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REVIEW

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Harnessing mesenchymal stem/stromal cells-based therapies for rheumatoid arthritis: mechanisms, clinical applications, and microenvironmental interactions

Ying-Feng Gao[1†], Na Zhao[1†] and Cheng-Hu Hu[1*]

Abstract

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by persistent joint inflammation and progressive bone destruction. Current conventional treatments, including nonsteroidal anti-inflammatory drugs, glucocorticoids, and disease-modifying antirheumatic drugs, often fail to achieve sustained remission in many patients. Mesenchymal stem/stromal cells (MSCs) have emerged as a promising therapeutic option for RA due to their immunomodulatory and regenerative properties. However, challenges such as poor migration and homing, low survival rate, heterogeneity, and impaired potency under pathological microenvironment need to be resolved to promote the use of MSCs in the clinic in the future. This review comprehensively examines the role of MSCs and their interaction with the microenvironment in the treatment of RA. We analyze completed and ongoing clinical trials involving MSC-based therapies for RA, summarize strategies to enhance the therapeutic effect of MSCs on RA, and propose standardized parameters to optimize clinical applications. By addressing these critical aspects, this review aims to advance the development of MSC-based therapies for RA.

Keywords Mesenchymal stem/Stromal cells, Microenvironment, Rheumatoid arthritis, Clinical application, Bioengineering, Pre-activation, Co-administration, Extracellular vesicles, Immunomodulation, Tissue regeneration

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease in which a person's immune system attacks the lining of joints throughout the body [1–3]. The general features of RA are demonstrated in Fig. 1. The main pathologic feature of RA is proliferation of synovial hyperplasia, inflammatory cell infiltration in the stroma, as well as angiogenesis, pannus formation, and destruction of cartilage and bone tissue. The diagnosis of RA relies on physical examination, serological markers, imaging examination, and differential diagnosis guided by the American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) classification criteria [4, 5]. Globally, the prevalence of RA ranged from 0.25–1% [6], with three to five times more in women than in men [1, 6, 7]. In the reporting of Global Epidemiology of Rheumatoid Arthritis 2017 Data, the global prevalence rate, incidence rate, and age of onset of RA have all increased, but the disease severity and mortality rate show a downward trend [6].

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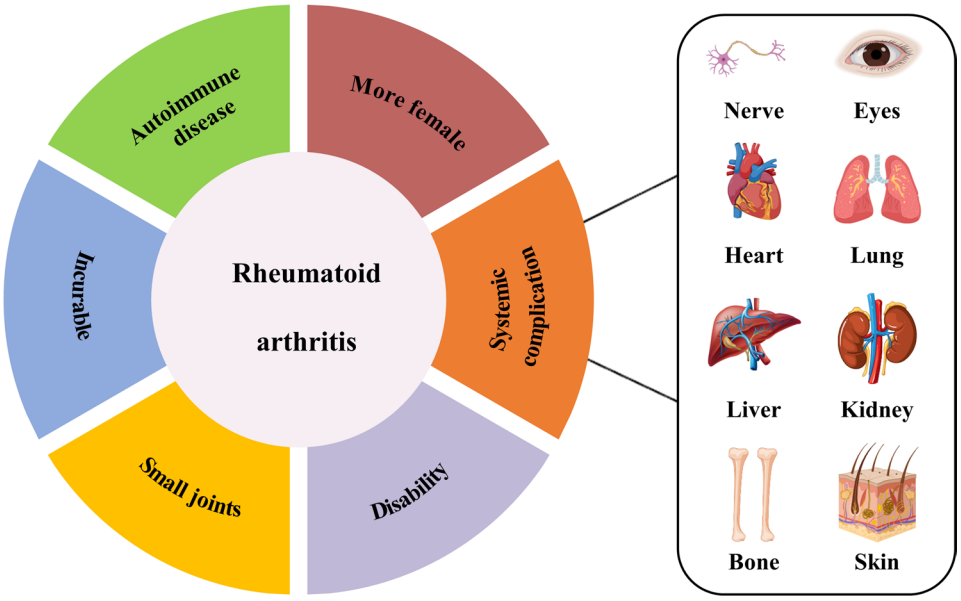

Fig. 1 General features of RA. RA is an incurable autoimmune disease that occurs most frequently in women, usually in the small joints of hands, wrists, and feet, and repeatedly in a symmetrical distribution, with systemic complications that ultimately lead to disability. Created with MedPeer (medpeer.cn)

The cause of RA remains unknown, but certain risk factors are associated with an increased likelihood of developing RA, including environmental and genetic factors (Fig. 2). Indeed, more than 100 susceptibility genes have been identified that contribute to the risk of disease and overwhelmingly implicate immune pathways [8, 9], particularly HLA-DRB1, PTPN22, CTLA4, and PADI4 are related to the occurrence of RA [10–12]. Epigenetic factors, such as DNA methylation and histone acetylation, also contribute to RA, probably by integrating genetic and environmental effects [13]. Environmental factors are also key points in causing RA, such as respiratory exposure, oral health, hormonal factors, intestinal health, and personal lifestyle and habits, which directly affect the post-transcriptional modification of certain genes or indirectly affect susceptibility genes via epigenetic mechanisms [6]. The interaction of environmental factors, susceptibility genes, and epigenetics will drive changes in the relative levels and expression of coded proteins, which can lead to loss of tolerance. As a hypothesis, it is generally accepted that RA originates from a high-risk genetic background that, in combination with epigenetic markers and environmental factors, causes new epitopes [14]. It triggers a cascade of events that induces immune cell infiltration of synovium and proliferation and invasion of synoviocytes, leading to the production of high levels of inflammatory cytokines, chemokines, matrix-degrading enzymes, and receptor activator of nuclear factor k B ligand (RANKL), which ultimately causes chronic and destructive arthritis [15–18] (Fig. 2).

