EVs 100-400 nm Ultracentrifugation (100000 g 2 h) TEM, western blotting, and NTA Mouse BM-HSPCs | Mouse BM-HSPCs | Induce the decreased expansion of HSPCs and increased differentiation of HSPCs to myeloid progenitors ex vivo | | 164 |
| Murine BM-MSCs and human BM-MSCs | EVs mean 249nm Ultracentrifugation (100000 g 1 h) TEM, western blotting, and NTA Murine BM-HSCs | Murine BM-HSCs | Decrease radiation injury to murine HSCs cell line ex vivo. Partially decrease radiation injury to murine marrow HSCs in vivo | | 145 |
| Human UCB-MSCs | EVs 30-100 nm Ultracentrifugation (100000 g 2 + 2 h) Flowcytometry and TEM | Mouse BM-HSCs | Decrease acute GVHD and increase the survival of recipient mice that undergo allogeneic HSCT. Show immunosuppressive effects ex vivo | | 25 |
| Human BM-MSCs | Exosome Size not shown Exosome isolation kit Flowcytometry and electron microscopy | - | Attenuated GVHD in a mouse model via the exosome-associated adenosine signaling pathway in Th1 cells. | | 111 |
| Human BM-MSCs | Exosome 99-123 nm (ZetaView®) 133-138 nm (NanoSight®) Ultracentrifugation (100000 g 2 h) TEM, western blotting, and NTA | - | Decrease symptoms of the therapy-resistant acute GVHD patients. In vivo and ex vivo decrease of pro-inflammatory cytokines | | 146 |

EVs: extracellular vesicles; MVs: microvesicles; UCB-MSCs: umbilical cord blood-derived mesenchymal stem cells; BM-MSCs: bone marrow-derived mesenchymal stem cells; AD-MSCs: adipose tissue-derived mesenchymal stem cells; TEM: transmission electron microscopy; NTA: nanoparticle tracking analysis; BM-HSPCs: bone marrow derived hematopoietic stem and progenitor cells; BM-HSCs: bone marrow derived hematopoietic stem cells; HPC: hematopoietic progenitor cells; GVHD: graft versus host disease; HSCT: hematopoietic stem cell transplantation.

In addition to healthy tissues, MSC-EVs obtained from patient tissues could significantly control the determination of HSCs fate. In this regard, a group recently showed that MSC-MVs of myelodysplastic syndrome (MDS) patients have different cargo compared with MSC-EVs from healthy donors and could adjust CD34+ hematopoietic progenitor cells (HPCs) characteristics such as increasing cell viability and clonogenicity and changing of miRNA and gene expression pattern in the co-culture system.148 As well as, investigations on MSC-EVs of myeloproliferative neoplasm (MPN) patients, showed that their total miRNA content especially miRNA155 increased compared with healthy donors. MSC-EVs from both MPN patients and healthy donors could increase cell viability of CD34+ HPCs and MSC-EVs from MPN patients could increase the number of granulocyte-monocyte colony forming unit (CFU-GM) from neoplastic CD34+ HPCs in the co-culture system.149

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Conclusion
Many studies have been conducted on the co-culture and co-transplantation of MSCs with HSCs to improve HSCs expansion and the clinical potential of HSCT, prevention and treatment of GVHD, which emerging results showed different effects. During co-culture and co-transplantation with MSCs, HSCs undergo of either expansion or differentiation. Controlling the balance between expansion and differentiation of HSCs for HSCT and GVHD objectives is essential. For HSCT and GVHD objectives, it is essential to decrease differentiation and increase expansion and further studies are needed to answer this problem. However, there are some drawbacks about MSCs clinical applications such as increased incidence of pneumonia-related death in HSCT patients, the increased tumor progression, the uncontrolled differentiation of MSCs that led to ectopic tissue formation, pulmonary embolism due to intravenous infusion, and the undesired long-term side effects. Which challenged their clinical applications. Therefore, some recent studies used MSC-EVs because of the advantages that mentioned in the introduction, instead of MSCs for cell-based therapy in the field of HSCT's expansion, engrafment, and GVHD. MSC-EVs in these studies showed that they can use as a practical alternative for improving HSCs expansion and engrafment, decreasing differentiation of HSCs and GVHD following HSCT. Their effects were lower than MSCs. These effects are probably due to the fact that MSCs exert their effects in the cell-based therapy by employing both intercellular direct contact and paracrine processes. Whereas the well-defined molecular and cellular mechanisms that led to the supportive effect of MSC-EVs on hematopoeisis are still unknown, controversial, and probably are different from one condition to another. Moreover, at present, differential ultracentrifugation is the most widely used and gold standard method for isolation of MSC-EVs that performed through different protocols and therefore led to different results. For example, different populations of MSC-EVs obtained through different protocols of differential ultracentrifugation that they are different in bioactive cargoes. Additionally, MSCs heterogeneity, gender, and donor age may influence on MSC-EVs biological effects and cargoes because of these factors showed different effects on the functional features of MSCs. Therefore, further studies are needed to obtain comprehensive knowledge about MSC-EVs roles in the hematopoeisis and their isolation methods. In the future, developing the combination of MSCs and MSC-EVs with factors that enhance self-renewal, block differentiation, and promote homing for HSCs engrafment purposes is essential. Moreover, we must have comprehensive knowledge about the changes that are occurring in the HSCs after inducing by MSC-EVs, the complete bioactive cargo that packaged into the MSC-EVs, the safety of MSC-EVs, and optimal dosage of MSC-EVs. Finally, methods for improving the storage, collection, purifying, and large-scale production of MSC-EVs that are cost-effective, less time-consuming and less labor-intensive should be developed and standardized.

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Ethical Issues
Not applicable.

Conflict of Interest
The authors report no conflicts of interest in this review.

