Front. Med. 2019, 13(2): 138–151
https://doi.org/10.1007/s11684-018-0627-y

REVIEW

Mesenchymal stem cells and immune disorders: from basic science to clinical transition

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Abstract **As a promising candidate seed cell type in regenerative medicine, mesenchymal stem cells (MSCs) have attracted considerable attention. The unique capacity of MSCs to exert a regulatory effect on immunity in an autologous/allergenic manner makes them an attractive therapeutic cell type for immune disorders. In this review, we discussed the current knowledge of and advances in MSCs, including its basic biological properties, i.e., multilineage differentiation, secretome, and immunomodulation. Specifically, on the basis of our previous work, we proposed three new concepts of MSCs, i.e., "subtotipotent stem cell" hypothesis, MSC system, and "Yin and Yang" balance of MSC regulation, which may bring new insights into our understanding of MSCs. Furthermore, we analyzed data from the Clinical Trials database (http://clinicaltrials.gov) on registered clinical trials using MSCs to treat a variety of immune diseases, such as graft-versus-host disease, systemic lupus erythematosus, and multiple sclerosis. In addition, we highlighted MSC clinical trials in China and discussed the challenges and future directions in the field of MSC clinical application.**

Keywords mesenchymal stem cell; clinical transition; immune disorders

Introduction

Over the past 10 years, few cells have attracted considerable attention from scientists and physicians as mesenchymal stem cells (MSCs), a type of adult stem cells first discovered in 1968 by Friedenstein and colleagues. They observed an adherent fibroblast-like population in the bone marrow that is capable of differentiating into adipocytes, chondrocytes, and osteocytes [1]. In 1991, Caplan *et al*. called these cells "mesenchymal stem cells." Since then, the term "MSC" has become popular. Caplan's work showed that MSCs were involved in bone and cartilage turnover and that the surrounding conditions played a pivotal role in MSC differentiation. They also indicated that the study of MSCs, whatever its origin, paves the road for the emergence of a novel therapeutic strategy of self-cell repair [2]. Subsequently, the multilineage potential of MSCs to differentiate into the adipocytic, chondrocytic, or osteocytic lineages was definitively demonstrated [3]. Since then, MSCs have been intensively investigated. However, different laboratories use different methods to culture MSCs, which makes it difficult to compare experimental results. Thus, in 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a set of standards to define human MSCs for laboratory-based scientific investigations and preclinical studies [4]: (1) MSCs must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks. (2) Of the MSC population, $\geqslant$ 95% must express CD105, CD73, and CD90, as measured by flow cytometry. In addition, these cells must lack the expression ($\leqslant$ 2% positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA class II. (3) The cells must be able to differentiate into osteoblasts, adipocytes, and chondroblasts under standard *in vitro* differentiating conditions [4] (Table 1).

MSCs could be isolated from almost every tissue type, including bone marrow, adipose tissue, placenta, umbilical cord (UC) blood, amniotic fluid, and liver. Currently, the main source of MSCs for most preclinical and clinical studies is bone marrow. Although only approximately 0.01% to 0.001% of the total nucleated cells within isolated bone marrow aspirates are MSCs, they can be

Received September 20, 2017; accepted December 12, 2017
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Table 1 Basic characteristics of MSCs

No.	Basic characteristics of MSCs
1	Plastic-adherent
2	CD105, CD73, and CD90 positive; CD45, CD34, CD14 CD11b, CD79a, CD19, and HLA class II negative
3	Osteogenic, chondrogenic, and adipogenic differentiation capacities

rapidly culture-expanded for up to 40 population doublings in approximately two to three months [5]. Compared with normal bone marrow, adipose and birth-associated tissues, including placenta, are relatively easy to obtain as a tissue source for MSCs. It is important to realize that MSCs from different sources may have different proliferative and differentiation potentials [6].

One of the most intriguing biological characteristics of MSCs is their immunomodulatory property, which lays the therapeutic basis of the use of MSCs for a number of immune disorders. In this review, we discussed the current knowledge of MSCs, focusing on its clinical translation in a variety of immune disorders, as well as the challenges and future directions.

Basic research on the biological characteristics of MSC

Basic research has made significant progress in understanding the biological characteristics of MSCs. Generally, MSCs have the capability to produce a number of cell types, home to sites of injury/inflammation, secrete bioactive molecules, and modulate immune or inflammation responses, thus contributing to tissue repair and homeostasis maintenance.

Multilineage differentiation

Although MSCs tend to generate mesenchymal lineages, they also have the capability to differentiate into cells of three germ layers, i.e., mesoderm, ectoderm, and endoderm. Recently, studies have reported different methods to induce cardiomyocyte differentiation of MSCs [7,8]. Evidence indicates that MSCs have the potential to regenerate hepatic-like cells [9–11]. MSCs maintained on decellularized cell-deposited extracellular matrix can differentiate into mature hepatocyte more efficiently [12]. MSC-derived hepatocyte-like cells have the capability to take up low-density lipoprotein [13]. In a partially hepatectomized model rat, human MSCs can survive and differentiate into hepatocyte-like cells *in vivo* [14]. MSC-derived hepatocytes are capable of expressing albumin when transplanted into a CCl4-injured SCID mouse model [15]. MSCs also have the capacity to produce pancreas cell lineages. MSCs can differentiate into islet-like cells after stepwise addition of activin A, EGF bFGF, and ITS, which can function as normal pancreatic cells *in vitro* and *in vivo* [16]. Tonsil-derived MSCs can generate islet-like cells similar to ADSCs [17]. Pancreatic extract or coculture with pancreatic adult stem cells can efficiently induce MSC differentiation into functional islet-like cells without any gene manipulation [18–20]. Insulin-producing cells derived from MSCs can ameliorate STZ-induced diabetic hyperglycemia [21]. MSCs can also generate lung epithelial-like cells and repair bleomycin-induced lung injury [22,23]. When seeded on a chitosan-coated surface or cultured in the serum-free medium, MSCs can form spheres containing $19.5\% \pm 2.6\%$ or $51\% \pm 13.22\%$ nestin-positive cells, respectively [24,25]. However, no functional detections were achieved, particularly during electrophysiology analysis. Recently, a three-step NSC-inducing protocol was established, in which MSC-derived neural stem cells can further differentiate into astrocytes, oligodendrocytes, and functional neurons [26]. Although MSCs possess multilineage differentiation capacity, growing evidence indicates that specific differentiation of MSCs in damaged tissues is only a small part of the mechanism responsible for the efficacy of MSCs in disease treatment.

MSC secretome

MSCs can secrete multiple bioactive factors, including cytokines, chemokines, inflammatory factors, and extracellular vesicles (EVs; e.g., exosomes and microvesicles), which are commonly referred to as MSC secretome. MSC secretome has diverse cellular functions, such as promoting angiogenesis, anti-apoptosis, anti-fibrosis, anti-oxidation, immunomodulation, and hematopoietic support [27]. The soluble factors of MSC secretome isolated from different tissues may be different but most often have a core of cytokines, such as CCL2, CCL5, bFGF, insulin-like growth factor-1 (IGF-1), IL-6, TGF-β, vascular endothelial growth factor (VEGF), and TNFR1, which are involved in tissue development, differentiation, apoptosis, tumor growth, and metastasis [28–31]. Adipose-tissue-derived MSCs secrete higher levels of IGF-1, VEGF-D, and IL-8 than BM-MSCs, whereas other factors, such as nerve growth factor (NGF), VEGF-A, bFGF, and angiogenin, were expressed at comparable levels between them [32]. Soluble factors that account for MSC immunomodulatory functions are IL-6, IL-10, PGE2, HGF, DO, NO, TGF-β, and human HLA-G. MSCs can also be an attractive cellular source for brain disorders because of the production of multiple neurotrophic factors, such as brain-derived neurotrophic factor, NGF, or glial-derived neurotrophic factor [33]. MSCs also secrete EVs, which have the immunomodulatory traits of MSCs, and deliver a variety of small molecules to the surrounding cells [34], which are conducive to intercellular communication activities and lead to functional changes in the

recipient cells [35]. MSC-derived EVs enhanced angio-neurogenesis and contributed to the improvement of impaired neurological functions [36]. We observed that exosomes secreted by hADSCs could transfer miR-125a to endothelial cells and promote angiogenesis [37]. In the animal model of allogeneic hematopoietic stem cell transplantation (HSCT), EVs released from human-umbilical-cord-derived MSC prevent life-threatening acute graft-versus-host disease (GVHD) [38].