Current approaches in RA treatment

The current treatment strategy for RA is based on a treat-to-target approach, which requires tight monitoring of the disease activity and prompt correction of treatment when the target is not reached. The two most influential organizations for rheumatology worldwide updated versions of recommendations and guidelines for the management of RA, the ACR in 2021 [19] and EULAR in 2022 [20]. Current therapies target either specific immune cells, their secretory products, or specific signaling pathways using small molecule inhibitors. The current treatment target is remission or, at the very least, low disease activity. In clinical RA treatment, the commonly used drugs are nonsteroidal anti-inflammatory drugs (NSAIDs) [21, 22], glucocorticoids (GCs) [23, 24], disease-modifying antirheumatic drugs (DMARDs) [25], including conventional synthetic (csDMARDs) [26–30], biologic DMARDs (bDMARDs) [31–40] and its biosimilar, and targeted synthetic DMARDs (tsDMARDs) [41–43], namely the Janus kinase (JAK) inhibitors [44–48]. The principal approved drugs for RA treatment are highlighted in Table 1.

RA remains an incurable disease, but since around the turn of the millennium, with the advent of bDMARDs and tsDMARDs, remission has become an attainable goal. However, many patients still experience clinically meaningful levels of remaining pain,

about 20–30% of moderate-to-severe RA patients are unresponsive to current treatment strategies [49]. Besides, long-term use of traditional drugs for RA can cause serious side effects, such as metabolic disorders and increased risk of infection [50–54], as well as drug

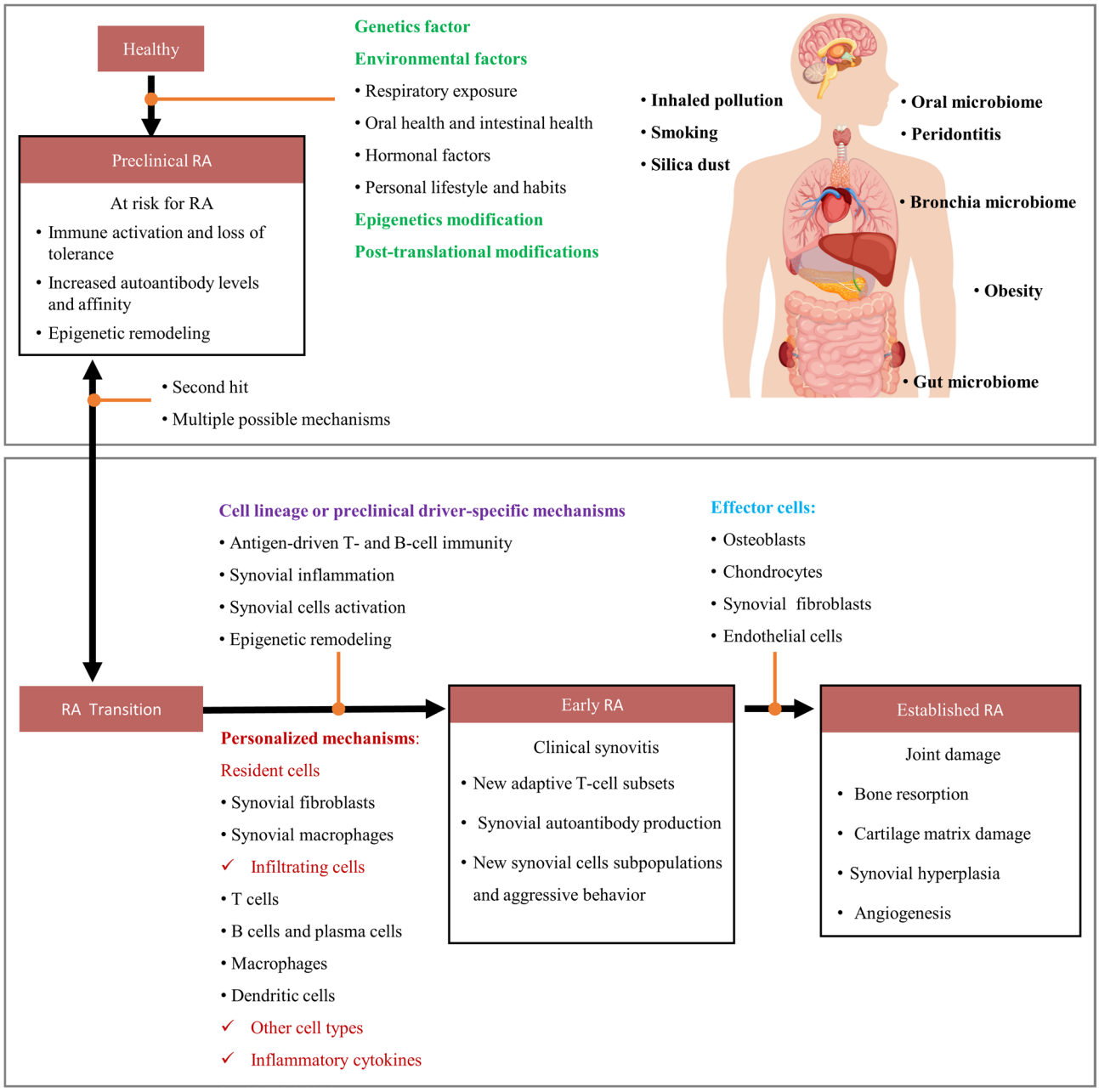

Fig. 2 Initiation and Progression of RA. RA progresses from a healthy state to preclinical RA (at risk for RA) to the RA transition to early synovitis and finally to established, destructive disease. The pathway is not unidirectional, since persons in the disease stage before synovitis who are positive for antibodies against citrullinated peptides (ACPAs) can become ACPA-negative, and in some ACPA-positive persons, disease never develops. Although each disease state has a characteristic clinical phenotype, multiple pathways and mechanisms can contribute to pathogenesis for an individual patient. Created with MedPeer (medpeer.cn)

resistance in some patients. So, it's critically needed to find an effective and safe therapeutic approach.