References
1. Saleh M, Shamsasanjan K, Movassaghpourakbari A, Akbarzadehaleh P, Molaiepour Z. The impact of mesenchymal stem cells on differentiation of hematopoietic stem cells. Adv Pharm Bull 2015;5(3):299-304. doi: 10.15171/apb.2015.042
2. Wang LD, Wagers AJ. Dynamic niches in the origination and differentiation of haematopoietic stem cells. Nat Rev Mol Cell Biol 2011;12(10):643-55. doi: 10.1038/nrm3184
3. Mosaad YM. Hematopoietic stem cells: An overview. Transfus Apher Sci 2014;51(3):68-82. doi: 10.1016/j.transci.2014.10.016
4. Gang EJ, Jeong JA, Hong SH, Hwang SH, Kim SW, Yang IH, et al. Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood. Stem Cells 2004;22(4):617-24. doi: 10.1634/stemcells.22-4-617
5. Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. Mol Ther 2015;23(5):812-23. doi: 10.1038/mt.2015.44
6. Mohammadian M, Shamsasanjan K, Lotfi Nezhad P, Talebi M, Jahedi M, Nickkhhah H, et al. Mesenchymal stem cells: New aspect in cell-based regenerative therapy. Adv Pharm Bull 2013;3(2):433-7. doi: 10.5681/apb.2013.070
7. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 2012;12(5):383-96. doi: 10.1038/nri3209
8. Aqmasheh S, Shamsasanjan K, Akbarzadehaleh P, Pashoutan Sarvar D, Timari H. Effects of mesenchymal stem cell derivatives on hematopoiesis and hematopoietic stem cells. Adv Pharm Bull 2017;7(2):165-77. doi: 10.15171/apb.2017.021
9. Fierabracci A, Del Fattore A, Luciano R, Muraca M, Teti A, Muraca M. Recent advances in mesenchymal stem cell immunomodulation: The role of microvesicles. Cell Transplant 2015;24(2):133-49. doi: 10.3727/096368913x675728
10. Maukus M, Jorgensen C, Noel D. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secrectome and exosomes. Biochimie 2013;95(12):2229-34. doi: 10.1016/j.biochi.2013.04.017
11. Li T, Wu Y. Paracrine molecules of mesenchymal stem cells for hematopoietic stem cell niche. Bone Marrow Res 2011;2011:353878. doi: 10.1155/2011/353878

12. Xie H, Sun L, Zhang L, Liu T, Chen L, Zhao A, et al. Mesenchymal stem cell-derived microvesicles support ex vivo expansion of cord blood-derived cd34(+) cells. Stem Cells Int 2016;2016:6493241. doi: 10.1155/2016/6493241

13. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010;466(7308):829-34. doi: 10.1038/nature09262

14. Jing D, Fonseca AV, Alakel N, Fierro FA, Muller K, Bornhauser M, et al. Hematopoietic stem cells in coculture with mesenchymal stromal cells--modeling the niche compartments in vitro. Haematologica 2010;95(4):542-50. doi: 10.3324/haematol.2009.010736

15. Liang X, Ding Y, Zhang Y, Tse HF, Lian Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: Current status and perspectives. Cell Transplant 2014;23(9):1045-59. doi: 10.3727/096368913x667709

16. Camussi G, Deregibus MC, Tetta C. Paracrine/endocrine mechanism of stem cells on kidney repair: Role of microvesicle-mediated transfer of genetic information. Curr Opin Nephrol Hypertens 2010;19(1):7-12. doi: 10.1097/MNH.0b013e3283323b6f

17. Pashoustan Sarvar D, Shamsasenjan K, Akbarzadehlah P. Mesenchymal stem cell-derived exosomes: New opportunity in cell-free therapy. Adv Pharm Bull 2016;6(3):293-9. doi: 10.15171/apb.2016.041

18. Chen J, Li C, Chen L. The role of microvesicles derived from mesenchymal stem cells in lung diseases. Biomed Res Int 2015;2015:985814. doi: 10.1155/2015/985814

19. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009;9(8):581-93. doi: 10.1038/nri2567

20. Zoller M. Tetraspanins: Push and pull in suppressing and promoting metastasis. Nat Rev Cancer 2009;9(1):40-55. doi: 10.1038/nrc2543

21. Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C, et al. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. Nephrol Dial Transplant 2011;26(5):1474-83. doi: 10.1093/ndt/gfr015

22. Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, et al. Exosome secreted by msc reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010;4(3):214-22. doi: 10.1016/j.scr.2009.12.003

23. Gyorgy B, Hung ME, Breakefield XO, Leonard JN. Therapeutic applications of extracellular vesicles: Clinical promise and open questions. Ann Rev Pharmacol Toxicol 2015;55:439-64. doi: 10.1146/annurev-pharmaco-010814-124630

24. De Luca L, Trino S, Laurennaza I, Simeon V, Calice G, Raimondo S, et al. Mirnas and pirnas from bone marrow mesenchymal stem cell extracellular vesicles induce cell survival and inhibit cell differentiation of cord blood hematopoietic stem cells: A new insight in transplantation. Oncotarget 2016;7(6):6676-92. doi: 10.18632/oncotarget.6791

25. Wang L, Gu Z, Zhao X, Yang N, Wang F, Deng A, et al. Extracellular vesicles released from human umbilical cord-derived mesenchymal stem cells prevent life-threatening acute graft-versus-host disease in a mouse model of allogeneic hematopoietic stem cell transplantation. Stem Cells Dev 2016;25(24):1874-83. doi: 10.1089/scd.2016.0107

26. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: Composition, biological relevance, and methods of study. Bioscience 2015;65(8):783-97. doi: 10.1038/biov084

27. Lai RC, Tan SS, Teh BJ, Sze SK, Arslan F, de Kleijn DP, et al. Proteolytic potential of the msc exosome proteome: Implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics 2012;2012:971907. doi: 10.1155/2012/971907

28. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gaine L, Sena-Estes M, et al. Glioblastoma microvesicles transport rna and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008;10(12):1470-6. doi: 10.1038/ncl1800

29. Lou G, Chen Z, Zheng M, Liu Y. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp Mol Med 2017;49(6):e346. doi: 10.1038/emm.2017.63

30. Mulcay LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles 2014;3: doi: 10.3402/jev.v3.24641

31. van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 2012;64(3):676-705. doi: 10.1124/pr.112.005983

32. Kilpinen L, Impola U, Sankkila L, Ritamo I, Aatonen M, Kilpinen S, et al. Extracellular membrane vesicles from umbilical cord blood-derived msc protect against ischemic acute kidney injury, a feature that is lost after inflammatory conditioning. J Extracell Vesicles 2013;2: doi: 10.3402/jev.v2i0.21927

33. Lespagnol A, Dufaut D, Beehman C, Blanco L, Fiucci G, Marine JC, et al. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in tsap6/steap3-null mice. Cell Death Differ 2008;15(11):1723-33. doi: 10.1038/cdd.2008.104

34. Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, et al. Exodus mediates the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension.
Circulation 2012;126(22):2601-11. doi: 10.1161/circulationaha.112.114173

35. Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci 2014;15(3):4142-57. doi: 10.3390/ijms15034142

36. Sabin K, Kikyo N. Microvesicles as mediators of tissue regeneration. Transl Res 2014;164(3):286-95. doi: 10.1016/j.trsl.2013.10.005

37. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 2011;29(4):341-5. doi: 10.1038/nbt.1807

38. Wang B, Yao K, Huuskes BM, Shen HH, Zhuang J, Godson C, et al. Mesenchymal stem cells deliver exogenous microrna-let7c via exosomes to attenuate renal fibrosis. Mol Ther 2016;24(7):1290-301. doi: 10.1038/mt.2016.90

39. Pascucci L, Cocce V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: A new approach for drug delivery. J Control Release 2014;192:262-70. doi: 10.1016/j.jconrel.2014.07.042

40. Yuan Z, Kolluri KK, Gowers KH, Janes SM. Trail delivery by mes-cs-derived extracellular vesicles is an effective anticancer therapy. J Extracell Vesicles 2017;6(1):1265291. doi: 10.1080/20013078.2017.1265291

41. Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells Dev 2013;22(6):845-54. doi: 10.1089/scd.2012.0395

42. Tan CY, Lai RC, Wong W, Dan YY, Lim SK, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 2014;5(3):76. doi: 10.1186/scrt465

43. Bruno S, Grange C, Collino F, Deregibus MC, Cantaluppi V, Biancone L, et al. Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. PLoS One 2012;7(3):e33115. doi: 10.1371/journal.pone.0033115

44. Zou X, Zhang G, Cheng Z, Yin D, Du T, Ju G, et al. Microvesicles derived from human wharton's jelly mesenchymal stromal cells ameliorate renal ischemia-reperfusion injury in rats by suppressing cx3ccl1. Stem Cell Res Ther 2014;5(2):40. doi: 10.1186/scrt428

45. Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, et al. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. J Neurosurg 2015;122(4):856-67. doi: 10.3171/2014.11.jns14770

46. Shigemoto-Kuroda T, Oh JY, Kim DK, Jeong HJ, Park SY, Lee HJ, et al. Msc-derived extracellular vesicles attenuate immune responses in two autoimmune murine models: Type 1 diabetes and uveoretinitis. Stem Cell Reports 2017;8(5):1214-25. doi: 10.1016/j.stemcr.2017.04.008

47. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells Dev 2015;24(14):1635-47. doi: 10.1089/scd.2014.0316

48. Pelosi E, Castelli G, Testa U. Human umbilical cord is a unique and safe source of various types of stem cells suitable for treatment of hematological diseases and for regenerative medicine. Blood Cells Mol Dis 2012;49(1):20-8. doi: 10.1016/j.bcmd.2012.02.007

49. Herve P. Donor-derived hematopoietic stem cells in organ transplantation: Technical aspects and hurdles yet to be cleared. Transplantation 2003;75(9 Suppl):55s-7s. doi: 10.1097/01.tp.0000076954.60639.9c

50. Gluckman E. History of cord blood transplantation. Bone Marrow Transplant 2009;44(10):621-6. doi: 10.1088/bmt.2009.280

51. Zhong XY, Zhang B, Asadollahi R, Low SH, Holzgreve W. Umbilical cord blood stem cells: What to expect. Ann N Y Acad Sci 2010;1205:17-22. doi: 10.1111/j.1749-6632.2010.05659.x

52. Norkin M, Lazarus HM, Wingard JR. Umbilical cord blood graft enhancement strategies: Has the time come to move these into the clinic? Bone Marrow Transplant 2013;48(7):884-9. doi: 10.1038/bmt.2012.163

53. Sideri A, Neokleous N, Brunet De La Grange P, Guerton B, Le Bousse Kerdilles MC, Uzan G, et al. An overview of the progress on double umbilical cord blood transplantation. Haematologica 2011;96(8):1213-20. doi: 10.3324/haematol.2010.038836

54. Fan X, Gay FP, Ong SY, Ang JM, Chu PP, Bari S, et al. Mesenchymal stromal cell supported umbilical cord blood ex vivo expansion enhances regulatory t cells and reduces graft versus host disease. Cytotherapy 2013;15(5):610-9. doi: 10.1016/j.jcyt.2012.12.007

55. Dahlberg A, Delaney C, Bernstein ID. Ex vivo expansion of human hematopoietic stem cells and progenitor cells. Blood 2011;117(23):6083-90. doi: 10.1182/blood-2011-01-283606