Immunomodulation

One important feature of MSC is their capability to regulate the adaptive and innate immune systems by interacting with a wide spectrum of immune cells, such as T and B lymphocytes, NK cells, and dendritic cells (DCs). We have discussed such interactions in a review article that we published previously [39]. Here, taking DCs as an example, our group determined that MSCs could modify the expression of several important surface markers in the process of DC differentiation and maturation [40]. Moreover, we reported that, when cocultured with MSCs, the proportion of cells with cDC phenotype is obviously decreased, whereas the proportion of cells with pDC phenotype is upregulated [41]. Notably, MSCs could induce maDCs into a novel type of regulatory DC, bringing new insights into the possible application of MSCs in organ transplantation and/or immune disease treatment [42]. Although the immunoregulation of MSCs is context dependent, most of the time, they mediate immunoregulation through the direct actions on immune cells or the recruitment of other immunoregulatory populations.

New concepts of MSCs

Our laboratory has been focusing on the basic biological properties of MSCs for more than 10 years and has conceived some new concepts that might bring more insights into our understanding of the therapeutic effects of MSCs.

"Subtotipotent stem cell" hypothesis

We hypothesized that an undefined subfraction of embryonic-like stem cells are left over in a number of tissues even after a fetus is formed. We called them subtotipotent stem cells. They have the capacity to produce cells with three germ layers. We proved this hypothesis by isolating MSC from human fetal bone marrow, called $Flk1^{+}CD31^{-}CD34^{-}$ stem cells, which could produce cells with three germ layers, e.g., endothelial cells, hepatocyte-like cells, and neurons at the single-cell level. Recent studies indicate that adult cells could be converted by small molecules or chemicals to cells of other lineages [43–50]. We postulate that the "subtotipotent stem cell" hypothesis might explain some of the observed lineage reprogramming phenomenon.

MSC system

We propose the concept of the MSC system (Fig. 1). The MSC system is composed of all MSCs derived from different stages of embryonic development, from postembryonic subtotipotent stem cells to progenitors [51]. The postembryonic subtotipotent stem cells are leftover cells during embryonic development, which are on top of the system. The progenitors include all of the subsets of MSCs, such as CAR cells and pericytes, that are included as long as they share a similar set of phenotypic markers. The MSC system has three important biological characteristics, i.e., stem cell properties including multipotency and self-renewal, low immunogenicity and immunomodulatory functions, and microenvironment and tissue balance. The establishment of the MSC system is of considerable significance because (1) it entirely explains the three important biological characteristics of MSCs; (2) it is a more comprehensive view of MSCs and could better explain the heterogeneity of MSCs in differentiation potential and immunomodulatory functions; and (3) it could provide tissue-specific stem cells for clinical application with high efficiency and safety.

"Yin and Yang" balance of MSC regulation

MSCs can be attracted to injury or inflammation sites, where they modulate local inflammatory processes and promote repair or regeneration. MSCs can be polarized toward a pro-inflammatory MSC1 or an immunosuppressive MSC2 according to stimulation factors [52]. We postulate that MSCs maintain the "Yin and Yang" balance of immunoregulation by modulating the proportion of MSC1 and MSC2, which could subsequently influence the balance of other immune cell types, such as macrophage, T cells, DCs, and B cells (Fig. 2).

MSC therapy for immune disorders

Database from clinical trials

Several clinical trials were reviewed for studying the safety and efficacy of MSCs. Immune-mediated diseases, including autoimmune diseases and other diseases with immune dysfunction and imbalanced immune regulation, are difficult to treat. Based on their capability to regulate immune responses and promote tissue repair, clinical applications of MSCs indicate a new prospective approach of treatment for immune-related diseases. The Clinical Trials database (http://clinicaltrials.gov) indicated that the

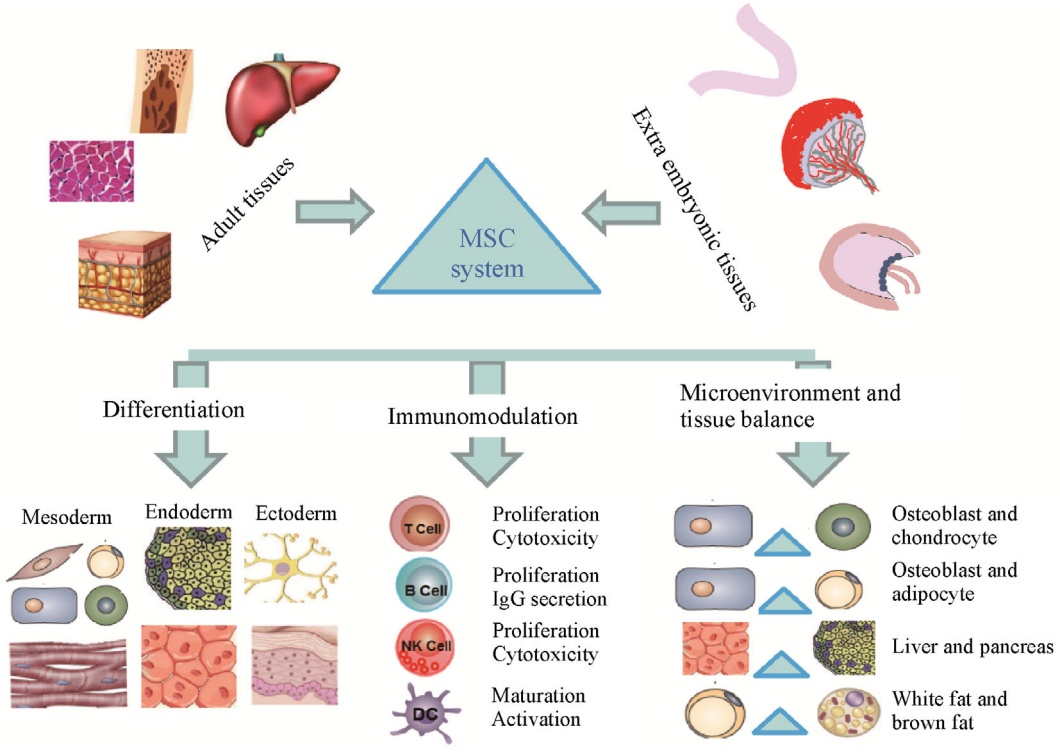

Fig. 1 Concept of the MSC system.

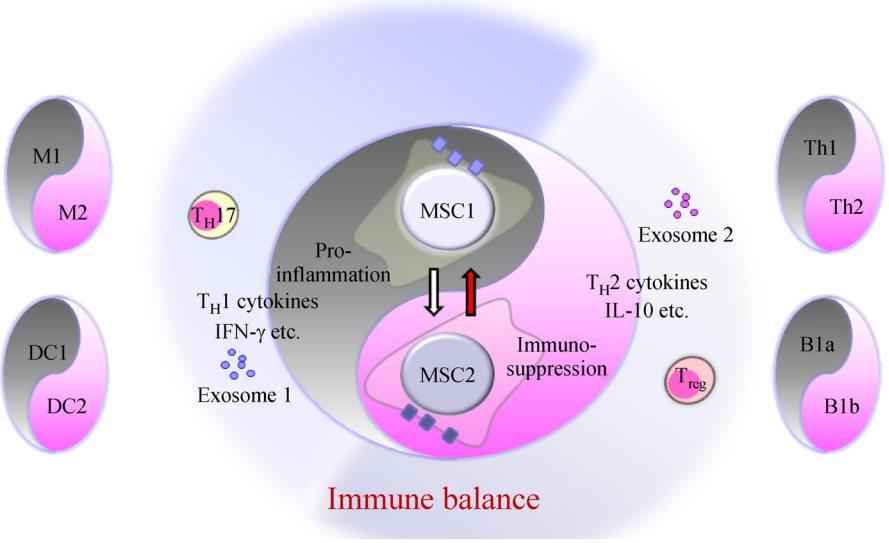

Fig. 2 "Yin and Yang" balance of MSC regulation.

trials using MSCs for immune-mediated diseases have increased annually since 2004, especially in 2010. Notably, the growth of clinical trials slowed down in 2011, which might be influenced by the new federal financial aid policies implemented in August 2010 in the United States (Fig. 3A). By October 18, 2016, 125 clinical trials have focused on immune-mediated diseases of MSCs, making up approximately one fifth of the entire number of clinical trials of MSCs. These trials overlay immune-mediated diseases targeting different organs and transplant rejection, as well some typical autoimmune diseases and HIV (Fig. 3B). According to the Clinical Trials database, the