Mesenchymal stem/ stromal cells (MSCs) are multipotent progenitor cells possessing self-renewal ability (limited in vitro), differentiation potential into mesenchymal lineages, according to the International Society for Cell and Gene Therapy [55]. In general, MSCs can be isolated from specific tissue in human body, such as dental pulp, bone marrow (BM), peripheral blood, adipose tissue (AD), lungs, hair, or the heart, and perinatal tissues, such as umbilical cord (UC), UC blood (UCB), and placental structure [56]. MSCs are a group of non-hematopoietic stromal cells with immunomodulatory and inhibitory potential, due to interaction with various innate and adaptive immune cells to suppress deregulated humoral immunity and regulate imbalanced cell responses. MSCs

Table 1 Summary of current approaches for RA treatment

Class	Target	Structure	Mechanisms of action	Administration/dose	Management of RA	Approved
NSAIDs	COX	Small chemical molecules	inhibiting PGs synthesis by suppressing COX activity and exerting antipyretic and analgesic effects	Oral, The addition of NSAID to either standard DMARD monotherapy or combinations of synthetic DMARDs	Relieving pain, swelling, and stiffness of the joints caused by RA	FDA
GCs	Genomic and non-genomic pathways	Small chemical molecules	direct impact on many aspects of cellular immunity such as antibody recognition, immune activation, cell proliferation, and immune effects	Oral, intramuscular, intravenous, intra-articular (dose depends on route of administration and clinical indication)	Bridging therapy when DMARD therapy is initiated or switched and treating flares; in some patients is sometimes used as a long-term maintenance therapy (similar to a DMARD)	FDA
DMARDs						
csDMARDs					First-line therapy in patients who are naive to DMARDs	
Methotrexate	Unknown	Small chemical molecules	Affects multiple cell types; inhibits several pathways, including adenosine metabolism	Oral, SC, IM (15–25 mg/week)	First choice among csDMARDs	FDA
Leflunomide	DHDDH	Small chemical molecules	Inhibits DHDDH and pyrimidine metabolism and may inhibit expansion of activated leukocytes	Oral (20 mg/day)	Monotherapy if methotrexate is contraindicated (combination therapy with methotrexate uncommon)	FDA
Sulfasalazine	Unknown	Small chemical molecules	potentially inhibits inflammatory cytokines and chemokines and alters adenosine metabolism	Oral (2–3 g/day)	Combination therapy with methotrexate (or monotherapy if methotrexate is contraindicated)	FDA
Hydroxychloroquine	Unknown	Small chemical molecules	Possibly stabilizes macrophage lysosomes; modulates TLR7 and TLR9 activity	Orally (200–400 mg/day)	Combination therapy with methotrexate (or monotherapy if methotrexate is contraindicated in patients with low disease activity)	FDA
Iguratimod	Unknown	Small chemical molecules	Decreasing the production of immunoglobulins and cytokines, thereby mediating T lymphocytes subsets; Stimulates osteoblast differentiation promotion and reduces osteoclastogenesis	Orally (50 mg/day)	Combination therapy with methotrexate (or monotherapy if methotrexate is contraindicated)	China
tsDMARDs						

Table 1 (continued)

Class	Target	Structure	Mechanisms of action	Administration/dose	Management of RA	Approved
Tofacitinib	JAK 1,2,3	Small chemical molecules	Interrupt cytokine networks through blockade of JAK–STAT pathway, inhibiting FLS activation, leukocyte maturation, and autoantibody production	Orally (10 mg/day)	In patients who have had at least one conventional synthetic DMARD, after at least one TNF inhibitor (ACR), or as first-line therapy (EULAR) in selected populations*; might have some advantages in monotherapy compared with other bDMARDs	FDA
Baricitinib	JAK 1,2			Orally (2–4 mg/day)		FDA
Upadacitinib	JAK 1,2[†]			Orally (15 or 30 mg/day)		FDA
Filgotinib	JAK 1			Orally (200 mg/day)		EU and Japan
Peficitinib	JAK 1,2,3			Orally (100 or 150 mg/day)		Japan
bDMARDs					First-line therapy in patients who have had at least one unsuccessful csDMARDs	
Etanercept	TNF-α	Receptor construct	Blockade of TNF; inhibits activation of leukocytes, FLS, endothelial cells, and osteoclasts, preventing matrix degradation and production of pro-inflammatory molecules	SC injection (50 mg/week)	Commonly used as first-line therapy among bDMARDs	FDA
Infliximab		Chimeric monoclonal antibody		IV (3 mg/kg at week 0, 2, and 6, and every 8 weeks); SC (120 mg every 2 weeks)		FDA
Adalimumab		Human monoclonal antibodies		SC injection (40 mg every two week)		FDA
Golimumab		Human monoclonal antibodies		IV (2 mg/kg at week 0, 4, and every 8 weeks); SC (50 mg every 4 weeks)		FDA
Certolizumab		Fab' fragment of humanized monoclonal antibody		SC injection (400 mg at weeks 0, 2 and 4, followed by 200 mg every 2 weeks)		FDA
Anakinra	IL-1R	Receptor antagonist construct	blocks interleukin-1 binding to receptor; inhibits activation of leukocytes, FLS, endothelial cells, and osteoclasts, preventing matrix degradation	SC injection (75–150 mg or 0.04–2 mg/kg)	In patients, who have failed one or more DMARDs	FDA
Tocilizumab	IL-6R	Human monoclonal antibodies	blocks interleukin-6 binding to receptor; inhibits B-cell differentiation; activation of leukocytes, osteoclasts, and acute-phase reactant elevation; lipid alterations	IV injection (8 mg/kg once every 4 weeks) or SC injection (162 mg/week)	First-line therapy in patients who have had at least one csDMARDs; might have some advantages compared with bDMARDs in monotherapy	FDA
Sarilumab		Human monoclonal antibodies		SC injection (200 mg/ twice a week)		FDA

Table 1 (continued)