56. Lo Iacono M, Anzalone R, La Rocca G, Baiamonte E, Maggio A, Acuto S. Wharton's jelly mesenchymal stromal cells as a feeder layer for the ex vivo expansion of hematopoietic stem and progenitor cells: A review. Stem Cell Rev 2017;13(1):35-49. doi: 10.1007/s12015-016-9702-4

57. Shpall EJ, Quinones R, Giller R, Zeng C, Baron AE, Jones RB, et al. Transplantation of ex vivo expanded cord blood. Biol Blood Marrow Transplant 2002;8(7):368-76.
cortical blood (ucb) transplantation with ex vivo-expanded ucb cells: Results of a phase 1 trial using the aastromreplicell system. Blood 2003;101(12):5061-7. doi: 10.1182/blood-2001-12-0290

59. Fajardo-Orduña GR, Mayani H, Montesinos JJ. Hematopoietic support capacity of mesenchymal stem cells: Biology and clinical potential. Arch Med Res 2015;46(8):589-96. doi: 10.1016/j.arcmed.2015.10.001

60. Wang JF, Wang LJ, Wu YF, Xiang Y, Xie CG, Jia BB, et al. Mesenchymal stem/progenitor cells in human umbilical cord blood as support for ex vivo expansion of cd34(+) hematopoietic stem cells and for chondrogenic differentiation. Haematologica 2004;89(7):837-44.

61. Jang YK, Jung DH, Jung MH, Kim DH, Yoo KH, Sung KW, et al. Mesenchymal stem cells feeder layer from human umbilical cord blood for ex vivo expanded growth and proliferation of hematopoietic progenitor cells. Ann Hematol 2006;85(4):212-25. doi: 10.1007/s00277-005-0437-3

62. Wolenda T, Bork S, Horn P, Wein F, Saffrich R, Diehlmann A, et al. Co-culture with mesenchymal stromal cells increases proliferation and maintenance of haematopoietic progenitor cells. J Cell Mol Med 2010;14(1-2):337-50. doi: 10.1111/j.1582-4934.2009.00776.x

63. da Silva CL, Goncalves R, Porada CD, Ascensao JL, Zanjani ED, Cabral JM, et al. Differences amid bone marrow and cord blood hematopoietic stem/progenitor cell division kinetics. J Cell Physiol 2009;220(1):102-11. doi: 10.1002/jcp.21736

64. Kadekar D, Kale V, Limaye L. Differential ability of mscs isolated from placenta and cord as feeders for supporting ex vivo expansion of umbilical cord blood derived cd34(+) cells. Stem Cell Res Ther 2015;6:201. doi: 10.1186/s13287-015-0194-y

65. Celebi B, Mantovani D, Pineault N. Irradiated mesenchymal stem cells improve the ex vivo expansion of hematopoietic progenitors by partly mimicking the bone marrow endosteal environment. J Immunol Methods 2011;370(1-2):93-103. doi: 10.1016/j.jim.2011.06.006

66. Pelagiadis I, Stiakaki E, Choulaki C, Kalmanti M, Dimitriou H. The role of children's bone marrow mesenchymal stromal cells in the ex vivo expansion of autologous and allogeneic hematopoietic stem cells. Cell Biol Int 2015;39(10):1099-110. doi: 10.1002/cibi.10483

67. Khoury M, Drake A, Chen Q, Dong D, Leskov I, Fragoso MF, et al. Mesenchymal stem cells secreting angiopoietin-1 like-5 support efficient expansion of human hematopoietic stem cells without compromising their repopulating potential. Stem Cells Dev 2011;20(8):1371-81. doi: 10.1089/scd.2010.0456

68. Ong LM, Fan X, Chu PP, Gay FP, Bari S, Ang JM, et al. Cotransplantation of ex vivo expanded and unexpanded cord blood units in immunodeficient mice using insulin growth factor binding protein-2 augmented mesenchymal cell cocultures. Biol Blood Marrow Transplant 2012;18(5):674-82. doi: 10.1016/j.bbmt.2012.01.001

69. Amirizadeh N, Oodi A, Mehrasa R, Nikougoftar M. Apoptosis, dap kinase 1 expression and the influences of cytokine milieu and mesenchymal stromal cells on ex vivo expansion of umbilical cord blood-derived hematopoietic stem cells. Indian J Hematol Blood Transfus 2016;32(1):67-77. doi: 10.1007/s12288-015-0545-y

70. Mehrasa R, Vaziri H, Oodi A, Khoshshidfar M, Nikougoftar M, Golpour M, et al. Mesenchymal stem cells as a feeder layer can prevent apoptosis of expanded hematopoietic stem cells derived from cord blood. Int J Mol Cell Med 2014;3(1):1-10.

71. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001;98(8):2396-402.

72. In ’t Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FH, Willemsre R, et al. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood 2003;102(4):1548-9. doi: 10.1182/blood-2003-04-1291

73. Kellner J, Sivajothi S, McNiece I. Differential properties of human stromal cells from bone marrow, adipose, liver and cardiac tissues. Cytotherapy 2015;17(11):1514-23. doi: 10.1016/j.jcyt.2015.07.009

74. Robinson SN, Ng J, Niu T, Yang H, McMannis JD, Karandish S, et al. Superior ex vivo cord blood expansion following co-culture with bone marrow-derived mesenchymal stem cells. Bone Marrow Transplant 2006;37(4):359-66. doi: 10.1038/sj.bmt.1705258

75. Li N, Feugier P, Serrurier B, Latger JF, Stoltz JF, et al. Human mesenchymal stem cells improve ex vivo expansion of adult human cd34+ peripheral blood progenitor cells and decrease their allostimulatory capacity. Exp Hematol 2007;35(3):507-15. doi: 10.1016/j.exphem.2006.10.015