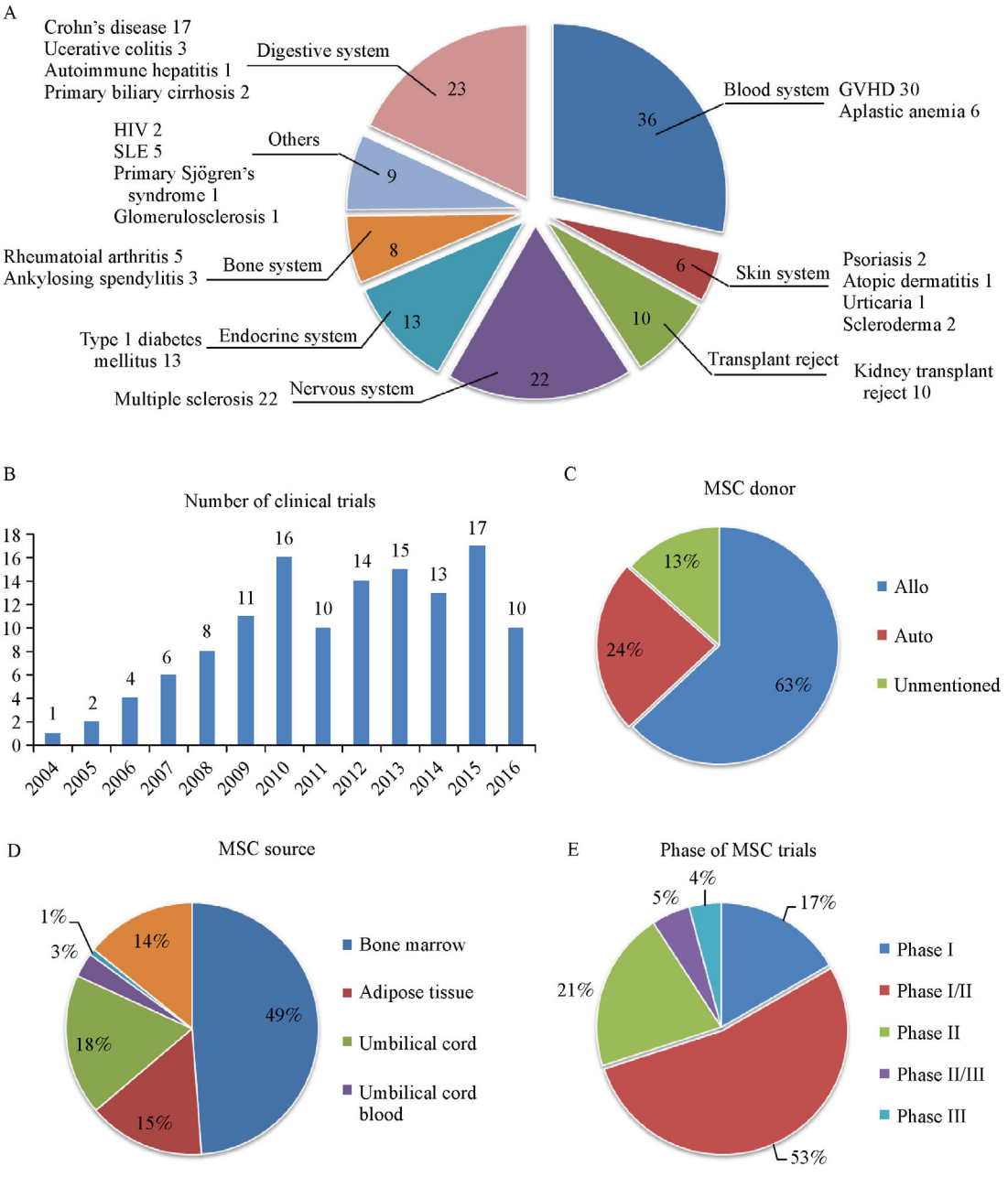

Fig. 3 Analysis of clinical trials of MSC on immune-related diseases (in October 17, 2016, $n = 125$). (A) clinical trials classified by disease types and different systems; (B) numbers of newly created clinical trials of MSC on immune-related disorders over these years; (C) clinical trials of MSCs classified by donors; (D) clinical trials of MSCs classified by tissue sources; and (E) clinical trials of MSCs classified by phases.

MSC donors and the tissue sources are analyzed in the clinical trials of immune-mediated diseases. Besides the 14% of the trials not mentioned, we conclude that approximately 62% of MSCs were derived from allo-donors, whereas 24% were from the patients themselves who received the cell treatment (Fig. 3C), making a roughly 2.6:1 rate between the two origins. Indeed, MSCs derived from allo-donors have been widely used in clinical trials of certain diseases, such as GVHD, transplant rejection, and most autoimmune diseases. However, in trials of multiple sclerosis (MS), an autoimmune disease making lesions in the central nervous system (CNS), auto-MSCs are usually obtained from the blood–brain barrier. Fig. 3D shows that the tissue sources of 30% of MSCs used

in the trials are not shown. Moreover, most MSCs are derived from the bone marrow, accounting for 49% of all trials. Of the trials, 15% used MSCs from adipose tissue, which is approximately similar to MSCs from UC (18%). MSCs from UC blood and menstrual blood accounted for 3% and 1%, respectively (Fig. 3D). Furthermore, allogeneic MSCs from BM are used more preferentially than those autologously derived [53–55]. Meanwhile, adipose-tissue-derived MSCs are acquired more easily autologously and are shown to be effective in treating GVHD, Crohn's disease, and systemic lupus erythematosus (SLE) [56–58]. The results from clinical trials showed that MSCs from different sources exhibited parallel therapeutic effects on immune-mediated diseases [59]. The majority of these trials are in Phase I/II (52%). Meanwhile, 16% of these trials are in Phase I (for safety studies) and 21% in Phase II studies (for efficacy in patients). Only a few are in Phase III or Phase II/III (Fig. 3E), accounting for 4% and 5%, respectively. Meanwhile, among these trials, only two trials for GVHD are in Phase III, indicating significant progress in the trials of these disease.

GVHD

GVHD, including acute GVHD (aGVHD) and chronic GVHD (cGVHD), is a refractory and even lethal disease usually occurring after allogeneic HSCT because of immune attacks against hosts by grafts. MSCs have been a promising treatment of GVHD for quite a few years. The first case of allogenic MSC injection into a patient with severe GVHD grade IV resulted in prominent response to the disease [60]. Thus, a growing number of GVHD trials have been conducted on MSCs over these years.

In the aGVHD setting, allogeneic BM-MSCs were usually infused to the patients, particularly with steroid-refractory GVHD [59,61,62]. The results showed that five of seven patients with steroid-refractory aGVHD improved completely, with rapid reductions in inflammatory cytokines, significantly long survival rate, and high baseline absolute lymphocyte [63]. Another study resulted in 17 of 24 patients (71%) responding to allogeneic BM-MSCs infusion [64]. Meanwhile, the occurrence rate of grades II to IV GVHD decreased significantly [65]. Furthermore, no differences were observed between MSC and non-MSC groups during aGVHD treatment and follow-up, including infection incidence (cytomegalovirus and Epstein–Barr virus), as well as tumor relapse [66]. Prophylaxis of aGVHD was also conducted by MSCs, resulting in a reduction of the incidence of aGVHD and an increase in the overall survival of those patients [67]. Notably, an increase in the number of immature myeloid DCs related to the reduction in mortality was observed in addition to improved overall survival [68].

GVHD occurring over 100 days after HSCT is called cGVHD. MSCs derived from either HLA-identical sibling donors or HLA-disparate donors were considered as a salvage and effective therapy for refractory cGVHD [69]. MSCs were infused as second-line or third-line treatment. In a study including seven patients, one patient improved completely and three patients responded partially, whereas three patients did not respond [70]. Yi *et al.* reported that three patients with cGVHD maintained stable disease during the observation period [71]. Pretreatment of MSCs resulted in a low incidence and severity of cGVHD, a high number of total T cells and $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T (Treg) cells, as well as increased levels of signal joint T cell receptor excision DNA circles [66]. However, Erbey *et al*. observed the limited treatment response on cGVHD by MSCs in aGVHD patients who had been treated with MSCs [72].