Class	Target	Structure	Mechanisms of action	Administration/dose	Management of RA	Approved
Rituximab	CD20	Chimeric monoclonal antibody	Binds CD20 and depletes B cells, inhibiting antigen presentation and autoantibody production	IV injection (1 gm twice separated by 2 weeks) with MTX and IV corticosteroid premedication	In patients, who have had at least one csDMARDs, usually after TNF inhibitors; ACR suggests use after inadequate response to TNF inhibitors or in patients with history of lymphoproliferative disorder	FDA
Abatacept	CD80/86	Receptor construct	Binds CD80 and CD86 and blocks T-cell costimulation, inhibiting naive T-cell activation	IV injection (2–10 mg/kg on days 1, 15 and 30, and then every 4 weeks)	First-line therapy in patients who have had at least one unsuccessful biological or csDMARDs	FDA

Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; COX, cyclooxygenase; PG, prostaglandin; FDA, Food and Drug Administration; GCs, glucocorticoids; DMARDs, disease-modifying antirheumatic drugs; csDMARDs, conventional synthetic DMARDs; IV, intravenous; SC, subcutaneous; IM, intramuscular; DHDDH, dihydroorotate dehydrogenase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; tsDMARDs, targeted synthetic DMARDs; STAK, signal transducer and activator of transcription. FLS, fibroblast like synoviocytes; bDMARDs, biologic DMARDs; TNF, tumor necrosis factor; IL-1R, interleukin-1 receptor; IL-6R, interleukin-6 receptor; CD, cluster of differentiation. MTX, methotrexate. *Risk factors for cardiovascular events and malignancies to consider before prescribing a JAK inhibitor: age older than 65 years, previous or current smoking, diabetes, obesity, hypertension, current or previous malignancy (other than non-melanoma skin cancer), and risk factors for thromboembolic events (e.g., history of myocardial infarction or heart failure, history of blood clots or inherited disorders of coagulation, combined contraceptives or hormonal replacement therapy, immobility, and undergoing major surgery). †Various assays showed different specificities for JAK 2 inhibition

have been regarded as an ideal source for therapeutics in autoimmune and hyperinflammatory diseases [57].

The clinical application of MSCs in the treatment of RA

There are 17 clinical trials for RA involving MSC intervention registered at https://clinicaltrials.gov/ as of Ja nuary 2024 (Fig. 3). In clinical trials of RA, most are in Phase I and Phase II, and there is only one in Phase II/III (Fig. 3A). Eight trials had been completed, and seven of them posted results (Fig. 3B). The others are mostly unknow or not yet recruiting; one is being recruiting and one has been terminated (Fig. 3B). Thus, limited data are available to evaluate the therapeutic effects of MSCs in RA. Therefore, we referred to these 12 published clinical trial articles on MSC treatment of RA [58–69], which overlapped with the 8 completed clinical trials that had results (Fig. 4). The clinical trial articles for RA treatment are highlighted in Table 2. Among the 12 articles, most of them are in Phase I and Phase II (Fig. 4A), and almost 83.33% of enrolled patients are refractory RA (Fig. 4B). The number of administrations is mainly a single administration, with multiple administrations consisting of 2 to 4 doses at intervals of 1 week, 1 month, and 3 months (Fig. 4C). MSC transplantation is performed according to patient body weight in most clinical trials ($1–4\times10^6$/kg) (Fig. 4D), while other clinical trials administer MSCs according to the quantity of cells ($2.5\times10^7–3\times10^8$ cells) (Fig. 4D). UC-MSCs, BM-MSCs, AD-MSCs and UCB-MSCs have been used in clinical trials for RA treatment (Fig. 4E). UC-MSCs are mainly suitable for allogeneic transplantation, and BM-MSCs and AD-MSCs are mainly suitable for autologous transplantation (Fig. 4F). However, invasive isolation of BM-MSCs causes injury and inflammation in donors, and the efficiency is low compared with MSCs from other sources [70]. Routes of administration selection for MSC transplantation in current published clinical trials is intravenous (IV) and intra-articular (IA) injection, with IV injection being the predominant route, and only one study used IV combined with IA [58] (Fig. 4G). No toxicity or serious adverse effects were observed in most clinical trials (Table 2). Only the study by Álvaro-Gracia et al. reported three severe adverse events (SAEs), all occurring in patients treated with MSCs: one lacunar infarction (severe), one peroneal nerve palsy (moderate intensity), and one case of fever (moderate intensity) [61]. They considered the lacunar infarction as a dose-limiting toxicity (DLT). Clinically relevant AEs (grades 3–5) related to MSC administration were considered DLTs. This event encompassed three consecutive SAEs (two events of generalized muscle weakness and one event of left hemihypoesthesia and paretic ataxic gait, finally diagnosed as lacunar infarction). These episodes were transient, and the patient recovered with minimal sequelae. It was deemed as likely related because there were no other apparent causes, even though the pathophysiology of this event is unclear. In general, the infusion of MSCs is a safe approach for treating RA patients.

The 12 articles revealed the exciting effects of MSCs on RA mainly by evaluating clinical symptoms, the disease activity score, and RA serology. MSCs can significantly