76. Klein C, Strobel J, Zingsem J, Richter RH, Goecke TW, Beckmann MW, et al. Ex vivo expansion of hematopoietic stem- and progenitor cells from cord blood in coculture with mesenchymal stroma cells from amnion, chorion, wharton's jelly, amniotic fluid, cord blood, and bone marrow. Tissue Eng Part A 2013;19(23-24):2577-85. doi: 10.1089/ten.tea.2013.0073

77. van der Garde M, van Pel M, Millan Rivero JE, de Graaf-Dijkstra A, Slot MC, Kleinvedy Y, et al. Direct comparison of wharton's jelly and bone marrow-derived mesenchymal stromal cells to enhance engraftment of cord blood cd34(+) transplants. Stem
80. Wagner W, Wein F, Roderburg C, Safirich R, Faber A, Krause U, et al. Adhesion of hematopoietic progenitor cells to human mesenchymal stem cells as a model for cell-cell interaction. Exp Hematol 2007;35(2):314-25. doi: 10.1016/j.exphem.2006.10.003
81. Alaker N, Jing D, Muller K, Bornhauser M, Ehninger G, Ordemann R. Direct contact with mesenchymal stromal cells affects migratory behavior and gene expression profile of cd133+ hematopoietic stem cells during ex vivo expansion. Exp Hematol 2009;37(4):504-13. doi: 10.1016/j.exphem.2008.12.005
82. Lazar-Karsten P, Dorn I, Meyer G, Lindner U, Driller B, Schlenke P. The influence of extracellular matrix proteins and mesenchymal stem cells on erythropoietic cell maturation. Vox Sang 2011;101(1):65-76. doi: 10.1111/j.1423-0410.2010.01453.x
83. Zhang Y, Chai C, Jiang XS, Teoh SH, Leong KW. Co-culture of umbilical cord blood cd34+ cells with human mesenchymal stem cells: Tissue Eng 2006;12(8):2161-70. doi: 10.1089/ten.ten.2006.12.2161
84. Leisten I, Kramann R, Ventura Ferreira MS, Bovi M, Neuss S, Ziegler P, et al. 3d co-culture of hematopoietic stem progenitor cells and mesenchymal stem cells in collagen scaffolds as a model of the hematopoietic niche. Biomaterials 2012;33(6):1736-47. doi: 10.1016/j.biomaterials.2011.11.034
85. Ferreira MS, Jahnep-Dechent W, Labude N, Bovi M, Hieronymus T, Zenke M, et al. Cord blood-hematopoietic stem cell expansion in 3d fibrin scaffolds with stromal support. Biomaterials 2012;33(29):6987-97. doi: 10.1016/j.biomaterials.2012.06.029
86. Ferreira MS, Schneider RK, Wagner W, Jahnep-Dechent W, Labude N, Bovi M, et al. Two-dimensional polymer-based cultures expand cord blood-derived hematopoietic stem cells and support engraftment of nsg mice. Tissue Eng Part C Methods 2013;19(1):25-38. doi: 10.1089/ten.TEC.2011.0706
87. Huang XB, Liu T, Meng WT, Zhi W. [osteoblasts differentiated from human marrow bone mesenchymal stem cells support hematopoietic stem/progenitor cells from umbilical cord blood]. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2006;14(3):552-6.
88. Huang X, Zhu B, Wang X, Xiao R, Wang C. Three-dimensional co-culture of mesenchymal stromal cells and differentiated osteoblasts on human bio-derived bone scaffolds supports active multi-lineage hematopoiesis in vitro: Functional implication of the biomimetic niche. Int J Mol Med 2016;38(4):1141-51. doi: 10.3892/immm.2016.7212
89. Huang X, Li C, Zhu B, Wang H, Luo X, Wei L. Co-cultured hbmnsacs and hucvcs on human bio-derived bone scaffolds provide support for the long-term ex vivo culture of hsc/hpcs. J Biomed Mater Res A 2016;104(5):1221-30. doi: 10.1002/jbm.a.35656
90. Triputa C, Pande G. Applications of human hematopoietic stem cells isolated and expanded from different tissues in regenerative medicine. Regen Med 2013;8(6):783-95. doi: 10.2217/rme.13.75
91. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with fanconi's anemia by means of umbilical-cord blood from an hla-identical sibling. N Engl J Med 1989;321(17):1174-8. doi: 10.1056/nejm198910263211707
92. Kallekleiv M, Larun L, Bruserud O, Hatfield KJ. Co-transplantation of multipotent mesenchymal stromal cells in allogeneic hematopoietic stem cell transplantation: A systematic review and meta-analysis. Cytotherapy 2016;18(2):172-85. doi: 10.1016/j.jcyt.2015.11.010
93. Cahn JY, Klein JP, Lee SJ, Milipied N, Blaise D, Antin JH, et al. Prospective evaluation of 2 acute graft-versus-host (gvhd) grading systems: A joint societe francaise de greffe de moelle et therapie cellulaire (sfgm-tc), dana farber cancer institute (dfci), and international bone marrow transplant registry (ibmtr) prospective study. Blood 2005;106(4):1495-500. doi: 10.1182/blood-2004-11-4557
94. Wingard JR, Majhail NS, Brazauskas R, Wan JH, et al. Prospective evaluation of 2 acute graft-versus-host (gvhd) grading systems: A joint societe francaise de greffe de moelle et therapie cellulaire (sfgm-tc), dana farber cancer institute (dfci), and international bone marrow transplant registry (ibmtr) prospective study. Blood 2005;106(4):1495-500. doi: 10.1182/blood-2004-11-4557
95. Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with fanconi's anemia by means of umbilical-cord blood from an hla-identical sibling. N Engl J Med 1989;321(17):1174-8. doi: 10.1056/nejm198910263211707
96. Bernardo ME, Fibbe WE. Mesenchymal stromal cells and hematopoietic stem cell transplantation. Immunol Lett 2015;168(2):215-21. doi: 10.1016/j.imlet.2015.06.013
97. Battiwalla M, Hematti P. Mesenchymal stem cells in hematopoietic stem cell transplantation. Cytotherapy 2009;11(5):503-15. doi: 10.1080/14653240903193806
98. Ball LM, Bernardo ME, Roelofs H, Lankester A, Cometa A, Egeler RM, et al. Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. *Blood* 2007;110(7):2764-7. doi: 10.1182/blood-2007-04-087056