In addition, the majority of allogenic MSCs were used in GVHD treatment, although Copland *et al*. proposed that MSCs from recipient patients and healthy donors represented feasible options for GVHD treatment [63].

SLE treatment

SLE is a multisystem autoimmune disease, which remains potentially fatal, particularly in treatment-refractory patients. BM-MSCs derived from SLE patients showed osteogenic impairment [73], increased frequencies of apoptosis and aging, and decreased levels of Bcl-2 expression, which may be related to the SLE pathogenesis. Data from the Clinical Trials database indicate that six of six trials on SLE by MSC are all using allo-MSCs of healthy donors, indicating that allo-MSC are likely to be superior to auto-MSC in treating SLE. MSC derived from UC increased Treg cells and decreased Th17 cells by regulating the levels of TGF-β and PGE2 in lupus patients [74]. Allogenic MSCs kept the CTX/glucocorticoid treatment-refractory SLE patients in a stable 12–18 months disease remission and an upregulation of Treg cells [73]. No obvious differences were observed between single and double allo-MSC treatment in disease remission or relapse, as well as serum indices in a SLE trial beyond one year follow-up [75]. All of these studies show that UC-MSCs are a promising treatment option for SLE patients [76].

Crohn's disease

MSCs are also used to treat Crohn's disease in recent studies. Crohn's disease is a chronic inflammatory disease mediated by autoimmune disorders causing gastrointestinal tract damage. Refractory patients with Crohn's disease do not respond to steroids or immunosuppressive agents. Locally injected adipose-tissue-derived MSCs (AT-MSCs) exhibited a safe and effective therapy response, particularly for perianal fistula in these patients [77–80]. In a safety study, five pregnant women with fistula received AT-

MSC therapy. Notably, AT-MSCs did not affect the pregnancy or the development of newborns [81]. Injection of 3×10^7 MSCs promoted the healing of perianal fistulas [80]. In a five-year follow-up study, the Crohn's disease activity index score initially increased significantly during first two years after BM-MSC infusion and subsequently decreased gradually; finally, the patients achieved remission [82]. MSCs for Crohn's disease treatment are often obtained from adipose tissue. Bone marrow is another source of MSCs [54,83]. Intralesional injection is usually used rather than other routes.

Organ transplantation

MSCs are also applied for organ transplantation, particularly kidney transplantation. BM-MSCs combined with a reduced tacrolimus dose treatment are administered to patients who survived with stable renal function lasting for one year, as well as no graft failure, donor-specific lymphocyte proliferation, and Treg priming responses. Meanwhile, the IL-10 level exhibited a high increase in the patients [84–86]. A pilot study also proposed the use of autologous MSCs for Treg cell expansion with decreasing T cell proliferation in patients with kidney transplantation [87].

MS

MS, an inflammatory disease in the CNS, is characterized by demyelinating and neurodegenerative disorder. No definite treatment is available for patients with MS thus far, and MSCs are becoming one of the most prospective therapy methods without obvious side effects [88,89]. Most of these clinic trials were conducted with MSCs derived from autologous bone marrow and administrated through intrathecal infusion. MSC therapy was able to improve or delay the progress of this disease in refractory MS one year after MSC injection [90]. MSCs promoted immunomodulation and neurological restoration and remyelination of the impaired axons. Evaluation after three to six months showed Expanded Disability Status Score improvement and an increase in the number of Treg cells [91,92].

Other immune-related disorders

Rheumatoid arthritis (RA) is a kind of autoimmune disease with chronic inflammation resulting in disorder of the cartilage and bone joints. Failures in clinical treatment including antirheumatic drugs, cortical hormones, and biological agents still remain in a proportion of patients with arthritis. A preliminary report showed an essentially negative response in four patients who received allogeneic or bone-marrow-derived MSCs [93]. A nonrandomized comparative trial was reported, in which 136 patients were infused allogeneic UC-MSC, whereas 36 patients were infused only the cell solvent as control. This treatment induced a significant remission of the disease, which was sustained for more than three months, with an increase in Treg cells in peripheral blood without side effects [94]. Similar results were obtained from a study of 10 patients with juvenile idiopathic arthritis, also known as juvenile RA [95]. MSCs are also applied in other immune-related disorders, such as systemic sclerosis [96–98], primary Sjögrens syndrome [99,100], ankylosing spondylitis [101], and dermatomyositis [102,103]; all exhibited a positive response in these pilot studies with no significant toxicity. However, injection of allo-MSCs in aplastic anemia patients was safe but resulted in no obvious clinical hematologic response or engraftment in the recipients in a recent report [104] (Table 2).

Generally, MSC treatment is a feasible and promising strategy in the treatment of many immune-related diseases based on inspiring data from clinics. However, many questions remain to be discussed. Recently, a meta-analysis involving 13 nonrandomized studies at moderate risk of bias indicated that survival neither differed with respect to MSC culture medium nor MSC infusion dose on aGVHD treatment [105], although more analyses of other diseases are needed. Further studies should consider the potential transformations of MSCs during culture and after infusion, the source of MSCs, the assessment of function

Table 2 Clinical trials/pilot studies of MSCs for other immune-related diseases within five years

Disease	Number	Dose	Source	Route	Result	References
RA	4	1×10^6/kg	Allo-BM	iv	Negative	[92]
RA	136	4×10^7 total	Allo-UC	iv	Remission	[93]
Juvenile RA	10	4×10^7 total	Allo-UC	iv	Effective	[94]
Aplastic anemia	4	2.7×10^6/kg	Allo-BM	iv, 2–5 times	Unimproved	[104]
Dermatomyositis	10	1×10^6/kg	Allo-BM/UC	iv	Effective	[102]
Ankylosing spondylitis	31	1×10^6/kg	Allo-BM	iv, 4 times	Improved	[101]
Systemic sclerosis	12	3.76×10^6 each finger	Allo-AT	Subcutaneous injection	Improved	[96]

RA, rheumatoid arthritis; iv, intravenous infusion; UC, umbilical cord; BM, bone marrow; AT, adipose tissue; allo, allogeneic.

persistence, and the underlying mechanisms of immunoregulation *in vivo* to optimize patient benefits.

Clinical translation of MSCs in China

The number of studies in the field of stem cells in China over the past few years has increased significantly because of the rapid increase in government funds and researchers' enthusiasm in the potential application of stem-cell-based therapy. Although some clinics in China offer patients unproven stem cell treatment only for a large sum of money, well-regulated clinical trials are undergoing nationwide and have obtained encouraging results. In 2004, our group in the Chinese Academy of Medical Science received the first approval from the Chinese State Food and Drug Administration (SFDA) to use MSCs to prevent GVHD. We performed two clinical trials and obtained exciting results. The first study determined that a new transplantation strategy combining haploidentical peripheral blood stem cells and MSCs could improve donor engraftment and prevent GVHD [106]. The second study was an open-label, randomized phase II clinical trial to assess the outcome of MSC coinfusion (3×10^5–5×10^5 cells/kg) during haploidentical HSCT. Within 100 days, the time to a platelet concentration of $> 50 \times 10^9$ cells/L was markedly faster in the treatment group than that in the control group (22 vs. 28 days; $P = 0.036$) [107]. In cooperation with us, Chen *et al*. from Nanjing University initiated the first clinical trial in China to treat acute myocardial infarction (MI) with MSCs. A total of 69 patients who underwent primary percutaneous coronary intervention after onset of acute MI were randomized to receive intracoronary injection of autologous MSCs or saline, and significant improvement of left ventricular function was observed in the MSC group [108].

Following these studies, many groups began to conduct clinical trials using MSCs to treat various diseases, including limb ischemia, liver disease, neurodegenerative disorders, and pulmonary arterial hypertension. A brief summary of some of these studies is shown in Table 3.

Perspectives and suggestions for future development of MSC clinical trials

Despite that significant progress has been made in preclinical and clinical studies utilizing MSCs, considerable challenges remain to be overcome before MSC therapy can finally move to clinical practice [109].

MSC production according to Good Manufacturing Practice (GMP)

MSCs used for clinical trial should be manufactured in compliance with GMP to ensure that the "MSC drug" is safe, reproducible, and efficient when administered to patients. All aspects of the manufacturing process should be defined, e.g., approved written procedures and instructions; qualified and trained production and quality control personnel; and full traceability of MSC preparation, storage, and transportation.

It is very important to have a consensus standard for MSC production, so that the results obtained from different clinical trials may be easier to compare.