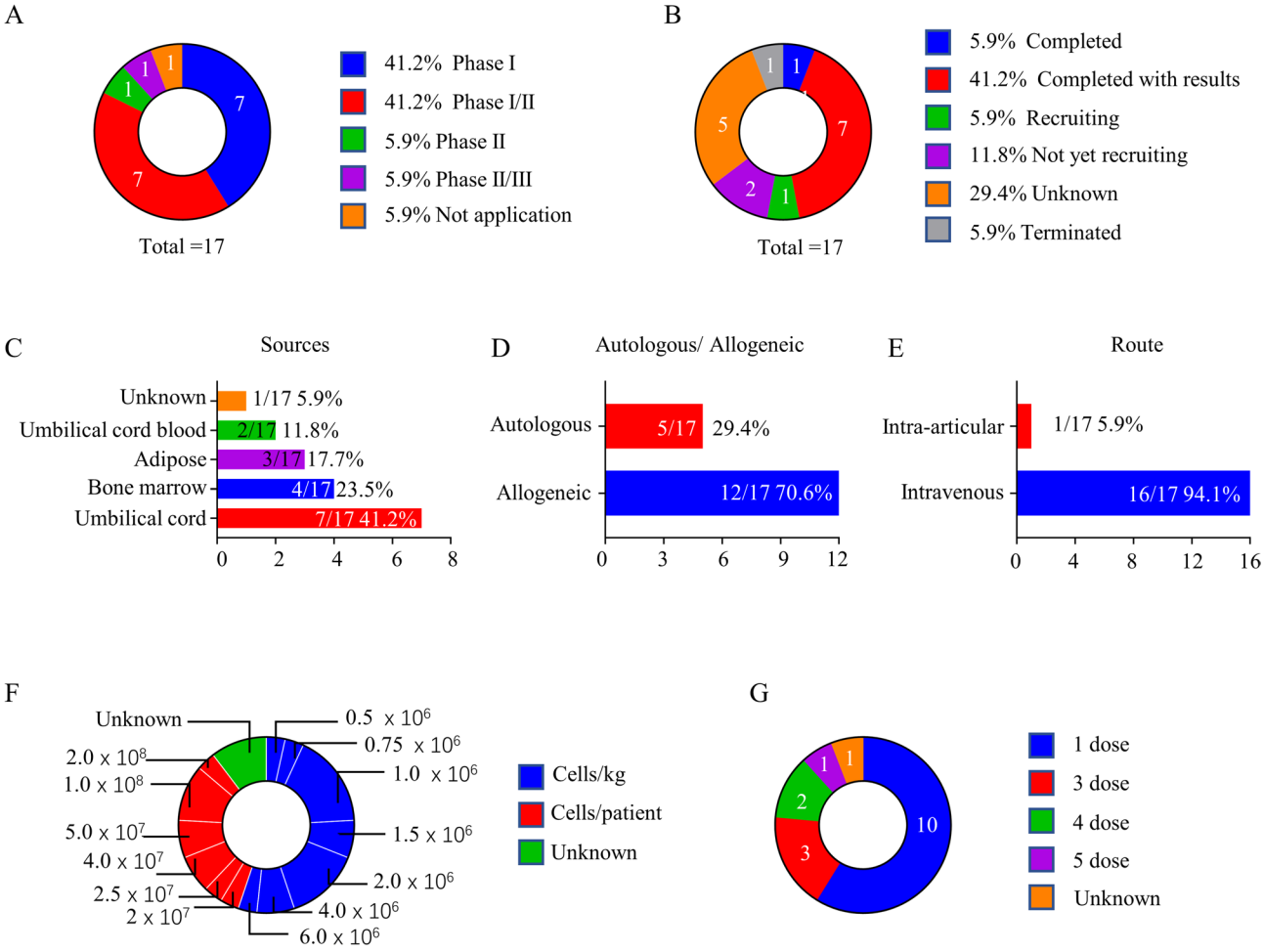

Fig. 3 Clinical trials of RA based on MSCs application

alleviate the clinical symptoms of RA patients, including increased walking distance, longer standing time, and pain relief; improve the joint disease activity score DAS28 and ACR20/50/70; reduce the serum levels of erythrocyte sedimentation rate (ESR), C reactive protein level (CRP), rheumatoid factor (RF), anti-cyclic citrullinated antibody (anti-CCP), IL-6, and TNF-α; and enhance joint function (Table 2). Most of these studies followed up with patients for more than 3 months and verified the safety of MSC transplantation (Table 2). Among the 12 articles, we believe that research by Xu's team about interferon (IFN)-γ as a key factor in determining the efficacy of MSC transplantation in the treatment of RA has significant clinical implication [65, 68], because it not only had the long follow-up and large sample size but also fully considered the impact of the patient's immune microenvironment on MSC treatment. Their study clarified that the combination of UCMSC transplantation with IFN-γ treatment synergistically improves the clinical outcomes of patients with RA. The 3-month follow-up results showed that compared with MSC transplantation alone, the combination of MSC transplantation with IFN-γ treatment significantly improved the clinical symptoms and disease activity of RA patients, with a 40% increase in clinical efficacy from 53.3 to 93.3%.

In conclusion, the 12 published clinical studies indicate that both autologous and allogeneic MSC transplantation are safe and effective for the treatment of refractory RA patients. Little to no serious adverse effects have been reported in RA patients during these clinical trials. The patients who received MSC transplantation showed a reduction in serum inflammatory markers, symptomatic improvement, and significant disease remission. However, the current clinical research lacks large-scale randomized controlled trials. most studies have been conducted on RA patients enrolled from a single center, and sometimes without inclusion of a placebo control. In addition, patient enrollment in some clinical trials for evaluation of safety and efficacy was low. In some cases, MSC-treated groups included three or four patients. Therefore, to confirm the clinical efficacy of MSC therapy on RA, a multiple-center, controlled trial should be