99. Bernardo ME, Ball LM, Cometa AM, Roelofs H, Zecca M, Avanzini MA, et al. Co-infusion of ex vivo-expanded, parental mscs prevents life-threatening acute graft-versus-host disease, but does not reduce the risk of graft failure in pediatric patients undergoing allogeneic umbilical cord blood transplantation. *Bone Marrow Transplant* 2011;46(2):200-7. doi: 10.1038/bmt.2010.87

100. Chen W, Li M, Su G, Zang Y, Yan Z, Cheng H, et al. Co-transplantation of hematopoietic stem cells and ccr4 gene-transduced mesenchymal stem cells promotes hematopoiesis. *Cell Biochem Biophys* 2015;71(3):1579-87. doi: 10.1007/s12013-014-0381-y

101. Shi M, Li J, Liao L, Chen B, Li B, Chen L, et al. Regulation of ccr4 expression in human mesenchymal stem cells by cytokine treatment: Role in homing efficiency in nod/scid mice. *Haematologica* 2007;92(7):897-904.

102. Han JY, Goh RY, Seo SY, Hwang TH, Kwon HC, Kim SH, et al. Cotransplantation of cord blood hematopoietic stem cells and culture-expanded and gm-csf-/scf-transfected mesenchymal stem cells in scid mice. *J Korean Med Sci* 2007;22(2):242-7. doi: 10.3346/jkms.2007.22.2.242

103. Chen T, Zhang P, Fan W, Qian F, Pei L, Xu S, et al. Co-transplantation with mesenchymal stem cells expressing a sdf-1/hoxb4 fusion protein markedly improves hematopoietic stem cell engraftment and hematogenesis in irradiated mice. *Am J Transl Res* 2014;6(6):691-702.

104. Lee SH, Kim DS, Lee MW, Noh YH, Jang IK, Kim DH, et al. A strategy for enhancing the engraftment of human hematopoietic stem cells in nod/scid mice. *Ann Hematol* 2013;92(12):1595-602. doi: 10.1007/s00277-013-1830-1

105. Nakao N, Nakayama T, Yahata T, Muguruma Y, Saito S, Miyata Y, et al. Adipose tissue-derived mesenchymal stem cells facilitate hematopoiesis in vitro and in vivo: Advantages over bone marrow-derived mesenchymal stem cells. *Am J Pathol* 2010;177(2):547-54. doi: 10.2353/ajpath.2010.091042

106. Milazzo L, Vulcano F, Barca A, Macioce G, Paldino E, Rossi S, et al. Cord blood cd34+ cells expanded on whatson's jelly multipotent mesenchymal stromal cells improve the hematopoietic engraftment in nod/scid mice. *Eur J Haematol* 2014;93(5):384-91. doi: 10.1111/ejh.12363

107. Huang GP, Pan ZJ, Jia BB, Zheng Q, Xie CG, Gu JH, et al. Ex vivo expansion and transplantation of hematopoietic stem/progenitor cells supported by mesenchymal stem cells from human umbilical cord blood. *Cell Transplant* 2007;16(6):579-85.

108. Fei XM, Wu YJ, Chang Z, Miao KR, Tang YH, Zhou XY, et al. Co-culture of cord blood cd34+ cells with human bm mesenchymal stromal cells enhances short-term engraftment of cord blood cells in nod/scid mice. *Cytotherapy* 2007;9(4):338-47. doi: 10.1080/14653240701291638

109. Fei XM, Wu YJ, Chang Z, Miao KR, Zhou XY, Pan QQ, et al. Human bone marrow stromal cells facilitate the cord blood cd34+ cells ex vivo expansion and short-term engraftment in nod/scid mice. *Zhonghua Xue Ye Xue Za Zhi* 2008;29(2):97-100.

110. de Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, Horowitiz M, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. *N Engl J Med* 2012;367(24):2305-15. doi: 10.1056/NEJMoa1207285

111. van der Garde M, Brand A, Slot MC, de Graaf-Dijkstra A, Zwaginga JJ, van Hensenbergen Y. No synergistic effect of cotransplantation of msc and ex vivo tpo-expanded cd34+ cord blood cells on platelet recovery and bone marrow engraftment in nod scid mice. *Stem Cells Dev* 2015;24(12):1448-56. doi: 10.1089/scd.2014.0543

112. Thakkar UG, Trivedi HL, Vanikar AV, Dave SD. Insulin-secreting adipose-derived mesenchymal stromal cells with bone marrow-derived hematopoietic stem cells from autologous and allogeneic sources for type 1 diabetes mellitus. *Cytotherapy* 2015;17(7):940-7. doi: 10.1016/j.jcyt.2015.03.608

113. Fernandez-Garcia M, Yanez RM, Sanchez-Dominguez R, Hernandez-Rodriguez M, Peces-Barba M, Herrera G, et al. Mesenchymal stromal cells enhance the engraftment of hematopoietic stem cells in an autologous mouse transplantation model. *Stem Cell Res Ther* 2015;6:165. doi: 10.1186/s13287-015-0155-5

114. Batorov EV, Shevela EY, Tikhonova MA, Batorova DS, Ushakova GY, Sizikova SA, et al. Mesenchymal stromal cells improve early lymphocyte recovery and t cell reconstitution after autologous hematopoietic stem cell transplantation in patients with malignant lymphomas. *Cell Immunol* 2015;297(2):80-6. doi: 10.1016/j.cellimm.2015.07.001