Optimization of parameters related to MSCs

Several parameters should be optimized before the clinical application to guarantee the quality of produced MSCs. (1) Optimal passage: Studies have proposed that transformation and senescence may occur in late-passage MSCs. Therefore, *in vitro* passage time should be carefully controlled to reduce the chance of MSC malignant transformation. We conducted the first stem cell clinical trial approved from SFDA in China, and we used MSCs within six passages. (2) Optimal route of administration: To date, systemic administration is the main route used for MSC delivery in animal disease models and clinical studies. A previous report suggested that, during systemic administration, most MSCs become trapped in the liver and lungs [110], which could reduce the number of MSCs homing to target sites for repair. Therefore, compared with systemic infusion, site-specific administration of MSCs may lead to better efficacy. Comparing different routes of MSC administration and standardizing MSC delivery according to disease types are important to achieve maximum therapeutic effects. (3) Optimal cell dose: The optimal dose of MSCs administered should differ according to disease type and severity. Generally, the widely used MSCs dosage is approximately 1×10^6/kg of body weight. We propose conducting clinical trials with dose-escalating MSCs to define optimal cell dose in a context-dependent manner [109].

Strategies to enhance MSC-based immunomodulation

MSCs are responsive to environmental factors, and exogenous or endogenous modifications of MSCs may enhance MSC-based immunomodulation. Long-term expression of certain genes through gene modification could significantly increase the MSC capacity.

For example, Payne and colleagues transplanted gene-modified MSCs overexpressing IL-4 into a mouse model of experimental autoimmune encephalomyelitis and observed protective effects, which were associated with a reduction in peripheral T cell responses and a shift from a pro- to an anti-inflammatory cytokine response [111]. However, genetically modified MSC may cause serious safety issues for clinical use. Therefore, transient strengthening of MSC-based immune modulation through pre-

Table 3 Examples of clinical trials performed in China

References	Weng *et al.* (2012) [113]	Zhao *et al.* (2015) [66]	Wang *et al.* (2014) [114]	Wang *et al.* (2013) [94]	Gu *et al.* (2014) [115]	Wu *et al.* (2013) [116]	Wang *et al.* (2013) [117]	Qiao *et al.* (2014) [118]	Li *et al.* (2014) [119]	Wang *et al.* (2014) [101]	Zheng *et al.* (2014) [120]	Wang *et al.* (2013) [121]
No. of patients	Treatment (n = 22)	Control (n = 19); treatment (n = 28)	Treatment (n = 10)	Treatment (n = 7)	Treatment (n = 81)	Control (n = 12); treatment (n = 8)	Treatment (n = 52)	Treatment (n = 8)	Control (n = 10); treatment (n = 13)	Treatment (n = 31)	Control (n = 6); treatment (n = 6)	Control (n = 20); treatment (n = 20)
Follow-up period	3 months	1 year	12 months	48 weeks	1 year	16.5 months (range, 8–27 months)	18 months	2 years follow-up	12 months	20 weeks	28 days follow-up	6 months
Disease	Refractory dry eye secondary to cGVHD	Refractory aGVHD	UDCA-resistant primary biliary cirrhosis	Primary biliary cirrhosis	Refractory lupus nephritis	Delayed hematopoietic reconstitution after cord blood transplantation	Cerebral palsy	Stroke	Multiple sclerosis	Active ankylosing spondylitis	Acute respiratory distress syndrome	Sequelae of traumatic brain injury
Outcome	54.55% of the patients exhibited improved clinical symptoms after MSC treatment	75% of the patients exhibited improved clinical symptoms after MSC treatment, which was derived from the bone marrow of a third-party donor	The life quality of the patients was improved for 12 months after BM-MSC treatment	Symptoms (e.g., fatigue and pruritus) were significantly improved after UC-MSC transplantation	Renal remission was observed in active LN patients after allogeneic MSC treatment	Neutrophil and platelet engraftment time was obviously short in patients after UC-MSC treatment	GMFM-88 and GMFM-66 scores were increased after MSC treatment	Neurological functions and daily living abilities were improved after MSC treatment	Overall symptoms were improved in patients after UC-MSC treatment	The average total inflammation extent was decreased in patients after MSC treatment	Clinical effect was weak after allogeneic adipose-derived MSCs transplantation	Neurological function and self-care were improved in patients after UC-MSC transplantation

conditioning with cytokines may be more clinically relevant. MSC, when pretreated with IFN-γ, was rapidly activated and could reduce GVHD more efficiently than a fivefold number of inactive MSC [112].

MSC therapy is rapidly developing, leading to exciting and promising as well as confusing and sometimes contradictory results. Thus, further results from large clinical trials are needed to confirm preclinical findings and human noncontrolled studies.

Acknowledgements

This work was supported by grants from the Key Program for Beijing Municipal Natural Science Foundation (No. 7141006), National Collaborative Innovation Program (for Biotherapy), Beijing Science and Technology Project (No. Z151100001615-063), National Key Research and Development Program (Nos. 2016YFA0101000 and 2016YFA0101003), and PUMC Youth Fund and the Fundamental Research Funds for the Central Universities (No. 3332013141).

Compliance with ethics guidelines

Shihua Wang, Rongjia Zhu, Hongling Li, Jing Li, Qin Han, and Robert Chunhua Zhao declare that they have no conflict of interest or financial conflicts to disclose. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968; 6(2): 230–247
2. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991; 9(5): 641–650
3. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284(5411): 143–147
4. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8(4): 315–317
5. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999; 96(19): 10711–10716
6. Salem HK, Thiemermann C. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells 2010; 28(3): 585–596
7. Han X, Wan Q, Wu W, Zheng A, Li L, Liu X. Activin A and BMP-4 induce cardiomyocyte-like cells differentiation of human amniotic epithelial cells. J Biomedical Eng (Sheng Wu Yi Xue Gong Cheng Xue Za Zhi) 2011; 28(6): 1217–1222 (in Chinese)
8. Asumda FZ, Chase PB. Nuclear cardiac troponin and tropomyosin are expressed early in cardiac differentiation of rat mesenchymal stem cells. Differentiation 2012; 83(3): 106–115
9. Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS. Liver from bone marrow in humans. Hepatology 2000; 32(1): 11–16
10. Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 2000; 31(1): 235–240
11. Bornstein R, Macias MI, de la Torre P, Grande J, Flores AI. Human decidua-derived mesenchymal stromal cells differentiate into hepatic-like cells and form functional three-dimensional structures. Cytotherapy 2012; 14(10): 1182–1192
12. He H, Liu X, Peng L, Gao Z, Ye Y, Su Y, Zhao Q, Wang K, Gong Y, He F. Promotion of hepatic differentiation of bone marrow mesenchymal stem cells on decellularized cell-deposited extracellular matrix. Biomed Res Int 2013; 2013: 406871
13. Xu D, Nishimura T, Zheng M, Wu M, Su H, Sato N, Lee G, Michie S, Glenn J, Peltz G. Enabling autologous human liver regeneration with differentiated adipocyte stem cells. Cell Transplant 2014; 23 (12): 1573–1584
14. Chen Z, Kuang Q, Lao XJ, Yang J, Huang W, Zhou D. Differentiation of UC-MSCs into hepatocyte-like cells in partially hepatectomized model rats. Exp Ther Med 2016; 12(3): 1775–1779
15. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage *in vitro* and *in vivo*. Biochem Biophys Res Commun 2005; 328(1): 258–264
16. Yu YB, Bian JM, Gu DH. Transplantation of insulin-producing cells to treat diabetic rats after 90% pancreatectomy. World J Gastroenterol 2015; 21(21): 6582–6590
17. Kim SY, Kim YR, Park WJ, Kim HS, Jung SC, Woo SY, Jo I, Ryu KH, Park JW. Characterisation of insulin-producing cells differentiated from tonsil derived mesenchymal stem cells. Differentiation 2015; 90(1–3): 27–39
18. Lee J, Han DJ, Kim SC. *In vitro* differentiation of human adipose tissue-derived stem cells into cells with pancreatic phenotype by regenerating pancreas extract. Biochem Biophys Res Commun 2008; 375(4): 547–551
19. Hefei W, Yu R, Haiqing W, Xiao W, Jingyuan W, Dongjun L. Morphological characteristics and identification of islet-like cells derived from rat adipose-derived stem cells cocultured with pancreas adult stem cells. Cell Biol Int 2015; 39(3): 253–263
20. Mehrfarjam Z, Esmaeili F, Shabani L, Ebrahimie E. Induction of pancreatic β cell gene expression in mesenchymal stem cells. Cell Biol Int 2016; 40(5): 486–500
21. Xin Y, Jiang X, Wang Y, Su X, Sun M, Zhang L, Tan Y, Wintergerst KA, Li Y, Li Y. Insulin-producing cells differentiated from human bone marrow mesenchymal stem cells *in vitro* ameliorate streptozotocin-induced diabetic hyperglycemia. PLoS One 2016; 11(1): e0145838
22. O'Dell JW. Spectral analysis of interviews conducted by Rogers and Ellis. Psychol Rep 1990; 67(3 Pt 1): 819–825
23. Wang C, Zhu H, Sun Z, Xiang Z, Ge Y, Ni C, Luo Z, Qian W, Han