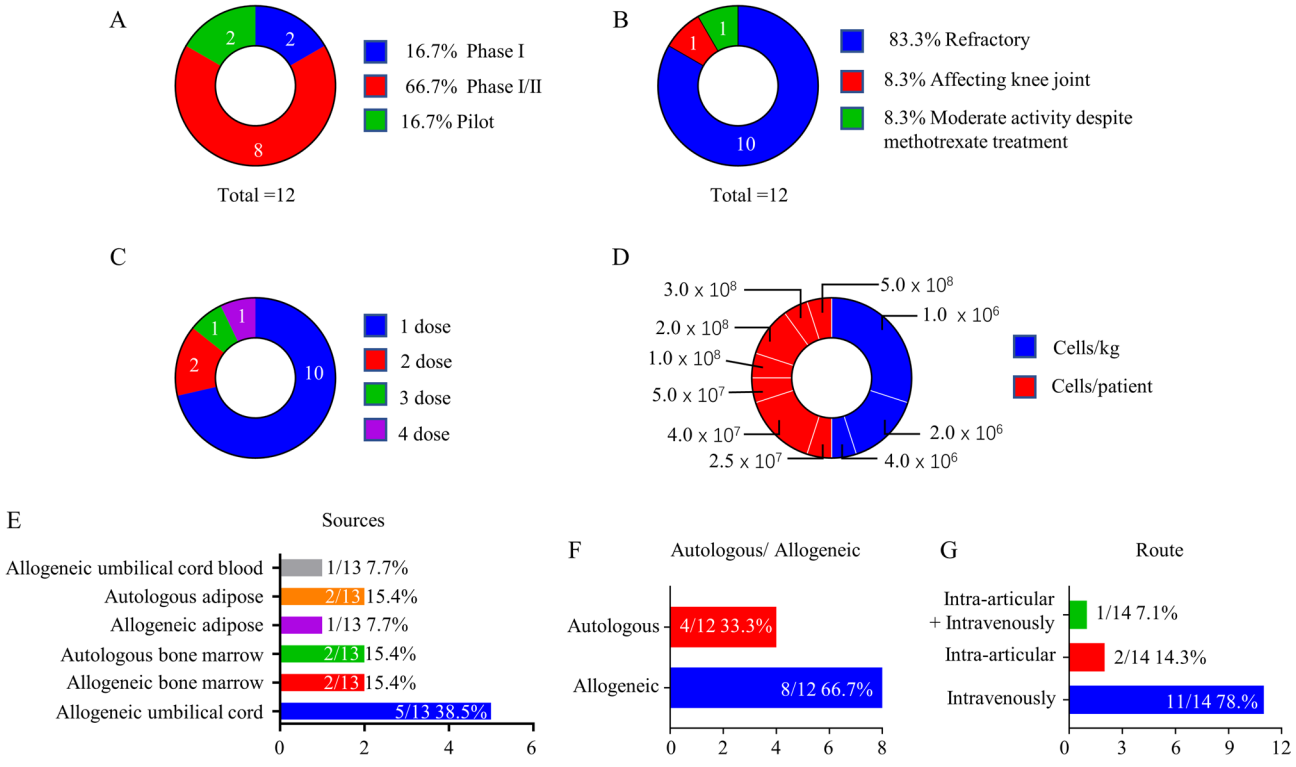

Fig. 4 Published clinical trials of RA based on MSCs application

conducted with the enrollment of a large number of RA patients.

Therapeutic mechanism of MSCs in RA

MSCs have strong tissue and organ regeneration ability as well as Immunomodulatory properties. MSCs function through multiple pathways [71]: (1) MSCs can differentiate and integrate into target tissues. (2) MSCs secrete a number of factors, including cytokines, angiogenic factors, and anti-apoptotic factors, as a paracrine effect. (3) They secrete extracellular vesicles (EVs) that are taken up by target cells and impact cellular function. (4) MSCs undergo apoptosis and are phagocytosed by tissue macrophages, altering macrophage function. (5) MSCs can act by cell-to-cell contact either by intercellular receptor interactions or via transfer of mitochondria.

Overall, the mechanism of MSCs in RA lies in two points: firstly, inhibiting the immune response, eliminating inflammation, and restoring the balance of the body's immunity; secondly, on the basis of immune regulation, promoting the proliferation of chondrocytes and osteoblasts and inhibiting osteoclasts' activity, thus promoting the repair of bone and cartilage. (Fig. 5).

Immune microenvironment regulation by MSCs in RA

Immunomodulatory properties of MSCs

Proverbially, MSCs can exert immunomodulatory effects on both innate and adaptive immune responses, and their immunomodulatory functions are exerted mainly through cell-to-cell contact and paracrine activity interacting with immune cells involving T cells, B cells, natural killer (NK) cells, macrophages, monocytes, dendritic cells (DCs), and neutrophils [57, 72]. All these mechanisms could contribute to the resolution of inflammation in RA.

Through direct actions, MSCs directly regulate various downstream pathways of immune cells by interacting with cell surface molecules and receptors, thereby affecting cell proliferation, effector production, and cell survival. The two main molecules on MSCs that are involved in such cell–cell interactions are the co-stimulatory molecule PDL1 [73, 74] and TNF ligand superfamily member 6 [75]. Through paracrine actions, the secretome of MSCs is a diverse repertoire of immunosuppressive molecules, growth factors, chemokines, extracellular vehicles (EVs), complement components, and various metabolites. MSCs produce a series of bioactive molecules, such as nitric oxide [76], indoleamine 2,3-dioxygenase (IDO) [77], tumor necrosis factor (TNF)-stimulated gene (TSG)-6 [78], prostaglandin E2 (PGE2) [79], interleukin (IL)-1 receptor antagonist [80], IL-6 [81], IL-10 [82], transforming growth factor (TGF)-β1 [83], heme oxygenase-1 [84], human leukocyte antigen-G5 [85], hepatocyte growth factor(HGF) [86], vascular endothelial growth factor [87], and an antagonistic variant of the chemokine, such as C-C chemokine ligand 9, CXC-chemokine