115. Wang Q, Qian S, Li J, Che N, Gu L, Wang Q, et al. Combined transplantation of autologous hematopoietic stem cells and allogeneic mesenchymal stem cells increases t regulatory cells in systemic lupus erythematosus with refractory lupus nephritis and leukopenia. *Lupus* 2015;24(11):1221-6. doi: 10.1177/0961203315583541

116. Park SK, Won JH, Kim HJ, Bae SB, Kim CK, Lee KT, et al. Co-transplantation of human mesenchymal stem cells promotes human cd34+ cells engraftment in a dose-dependent fashion in nod/scid mice. *J Korean Med Sci* 2007;22(3):412-9. doi: 10.3346/jkms.2007.22.3.412
117. Kim DH, Yoo KH, Yim YS, Choi J, Lee SH, Jung HL, et al. Cotransplanted bone marrow derived mesenchymal stem cells (MSC) enhanced engraftment of hematopoietic stem cells in a msc-dose dependent manner in nod/scid mice. J Korean Med Sci 2006;21(6):1004-9. doi: 10.3346/jkms.2006.21.6.1000

118. Liu RH, Li YQ, Zhou WJ, Shi YJ, Ni L, Liu GX. Supplementing mesenchymal stem cells improves the therapeutic effect of hematopoietic stem cell transplantation in the treatment of murine systemic lupus erythematosus. Transplant Proc 2014;46(5):1621-7. doi: 10.1016/j.transproceed.2014.03.003

119. 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 bedside. Cell Transplant 2013;22(4):723-9. doi: 10.3727/096368912x565217

120. Wang L, Zhang H, Guan L, Zhao S, Gu Z, Wei H, et al. Mesenchymal stem cells provide prophylaxis against acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation: A meta-analysis of animal models. Oncotarget 2016;7(38):61764-74. doi: 10.18632/oncotarget.11238

121. Reis M, Ogonek J, Qesari M, Borges NM, Nicholson L, Preussner L, et al. Recent developments in cellular immunotherapy for hsct-associated complications. Front Immunol 2016;7:500. doi: 10.3389/fimmu.2016.00500

122. Li ZY, Wang CQ, Lu G, Pan XY, Xu KL. 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 2014;70(1):115-22. doi: 10.1007/s12013-014-9866-y

123. Sudres M, Norol F, Trenado A, Gregoire S, Charlotte F, Levacher B, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol 2006;176(12):7761-7.

124. Han DM, Wang ZD, Zheng XL, Ding L, Yan HM, Xue M, et al. Co-infusion of mesenchymal stromal cells has no effect on relapse and infection in patients with leukemia undergoing haploidentical hematopoietic stem cell transplant. Leuk Lymphoma 2015;56(10):2965-8. doi: 10.3109/10428194.2015.1020061

125. Wen F, Zhang HJ, Chen Y, Yue Q, Liu Z, Zhang Q, et al. ScIl(+): mesenchymal stromal cells inhibit graft-versus-host disease in mice after bone marrow transplantation. Int Immunopharmacol 2015;26(1):50-7. doi: 10.1016/j.immuni.2015.03.001

126. Lim JY, Ryu DB, Lee SE, Park G, Min CK. Mesenchymal stem cells (MSCs) attenuate cutaneous sclerodermatous graft-versus-host disease (scl-gvhd) through inhibition of immune cell infiltration in a mouse model. J Invest Dermatol 2017;137(9):1895-904. doi: 10.1016/j.jid.2017.02.986

127. Gao L, Zhang Y, Hu B, Liu J, Kong P, Lou S, et al. Phase ii multicenter, randomized, double-blind controlled study of efficacy and safety of umbilical cord-derived mesenchymal stromal cells in the prophylaxis of chronic graft-versus-host disease after hla-haploidentical stem-cell transplantation. J Clin Oncol 2016;34(24):2843-50. doi: 10.1200/jco.2015.65.3642

128. Zhao K, Lou R, Huang F, Peng Y, Jiang Z, Huang K, et al. Immunomodulation effects of mesenchymal stromal cells on acute graft-versus-host disease after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2015;21(1):97-104. doi: 10.1016/j.bbmt.2014.09.030

129. Jitschin R, Mougiaakos D, Von Bahr L, Volkl S, Moll G, Ringden O, et al. Alterations in the cellular immune compartment of patients treated with third-party mesenchymal stromal cells following allogeneic hematopoietic stem cell transplantation. Stem Cells 2013;31(8):1715-25. doi: 10.1002/stem.1386

130. Clark FJ, Gregg R, Piper K, Dunnion D, Freeman L, Griffiths M, et al. Chronic graft-versus-host disease is associated with increased numbers of peripheral blood cd4+cd25high regulatory t cells. Blood 2004;103(6):2410-6. doi: 10.1182/blood-2003-06-2073

131. Amarnath S, Foley JE, Farthing DE, Gress RE, Laurence A, Eckhaus MA, et al. Bone marrow-derived mesenchymal stromal cells harness purinergic signaling to tolerize human th1 cells in vivo. Stem Cells 2015;33(4):1200-12. doi: 10.1002/stem.1934

132. Introna M, Lucchini G, Dander E, Galimberti S, Rovelli A, Balduzzi A, et al. Treatment of graft versus host disease with mesenchymal stromal cells: A phase i study on 40 adult and pediatric patients. Biol Blood Marrow Transplant 2014;20(3):375-81. doi: 10.1016/j.bbmt.2013.11.033

133. Dotoli GM, De Santis GC, Orellana MD, de Lima Prata K, Caruso SR, Fernandes TR, et al. Mesenchymal stromal cell infusion to treat steroid-refractory acute gyvd iiiviv after hematopoietic stem cell transplantation. Bone Marrow Transplant 2017;52(6):859-62. doi: 10.1038/bmt.2017.35

