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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

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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 angioneurogenesis 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

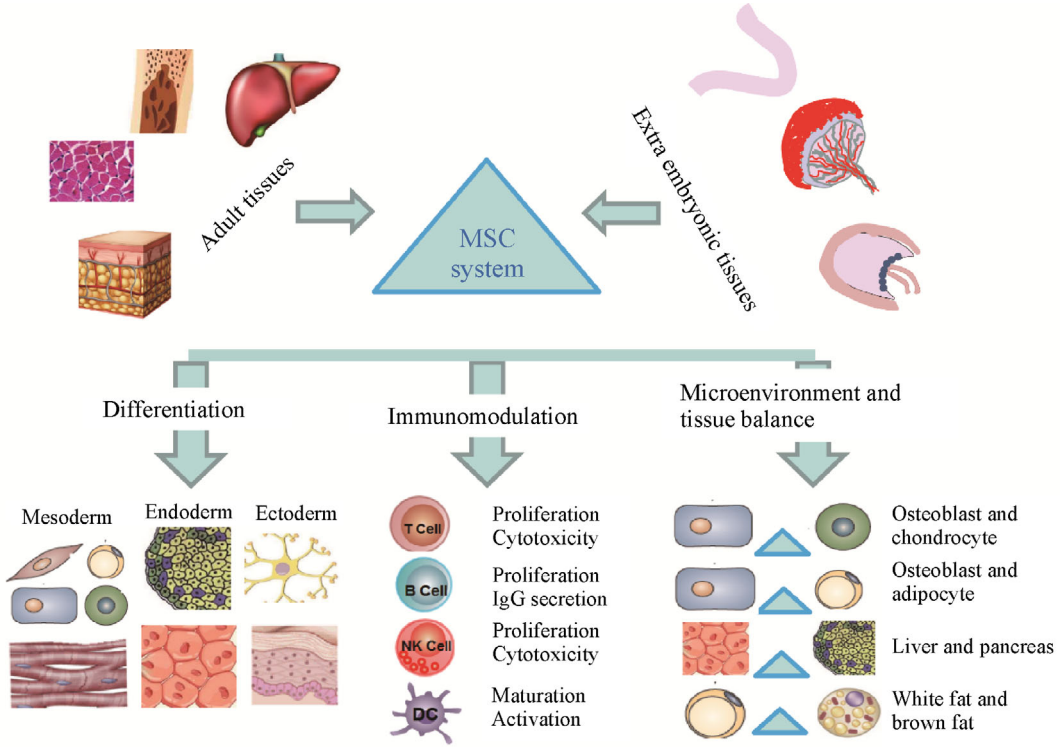

Fig. 1 Concept of the MSC system.

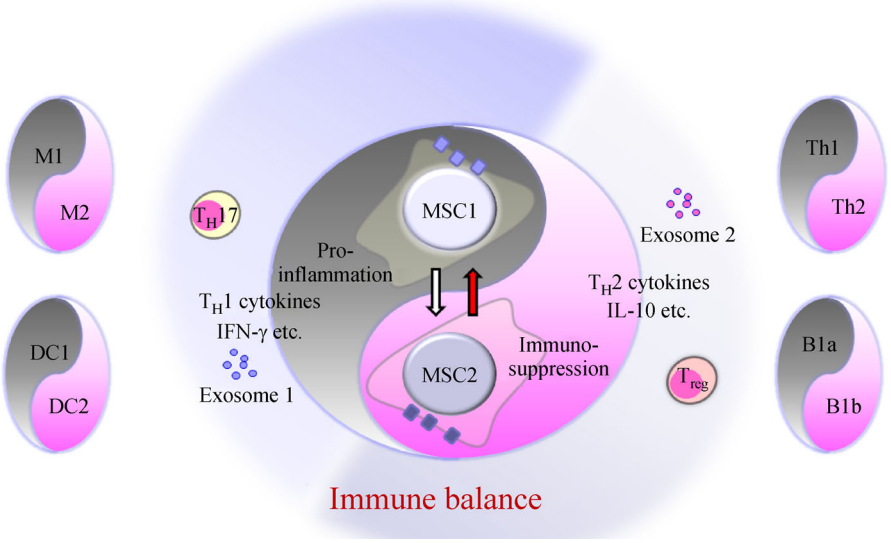

Fig. 2 "Yin and Yang" balance of MSC regulation.