X. Inhibition of Wnt/β-catenin signaling promotes epithelial differentiation of mesenchymal stem cells and repairs bleomycin-induced lung injury. Am J Physiol Cell Physiol 2014; 307(3): C234–C244

24. Hsueh YY, Chiang YL, Wu CC, Lin SC. Spheroid formation and neural induction in human adipose-derived stem cells on a chitosan-coated surface. Cells Tissues Organs 2012; 196(2): 117–128
25. Ahmadi N, Razavi S, Kazemi M, Oryan S. Stability of neural differentiation in human adipose derived stem cells by two induction protocols. Tissue Cell 2012; 44(2): 87–94
26. Feng N, Han Q, Li J, Wang S, Li H, Yao X, Zhao RC. Generation of highly purified neural stem cells from human adipose-derived mesenchymal stem cells by Sox1 activation. Stem Cells Dev 2014; 23(5): 515–529
27. Konala VB, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. Cytotherapy 2016; 18(1): 13–24
28. Gu Y, He M, Zhou X, Liu J, Hou N, Bin T, Zhang Y, Li T, Chen J. Endogenous IL-6 of mesenchymal stem cell improves behavioral outcome of hypoxic-ischemic brain damage neonatal rats by supressing apoptosis in astrocyte. Sci Rep 2016; 6: 18587
29. Zimmerlin L, Park TS, Zambidis ET, Donnenberg VS, Donnenberg AD. Mesenchymal stem cell secretome and regenerative therapy after cancer. Biochimie 2013; 95(12): 2235–2245
30. Ke F, Zhang L, Liu Z, Yan S, Xu Z, Bai J, Zhu H, Lou F, Cai W, Sun Y, Gao Y, Wang H, Wang H. Soluble tumor necrosis factor receptor 1 released by skin-derived mesenchymal stem cells is critical for inhibiting Th17 Cell differentiation. Stem Cells Transl Med 2016; 5(3): 301–313
31. Reikvam H, Brenner AK, Hagen KM, Liseth K, Skrede S, Hatfield KJ, Bruserud O. The cytokine-mediated crosstalk between primary human acute myeloid cells and mesenchymal stem cells alters the local cytokine network and the global gene expression profile of the mesenchymal cells. Stem Cell Res (Amst) 2015; 15(3): 530–541
32. Hsiao ST, Asgari A, Lokmic Z, Sinclair R, Dusting GJ, Lim SY, Dilley RJ. Comparative analysis of paracrine factor expression in human adult mesenchymal stem cells derived from bone marrow, adipose, and dermal tissue. Stem Cells Dev 2012; 21(12): 2189–2203
33. Lopatina T, Kalinina N, Karagyaur M, Stambolsky D, Rubina K, Revischin A, Pavlova G, Parfyonova Y, Tkachuk V. Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth *de novo*. PLoS One 2011; 6(3): e17899
34. Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 2012; 21(R1): R125–R134
35. Eldh M, Ekstrom K, Valadi H, Sjostrand M, Olsson B, Jernas M, Lotvall J. Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle RNA. PLoS One 2010; 5(12): e15353
36. Doeppner TR, Herz J, Gorgens A, Schlechter J, Ludwig AK, Radtke S, de Miroschedji K, Horn PA, Giebel B, Hermann DM. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. Stem Cells Transl Med 2015; 4(10): 1131–1143
37. Liang X, Zhang L, Wang S, Han Q, Zhao RC. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. J Cell Sci 2016; 129(11): 2182–2189
38. Wang L, Gu Z, Zhao X, Yang N, Wang F, Deng A, Zhao S, Luo L, Wei H, Guan L, Gao Z, Li Y, Liu D, Gao C. Extracellular vesicles released from human umbilical cord-derived mesenchymal stromal 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): 1874–1883
39. Wang S, Qu X, Zhao RC. Clinical applications of mesenchymal stem cells. J Hematol Oncol 2012; 5:19
40. Zhang W, Ge W, Li C, You S, Liao L, Han Q, Deng W, Zhao RC. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev 2004; 13(3): 263–271
41. Chen L, Zhang W, Yue H, Han Q, Chen B, Shi M, Li J, Li B, You S, Shi Y, Zhao RC. Effects of human mesenchymal stem cells on the differentiation of dendritic cells from $CD34^+$ cells. Stem Cells Dev 2007; 16(5): 719–731
42. Zhang B, Liu R, Shi D, Liu X, Chen Y, Dou X, Zhu X, Lu C, Liang W, Liao L, Zenke M, Zhao RC. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. Blood 2009; 113(1): 46–57
43. Hu W, Qiu B, Guan W, Wang Q, Wang M, Li W, Gao L, Shen L, Huang Y, Xie G, Zhao H, Jin Y, Tang B, Yu Y, Zhao J, Pei G. Direct conversion of normal and Alzheimer's disease human fibroblasts into neuronal cells by small molecules. Cell Stem Cell 2015; 17(2): 204–212
44. Thier M, Worsdorfer P, Lakes YB, Gorris R, Herms S, Opitz T, Seiferling D, Quandel T, Hoffmann P, Nothen MM, Brustle O, Edenhofer F. Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell 2012; 10(4): 473–479
45. Huang P, Zhang L, Gao Y, He Z, Yao D, Wu Z, Cen J, Chen X, Liu C, Hu Y, Lai D, Hu Z, Chen L, Zhang Y, Cheng X, Ma X, Pan G, Wang X, Hui L. Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell 2014; 14 (3): 370–384
46. Wang Q, Ye L, Liu H, Liu X, Li S, Chen Z. Reprogramming of bone marrow-derived mesenchymal stem cells into functional insulin-producing cells by chemical regimen. Am J Stem Cells 2012; 1(2): 128–137
47. Koch P. Direct conversion provides old neurons from aged donor's skin. Cell Stem Cell 2015; 17(6): 637–638
48. Han DW, Tapia N, Hermann A, Hemmer K, Hoing S, Arauzo-Bravo MJ, Zaehres H, Wu G, Frank S, Moritz S, Greber B, Yang JH, Lee HT, Schwamborn JC, Storch A, Scholer HR. Direct reprogramming of fibroblasts into neural stem cells by defined factors. Cell Stem Cell 2012; 10(4): 465–472
49. Han JK, Chang SH, Cho HJ, Choi SB, Ahn HS, Lee J, Jeong H, Youn SW, Lee HJ, Kwon YW, Oh BH, Oettgen P, Park YB, Kim HS. Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. Circulation 2014; 130(14): 1168–1178
50. Baeyens L, Lemper M, Leuckx G, De Groef S, Bonfanti P, Stange G, Shemer R, Nord C, Scheel DW, Pan FC, Ahlgren U, Gu G, Stoffers DA, Dor Y, Ferrer J, Gradwohl G, Wright CV, Van de

Casteele M, German MS, Bouwens L, Heimberg H. Transient cytokine treatment induces acinar cell reprogramming and regenerates functional β cell mass in diabetic mice. Nat Biotechnol 2014; 32(1): 76–83