134. Lucchini G, Introna M, Dander E, Rovelli A, Balduzzi A, Bonanomi S, et al. Platelet-lysate-expanded mesenchymal stromal cells as a salvage therapy for severe resistant graft-versus-host disease in a pediatric population. Biol Blood Marrow Transplant 2010;16(9):1293-301. doi: 10.1016/j.bbmt.2010.03.017

135. von Bonin M, Stolzel F, Goedecke A, Richter K, Wushek N, Holig K, et al. Treatment of refractory acute gyvd with third-party msc expanded in platelet lysate-containing medium. Bone Marrow Transplant 2009;43(3):245-51. doi: 10.1038/bmt.2008.316
136. Ball LM, Bernardo ME, Roelofs H, van Tol MJ, Contoli B, Zwagginga JJ, et al. Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease. *Br J Haematol* 2013;163(4):501-9. doi: 10.1111/bjh.12545

137. von Dalowski F, Kramer M, Wermke M, Wehner R, Rollig C, Alakel N, et al. Mesenchymal stromal cells for treatment of acute steroid-refractory graft versus host disease: Clinical responses and long-term outcome. *Stem Cells* 2016;34(2):357-66. doi: 10.1002/stem.2224

138. Peng Y, Chen X, Liu Q, Zhang X, Huang K, Liu L, et al. Mesenchymal stromal cells infusions improve refractory chronic graft versus host disease through an increase of CD5+ regulatory B cells producing interleukin 10. *Leukemia* 2015;29(3):636-46. doi: 10.1038/leu.2014.225

139. Muroi K, Miyamura K, Okada M, Yamashita T, Murata M, Ishikawa T, et al. Bone marrow-derived mesenchymal stem cells (r-MSC) for steroid-refractory grade III or IV acute graft-versus-host disease: A phase III/III study. *Int J Hematol* 2016;103(2):243-50. doi: 10.1007/s12185-015-1915-9

140. Zhou H, Guo M, Bian C, Sun Z, Yang Z, Zeng Y, et al. Efficacy of bone marrow-derived mesenchymal stem cells in the treatment of scleroderma-like chronic graft-versus-host disease: Clinical report. *Biol Blood Marrow Transplant* 2010;16(3):403-12. doi: 10.1016/j.bbmt.2009.11.006

141. Sanchez-Guijo F, Caballero-Velazquez T, Lopez-Villar O, Redondo A, Parody R, Martinez C, et al. Sequential third-party mesenchymal stromal cell therapy for refractory acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2014;20(10):1580-5. doi: 10.1016/j.bbmt.2014.06.015

142. Ratajczak J, Mieku K, Kucia M, Zhang J, Reca R, Dvorak P, et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: Evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* 2006;20(5):847-56. doi: 10.1038/sj.leu.2404132

143. Jiang J, Woulfe DS, Papoutsakis ET. Shear enhances thrombopoiesis and formation of microparticles that induce megakaryocytic differentiation of stem cells. *Blood* 2014;124(13):2094-103. doi: 10.1182/blood-2014-01-457927

144. Golovizkina NA, Verghese SC, Yoon YM, Taratula O, Marks DL, Kurre P. Mesenchymal stromal cell-derived extracellular vesicles promote myeloid-biased multipotent hematopoietic progenitor expansion via toll-like receptor engagement. *J Biol Chem* 2016;291(47):24607-17. doi: 10.1074/jbc.M116.745653

145. Wen S, Dooner M, Cheng Y, Papa E, Del Tatoo M, Pereira M, et al. Mesenchymal stromal cell-derived extracellular vesicles rescue radiation damage to murine marrow hematopoietic cells. *Leukemia* 2016;30(11):2221-31. doi: 10.1038/leu.2016.107

146. Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doepnner TR, et al. Msc-derived exosomes: A novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia* 2014;28(4):970-3. doi: 10.1038/leu.2014.41

147. Zhang B, Yin Y, Lai RC, Tan SS, Choo AB, Lim SK. Mesenchymal stem cells secrete immunologically active exosomes. *Stem Cells Dev* 2014;23(11):1233-44. doi: 10.1089/scd.2013.0479

148. Munition S, Ramos TL, Diez-Campelo M, Roson B, Sanchez-Abarca LI, Misiewicz-Krzemsinska I, et al. Microvesicles from mesenchymal stromal cells are involved in hpc-microenvironment crosstalk in myelodysplastic patients. *PLoS One* 2016;11(2):e0146722. doi: 10.1371/journal.pone.0146722

149. Ramos TL, Sanchez-Abarca LI, Roson B, Redondo A, Rodriguez C, Rodriguez H, et al. Extracellular vesicles play an important role in intercellular communication between bone marrow stroma and hematopoietic progenitor cells in myeloproliferative neoplasms. Proceedings of the 58th ASH Annual Meeting and Exposition; San Diego, CA, USA. 3–6 December 2016; p. 1957.

150. Forslow U, Blennow O, LeBlanc K, Ringden O, Gustafsson B, Mattsson J, et al. Treatment with mesenchymal stromal cells is a risk factor for pneumonia-related death after allogeneic hematopoietic stem cell transplantation. *Eur J Haematol* 2012;89(3):220-7. doi: 10.1111/j.1600-0609.2012.01824.x

151. Haarer J, Johnson CL, Soeder Y, Dahlke MH. Caveats of mesenchymal stem cell therapy in solid organ transplantation. *Transpl Int* 2015;28(1):1-9. doi: 10.1111/tri.12415

152. Castro-Manrreza ME, Montesinos JJ. Immunoregulation by mesenchymal stem cells: Biological aspects and clinical applications. *J Immunol Res* 2015;2015:394917. doi: 10.1155/2015/394917

153. Siegel G, Kluba T, Hermanutz-Klein U, Bieback K, Northoff H, Schafer R. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. *BMC Med* 2013;11:146. doi: 10.1186/1741-7015-11-146