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

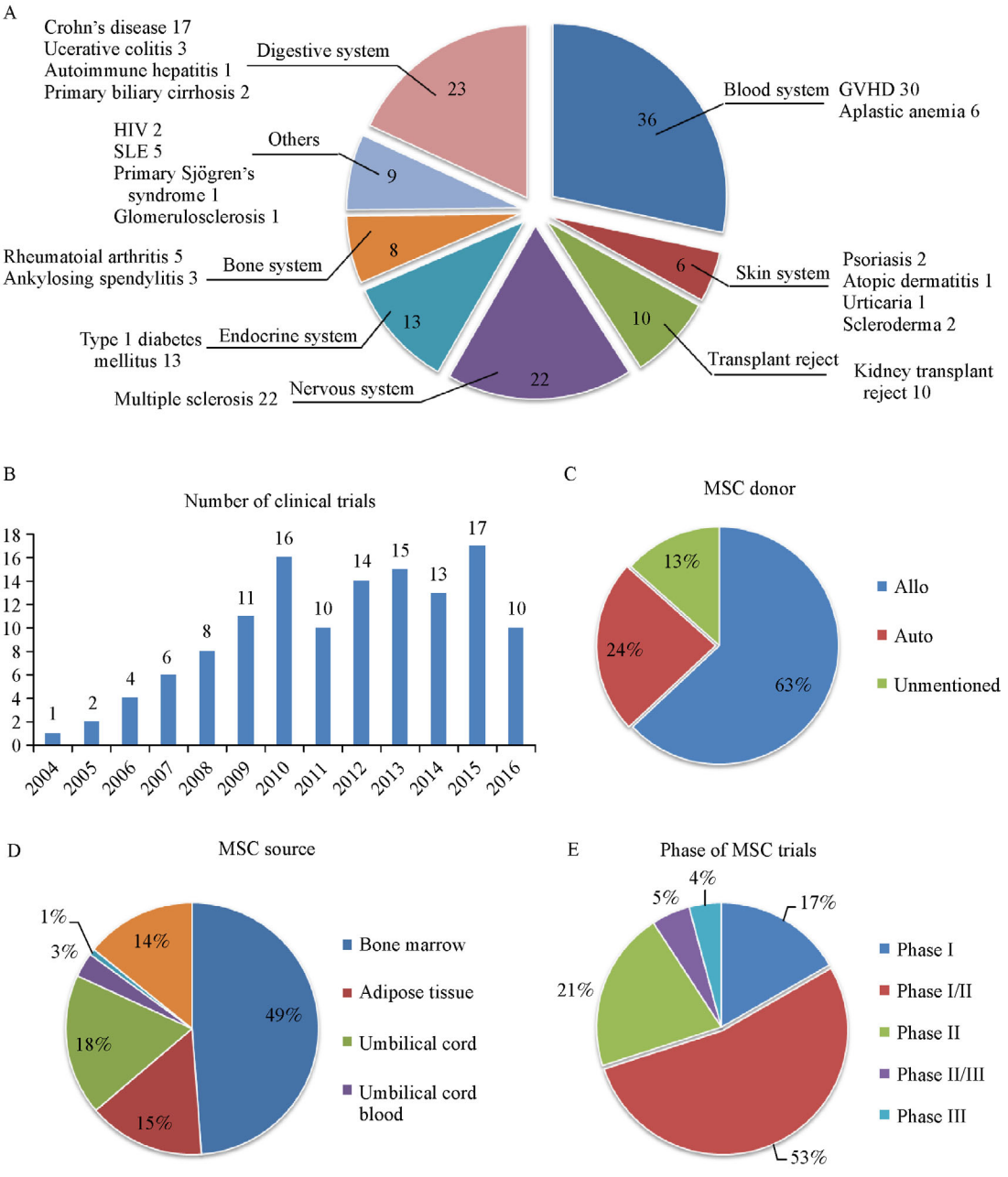

Fig. 3 Analysis of clinical trials of MSC on immune-related diseases (in October 17, 2016, $n = 125$). (A) clinical trials classified by disease types and different systems; (B) numbers of newly created clinical trials of MSC on immune-related disorders over these years; (C) clinical trials of MSCs classified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

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Compliance with ethics guidelines

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