51. Zhao CH. Concept of mesenchymal stem cells: bring more insights into functional research of MSC. J Exp Hematol (Zhongguo Shi Yan Xue Ye Xue Za Zhi) 2013; 21(2): 263–267 (in Chinese)
52. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 2010; 5(4): e10088
53. Carrion F, Nova E, Ruiz C, Diaz F, Inostroza C, Rojo D, Monckeberg G, Figueroa FE. Autologous mesenchymal stem cell treatment increased T regulatory cells with no effect on disease activity in two systemic lupus erythematosus patients. Lupus 2010; 19(3): 317–322
54. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, Kooy-Winkelaar EM, Koning F, Zwaginga JJ, Fidder HH, Verhaar AP, Fibbe WE, van den Brink GR, Hommes DW. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut 2010; 59(12): 1662–1669
55. Liang J, Zhang H, Hua B, Wang H, Lu L, Shi S, Hou Y, Zeng X, Gilkeson GS, Sun L. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. Ann Rheum Dis 2010; 69(8): 1423–1429
56. Sun L, Wang D, Liang J, Zhang H, Feng X, Wang H, Hua B, Liu B, Ye S, Hu X, Xu W, Zeng X, Hou Y, Gilkeson GS, Silver RM, Lu L, Shi S. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum 2010; 62(8): 2467–2475
57. Jiang R, Han Z, Zhuo G, Qu X, Li X, Wang X, Shao Y, Yang S, Han ZC. Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. Front Med 2011; 5(1): 94–100
58. Fang B, Song Y, Lin Q, Zhang Y, Cao Y, Zhao RC, Ma Y. Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant 2007; 11(7): 814–817
59. Kebriaei P, Isola L, Bahceci E, Holland K, Rowley S, McGuirk J, Devetten M, Jansen J, Herzig R, Schuster M, Monroy R, Uberti J. Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant 2009; 15(7): 804–811
60. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004; 363(9419): 1439–1441
61. Prasad VK, Lucas KG, Kleiner GI, Talano JA, Jacobsohn D, Broadwater G, Monroy R, Kurtzberg J. Efficacy and safety of *ex vivo* cultured adult human mesenchymal stem cells (Prochymal) in pediatric patients with severe refractory acute graft-versus-host disease in a compassionate use study. Biol Blood Marrow Transplant 2011; 17(4): 534–541
62. Wu KH, Chan CK, Tsai C, Chang YH, Sieber M, Chiu TH, Ho M, Peng CT, Wu HP, Huang JL. Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. Transplantation 2011; 91(12): 1412–1416
63. Copland IB, Qayed M, Garcia MA, Galipeau J, Waller EK. Bone marrow mesenchymal stromal cells from patients with acute and chronic graft-versus-host disease deploy normal phenotype, differentiation plasticity, and immune-suppressive activity. Biol Blood Marrow Transplant 2015; 21(5): 934–940
64. Sanchez-Guijo F, Caballero-Velazquez T, Lopez-Villar O, Redondo A, Parody R, Martinez C, Olavarria E, Andreu E, Prosper F, Diez-Campelo M, Regidor C, Villaron E, Lopez-Corral L, Caballero D, Canizo MC, Perez-Simon JA. Sequential third-party mesenchymal stromal cell therapy for refractory acute graft-versus-host disease. Biol Blood Marrow Transplant 2014; 20(10): 1580–1585
65. Maziarz RT, Devos T, Bachier CR, Goldstein SC, Leis JF, Devine SM, Meyers G, Gajewski JL, Maertens J, Deans RJ, Van't Hof W, Lazarus HM. Single and multiple dose MultiStem (multipotent adult progenitor cell) therapy prophylaxis of acute graft-versus-host disease in myeloablative allogeneic hematopoietic cell transplantation: a phase 1 trial. Biol Blood Marrow Transplant 2015; 21(4): 720–728
66. Zhao K, Lou R, Huang F, Peng Y, Jiang Z, Huang K, Wu X, Zhang Y, Fan Z, Zhou H, Liu C, Xiao Y, Sun J, Li Y, Xiang P, Liu Q. 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
67. Shipounova IN, Petinati NA, Bigildeev AE, Zezina EA, Drize NI, Kuzmina LA, Parovichnikova EN, Savchenko VG. Analysis of results of acute graft-versus-host disease prophylaxis with donor multipotent mesenchymal stromal cells in patients with hemoblastoses after allogeneic bone marrow transplantation. Biochemistry. Biokhimiia 2014; 79(12): 1363–1370
68. Te Boome LC, Mansilla C, van der Wagen LE, Lindemans CA, Petersen EJ, Spierings E, Thus KA, Westinga K, Plantinga M, Bierings M, Broers AE, Cuijpers ML, van Imhoff GW, Janssen JJ, Huisman C, Zeerleder S, Huls G, Boelens JJ, Wulffraat NM, Slaper-Cortenbach IC, Kuball J. Biomarker profiling of steroid-resistant acute GVHD in patients after infusion of mesenchymal stromal cells. Leukemia 2015; 29(9): 1839–1846
69. Weng JY, Du X, Geng SX, Peng YW, Wang Z, Lu ZS, Wu SJ, Luo CW, Guo R, Ling W, Deng CX, Liao PJ, Xiang AP. Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. Bone Marrow Transplant 2010; 45(12): 1732–1740
70. Perez-Simon JA, Lopez-Villar O, Andreu EJ, Rifon J, Muntion S, Diez Campelo M, Sanchez-Guijo FM, Martinez C, Valcarcel D, Canizo CD. Mesenchymal stem cells expanded *in vitro* with human serum for the treatment of acute and chronic graft-versus-host disease: results of a phase I/II clinical trial. Haematologica 2011; 96(7): 1072–1076
71. Yi HG, Yahng SA, Kim I, Lee JH, Min CK, Kim JH, Kim CS, Song SU. Allogeneic clonal mesenchymal stem cell therapy for refractory graft-versus-host disease to standard treatment: a phase I study. Korean J Physiol Pharmacol 2016; 20(1): 63–67
72. Erbey F, Atay D, Akcay A, Ovali E, Ozturk G. Mesenchymal stem cell treatment for steroid refractory graft-versus-host disease in children: a pilot and first study from Turkey. Stem Cells Int 2016; 2016: 1641402
73. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, Xu T, Le A, Shi S. Mesenchymal stem cell transplantation reverses multi-

organ dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 2009; 27(6): 1421–1432