Table 2 Published clinical trials for RA treatment with MSCs

Clinical Trial Identifier	Clinical phase	Country	Status of disease	Enrollment	Source of MSCs	Doses and route of administration	Follow-up	Clinical outcome	Adverse events	Ref
NCT01663116	Ib/IIa	Spain	Refractory RA	53	allogeneic AD-MSCs	1, 2 or 4x10^6 cells/kg of body weight; three IV injections, weekly	6 months	DAS28-ESR↓, CRP↓, ACR20 response after 1 month (20–45%) and 3 months (15–25%)	3 severe events: one lacunar infarction (severe), one peroneal nerve palsy (moderate intensity) and one case of fever (moderate intensity).	[61]
NCT01873625	I/II	Iran	RA (affecting knee joint)	30	autologous BM-MSCs	4.0x10^7 cells/patient; single IA injection	12 months	DAS28↓(NS), VAS↓, WOMAC↓, ESR↓ (NS), CRP↓(NS), Pain FWD↑, WD↑, Time to jelling↑, Standing time↑	None	[63]
NCT02221258	Ia	Korea	RA moderate Activity despite methotrexate treatment	9	allogeneic UCB-MSCs	2.5x10^7, 5x10^7, or 1x10^8 cells/patient; single IV injection	1 months	DAS28↓, VAS↓, HAQ↓, CRP↓, IL-1β↓, IL-6↓, IL-8↓, TNF-α↓	None	[64]
NCT03333681	I	Iran	Refractory RA	9	autologous BM-MSCs	1x10^6 cells/kg of body weight; single IV injection	12 months	DAS28-ESR↓, VAS↓, ESR↓, RF↓, CRP↓(NS), anti-CCP↓ (NS)	None	[66]
NCT03691909	I/IIa	USA	Refractory RA	15	autologous AD-MSCs	2x10^8 cells/patient; single IV injection	52 weeks	ACR66/68 scores for both S/TJC ↓, CRP ↓	None	[69]
NCT01547091	I/II	China	Active RA	172	Allogeneic UC-MSCs	4 x10^7 cells/patient; single IV injection or twice with 3 months' interval	8 months	DAS28↓, HAQ↓, CRP ↓, RF↓,TNF-α↓, IL-6↓; Repeated infusion enhances the therapeutic efficacy	None	[60]
NCT01547091	I/II	China	Refractory RA	64	Allogeneic UC-MSCs	4 x10^7 cells/patient; single IV injection	3 years	ESR↓, CRP↓, RF↓, HAQ↓, DAS28↓ after 1 year and 3 years; anti-CCP↓ after 3years;	None	[67]

Table 2 (continued)

Clinical Trial Identifier	Clinical phase	Country	Status of disease	Enrollment	Source of MSCs	Doses and route of administration	Follow-up	Clinical outcome	Adverse events	Ref
NCT01851070	Ib/IIa	USA	Refractory RA	48	Allogeneic BM-MPCs	1or 2 x10^6 cells/kg of body weight; single IV injection	3 months	higher ACR20, ACR50, ACR70 levels achieved	None	[62]
ChiCTR-ONC-16,008,770	I/II	China	Refractory RA	105	Allogeneic UC-MSCs	1x10^6 cells/kg of body weight; single IV injections	12 months	DAS28↓, HAQ↓, ERS↓, CRP↓, IL-6↓, TNF-α↓, RF↓ (NS), anti-CCP↓(NS), IL-10↑(NS)	Transient fever in 3 patients	[65]
ChiCTR-INR-17,012,462	I/II	China	Refractory RA	63	Allogeneic UC-MSCs	1x10^6 cells/kg of body weight with/without intramuscular infusion of IFN-γ; single IV injections	3 months	ERS↓, CRP↓, RF↓; 53.3% of patients with MSC monotherapy and 93.3% of patients with MSC combined with IFN-γ treatment achieved ACR20 response	None	[68]
Unidentified	Pilot	Korea	Refractory RA	3	Autologous AD-MSCs	Patient 1: two doses IV injection of 3x10^8 cells, 15 weeks' nterval; Patient 2: once 2x10^8 cells (IV injection) + 1x10^8 cells (IA injection); once 3.5x10^8 cells (IV injection) + 1.5x10^8 cells (IA injection), 3 months' interval; Patient 3: four doses IV injection of 2x10^8 cells, 4 weeks' interval	3–13 months	VAS↓, KWOMAC↓, RF↓, anti-CCP↓, CRP↓, standing time↑, WD↑, off steroids	None	[58]
Unidentified	Pilot	China	Refractory RA	4	Allogeneic BM-MSCs/ UC-MSCs	1x10^6 cells/kg of body weight; single IV injections; One patient received BM-MSCs, The other three received UC-MSCs;	23 mouths	ESR↓, DAS28↓, VAS↓ in 3 out of 4 patients; 2 out of 4 EULAR response but relapse at 7 and 23 months.	None	[59]

Abbreviations: Ref, References; RA, rheumatoid arthritis; AD, adipose tissue; MSC, mesenchymal stem cells; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; ACR, the American College of Rheumatology improvement criteria; BM, bone marrow; IA, intra-articular; VAS, Visual Analogue Scale; KWOMAC, Korean Western Ontario McMaster; pain FWD, pain-free walking distance; WD, walking distance; UCB, umbilical cord blood; IV, intravenous; HAQ, Health Assessment Questionnaire; IL, interleukin; TNF, tumor necrosis factor; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated antibody; S/TJC, swollen/tender joint count; UC, umbilical cord; MPCs, multipotent progenitor cells; IFN, interferon; EULAR, European league against rheumatism; increasing levels (↓); decreasing level(↑), non-significant (NS)