74. Wang D, Huang S, Yuan X, Liang J, Xu R, Yao G, Feng X, Sun L. The regulation of the Treg/Th17 balance by mesenchymal stem cells in human systemic lupus erythematosus. Cell Mol Immunol 2017; 14(5):423–431
75. Wang D, Akiyama K, Zhang H, Yamaza T, Li X, Feng X, Wang H, Hua B, Liu B, Xu H, Chen W, Shi S, Sun L. Double allogenic mesenchymal stem cells transplantations could not enhance therapeutic effect compared with single transplantation in systemic lupus erythematosus. Clin Dev Immunol 2012; 2012: 273291
76. Wang D, Niu L, Feng X, Yuan X, Zhao S, Zhang H, Liang J, Zhao C, Wang H, Hua B, Sun L. Long-term safety of umbilical cord mesenchymal stem cells transplantation for systemic lupus erythematosus: a 6-year follow-up study. Clin Exp Med 2017;17 (3):333–340
77. de la Portilla F, Alba F, Garcia-Olmo D, Herrerias JM, Gonzalez FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. Int J Colorectal Dis 2013; 28(3): 313–323
78. Forbes GM, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D, Cummins AG, Phillips M, Herrmann RP. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. Clin Gastroenterol Hepatol 2014; 12 (1): 64–71
79. Garcia-Arranz M, Dolores Herreros M, Gonzalez-Gomez C, de la Quintana P, Guadalajara H, Georgiev-Hristov T, Trebol J, Garcia-Olmo D. Treatment of Crohn's-related rectovaginal fistula with allogeneic expanded-adipose derived stem cells: a phase I–IIa clinical trial. Stem Cells Transl Med 2016; 5(11): 1441–1446
80. Molendijk I, Bonsing BA, Roelofs H, Peeters KC, Wasser MN, Dijkstra G, van der Woude CJ, Duijvestein M, Veenendaal RA, Zwaginga JJ, Verspaget HW, Fibbe WE, van der Meulen-de Jong AE, Hommes DW. Allogeneic bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. Gastroenterology 2015; 149(4): 918–927 e916
81. Sanz-Baro R, Garcia-Arranz M, Guadalajara H, de la Quintana P, Herreros MD, Garcia-Olmo D. First-in-human case study: pregnancy in women with Crohn's perianal fistula treated with adipose-derived stem cells: a safety study. Stem Cells Transl Med 2015; 4(6): 598–602
82. Ciccocioppo R, Gallia A, Sgarella A, Kruzliak P, Gobbi PG, Corazza GR. Long-term follow-up of Crohn disease fistulas after local injections of bone marrow-derived mesenchymal stem cells. Mayo Clin Proc 2015; 90(6): 747–755
83. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, Minelli A, Alvisi C, Vanoli A, Calliada F, Dionigi P, Perotti C, Locatelli F, Corazza GR. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut 2011; 60(6): 788–798
84. Peng Y, Ke M, Xu L, Liu L, Chen X, Xia W, Li X, Chen Z, Ma J, Liao D, Li G, Fang J, Pan G, Xiang AP. Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. Transplantation 2013; 95(1): 161–168
85. Pan GH, Chen Z, Xu L, Zhu JH, Xiang P, Ma JJ, Peng YW, Li GH, Chen XY, Fang JL, Guo YH, Zhang L, Liu LS. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study. Oncotarget 2016; 7(11): 12089–12101
86. Lee H, Park JB, Lee S, Baek S, Kim H, Kim SJ. Intra-osseous injection of donor mesenchymal stem cell (MSC) into the bone marrow in living donor kidney transplantation; a pilot study. J Transl Med 2013; 11: 96
87. Mudrabettu C, Kumar V, Rakha A, Yadav AK, Ramachandran R, Kanwar DB, Nada R, Minz M, Sakhuja V, Marwaha N, Jha V. Safety and efficacy of autologous mesenchymal stromal cells transplantation in patients undergoing living donor kidney transplantation: a pilot study. Nephrology (Carlton) 2015; 20(1): 25–33
88. Martino G, Franklin RJ, Baron Van Evercooren A, Kerr DA. Stem cell transplantation in multiple sclerosis: current status and future prospects. Nat Rev Neurol 2010; 6(5): 247–255
89. Freedman MS, Bar-Or A, Atkins HL, Karussis D, Frassoni F, Lazarus H, Scolding N, Slavin S, Le Blanc K, Uccelli A. The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. Mult Scler 2010; 16(4): 503–510
90. Bonab MM, Sahraian MA, Aghsaie A, Karvigh SA, Hosseinian SM, Nikbin B, Lotfi J, Khorramnia S, Motamed MR, Togha M, Harirchian MH, Moghadam NB, Alikhani K, Yadegari S, Jafarian S, Gheini MR. Autologous mesenchymal stem cell therapy in progressive multiple sclerosis: an open label study. Curr Stem Cell Res Ther 2012; 7(6): 407–414
91. Yamout B, Hourani R, Salti H, Barada W, El-Hajj T, Al-Kutoubi A, Herlopian A, Baz EK, Mahfouz R, Khalil-Hamdan R, Kreidieh NM, El-Sabban M, Bazarbachi A. Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. J Neuroimmunol 2010; 227(1–2): 185–189
92. Mohajeri M, Farazmand A, Mohyeddin Bonab M, Nikbin B, Minagar A. FOXP3 gene expression in multiple sclerosis patients pre- and post mesenchymal stem cell therapy. Iran J Allergy Asthma Immunol 2011; 10(3): 155–161
93. Liang J, Li X, Zhang H, Wang D, Feng X, Wang H, Hua B, Liu B, Sun L. Allogeneic mesenchymal stem cells transplantation in patients with refractory RA. Clin Rheumatol 2012; 31(1): 157–161
94. Wang L, Cong X, Liu G, Zhou J, Bai B, Li Y, Bai W, Li M, Ji H, Zhu D, Wu M, Liu Y. Human umbilical cord mesenchymal stem cell therapy for patients with active rheumatoid arthritis: safety and efficacy. Stem Cells Dev 2013; 22(24): 3192–3202
95. Wang L, Zhang Y, Li H, Hong J, Chen X, Li M, Bai W, Wang J, Liu Y, Wu M. Clinical observation of employment of umbilical cord derived mesenchymal stem cell for juvenile idiopathic arthritis therapy. Stem Cells Int 2016; 2016: 9165267
96. Keyszer G, Christopeit M, Fick S, Schendel M, Taute BM, Behre G, Muller LP, Schmoll HJ. Treatment of severe progressive systemic sclerosis with transplantation of mesenchymal stromal cells from allogeneic related donors: report of five cases. Arthritis Rheum 2011; 63(8): 2540–2542
97. Granel B, Daumas A, Jouve E, Harle JR, Nguyen PS, Chabannon C, Colavolpe N, Reynier JC, Truillet R, Mallet S, Baiada A, Casanova D, Giraudo L, Arnaud L, Veran J, Sabatier F, Magalon G. Safety, tolerability and potential efficacy of injection of autologous adipose-derived stromal vascular fraction in the fingers

of patients with systemic sclerosis: an open-label phase I trial. Ann Rheum Dis 2015; 74(12): 2175–2182

98. Scuderi N, Ceccarelli S, Onesti MG, Fioramonti P, Guidi C, Romano F, Frati L, Angeloni A, Marchese C. Human adipose-derived stromal cells for cell-based therapies in the treatment of systemic sclerosis. Cell Transplant 2013; 22(5): 779–795
99. Xu J, Wang D, Liu D, Fan Z, Zhang H, Liu O, Ding G, Gao R, Zhang C, Ding Y, Bromberg JS, Chen W, Sun L, Wang S. Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome. Blood 2012; 120(15): 3142–3151
100. Sada PR, Isenberg D, Ciurtin C. Biologic treatment in Sjogren's syndrome. Rheumatology (Oxford) 2015; 54(2): 219–230
101. Wang P, Li Y, Huang L, Yang J, Yang R, Deng W, Liang B, Dai L, Meng Q, Gao L, Chen X, Shen J, Tang Y, Zhang X, Hou J, Ye J, Chen K, Cai Z, Wu Y, Shen H. Effects and safety of allogenic mesenchymal stem cell intravenous infusion in active ankylosing spondylitis patients who failed NSAIDs: a 20-week clinical trial. Cell Transplant 2014; 23(10): 1293–1303
102. Wang D, Zhang H, Cao M, Tang Y, Liang J, Feng X, Wang H, Hua B, Liu B, Sun L. Efficacy of allogeneic mesenchymal stem cell transplantation in patients with drug-resistant polymyositis and dermatomyositis. Ann Rheum Dis 2011; 70(7): 1285–1288
103. Ra JC, Kang SK, Shin IS, Park HG, Joo SA, Kim JG, Kang BC, Lee YS, Nakama K, Piao M, Sohl B, Kurtz A. Stem cell treatment for patients with autoimmune disease by systemic infusion of culture-expanded autologous adipose tissue derived mesenchymal stem cells. J Transl Med 2011; 9:181
104. Cle DV, Santana-Lemos B, Tellechea MF, Prata KL, Orellana MD, Covas DT, Calado RT. Intravenous infusion of allogeneic mesenchymal stromal cells in refractory or relapsed aplastic anemia. Cytotherapy 2015; 17(12): 1696–1705
105. 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; 3(1): e45–e52
106. Guo M, Sun Z, Sun QY, Han Q, Yu CL, Wang DH, Qiao JH, Chen B, Sun WJ, Hu KX, Liu GX, Liu B, Zhao RC, Ai H. A modified haploidentical nonmyeloablative transplantation without T cell depletion for high-risk acute leukemia: successful engraftment and mild GVHD. Biol Blood Marrow Transplant 2009; 15(8): 930–937
107. Liu K, Chen Y, Zeng Y, Xu L, Liu D, Chen H, Zhang X, Han W, Wang Y, Zhao T, Wang J, Han Q, Zhao C, Huang X. Coinfusion of mesenchymal stromal cells facilitates platelet recovery without increasing leukemia recurrence in haploidentical hematopoietic stem cell transplantation: a randomized, controlled clinical study. Stem Cells Dev 2011; 20(10): 1679–1685
108. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 2004; 94(1): 92–95
109. Wang S, Qu X, Zhao RC. Clinical applications of mesenchymal stem cells. J Hematol Oncol 2012; 5(1): 19
110. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 2009; 4(3): 206–216
111. Payne NL, Dantanarayana A, Sun G, Moussa L, Caine S, McDonald C, Herszfeld D, Bernard CC, Siatskas C. Early intervention with gene-modified mesenchymal stem cells over-expressing interleukin-4 enhances anti-inflammatory responses and functional recovery in experimental autoimmune demyelination. Cell Adhes Migr 2012; 6(3): 179–189
112. Polchert D, Sobinsky J, Douglas G, Kidd M, Moadsiri A, Reina E, Genrich K, Mehrotra S, Setty S, Smith B, Bartholomew A. IFN-γ activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol 2008; 38(6): 1745–1755