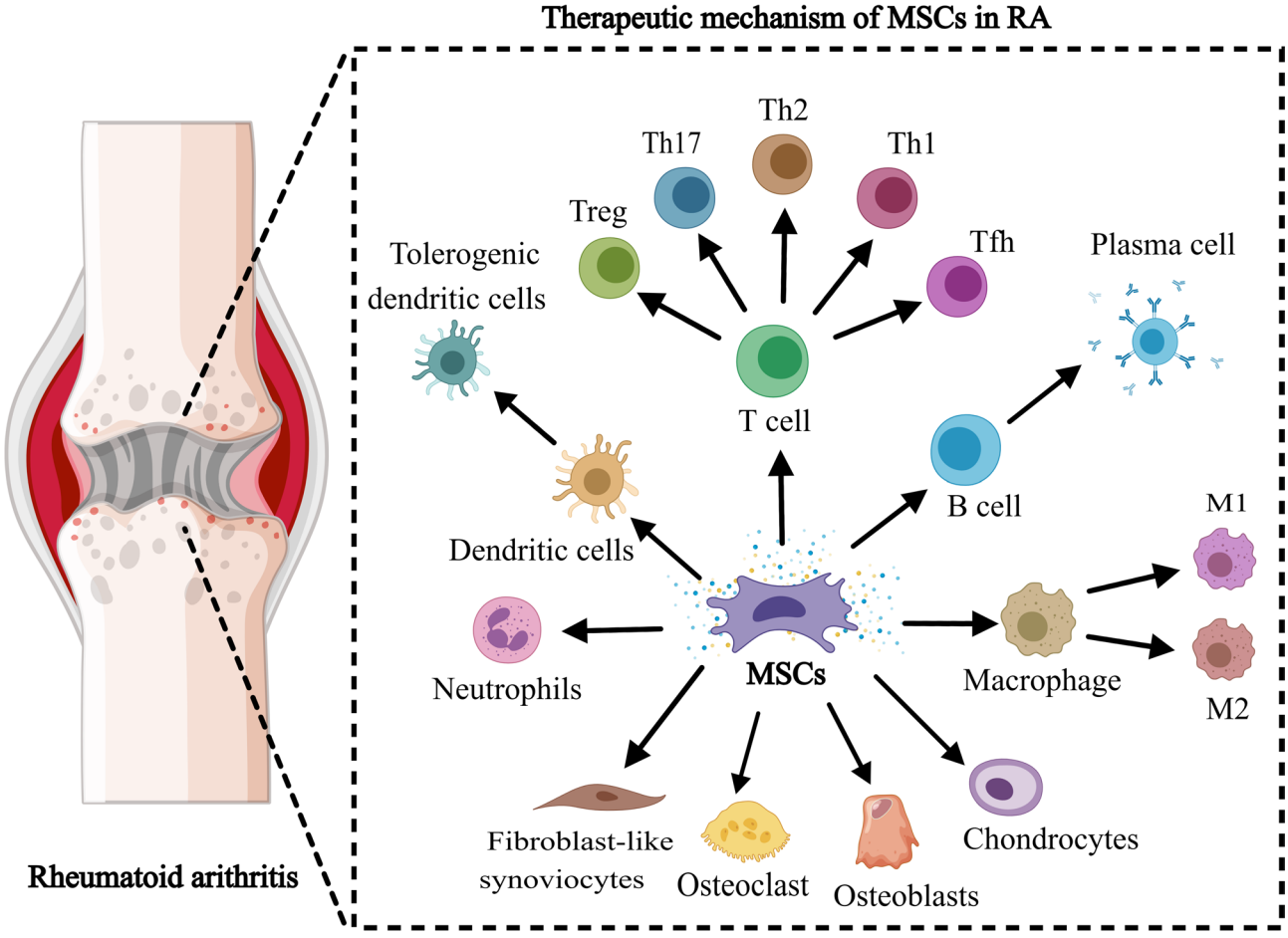

Fig. 5 Therapeutic mechanism of MSCs in RA. Created with MedPeer (medpeer.cn)

receptor (CXCR) 3, C-C chemokine receptor 5 [88]. These secretory factors contribute to immune modulation, tissue remodeling, and cellular homeostasis during regeneration.

However, the immunoregulatory properties of MSCs are not innate and immutable, but the plasticity is dependent on the induction of the inflammatory microenvironment. It reported that cytokines activate PI3K and AKT to initiate glycolysis, which is key to the production of high levels of chemokines, adhesion molecules, and effector molecules; apoptotic bodies can confer enhanced MSCs with enhanced immunomodulatory properties of MSCs; the extracellular matrix and scaffold also support the immunomodulatory functions of MSCs; and MSCs can also sacrifice themselves to fulfill the mission of immunosuppression [89]. In the presence of an inflammatory environment (high levels of IFN-γ and TNF-α), MSCs become Toll-like receptor (TLR) 3-primed anti-inflammatory phenotype of MSCs, also known as MSC2. In the absence of an inflammatory environment (low levels of IFN-γ and TNF-α), MSCs become the TLR4-primed pro-inflammatory phenotype of MSCs, also known as MSC1 [90]. As a whole, the immunomodulatory potency of MSCs is dynamically regulated by the kinds and concentrations of inflammatory mediators present in their microenvironment.

Immune tolerance reestablishment by MSCs in RA

RA is an autoimmune disease whose pathogenesis involves innate immunity, acquired immunity, cytokines, and intracellular signaling [3]. Utilizing the immunomodulatory function of MSC is to interact with immune cells associated with RA pathology to improve the patient's inflammatory microenvironment and remodel the patient's immune tolerance.

T cell T cells have a greater impact and play a crucial role in RA pathogenesis. T cells are activated and over-differentiated into Th1, Th17, and Tfh cells, releasing a variety of lymphokines that can drive synovial inflammation and joint destruction [91].

The immunomodulatory effect of MSCs to regulate T lymphocytes in vitro has been proved. Baharlou et al. co-cultured peripheral blood mononuclear cells (PBMCs)

of RA patients with AD-MSCs and confirmed that Th2 and Tregs transcription factors like GATA-binding protein-3 and forkhead box P3 (FoxP3) were upregulated, while Th1 and Th17 transcription factors like T-box 21 and retinoid-related orphan receptor γt were downregulated [92]. Vasilev et al.'s cultivation of RA patients' PBMCs with a conditioned medium of AD-MSCs confirmed that Th17 decreased and Treg increased [93]. Vohra et al. co-cultured T lymphocytes from synovial fluid (SF) and peripheral blood of RA patients with hUC-MSCs and confirmed that the proliferation and activation of lymphocytes from both peripheral blood and SF of RA patients were suppressed, the functions of activated clusters of differentiation (CD) 4^+ and $CD8^+$ T cells were downregulated, the secretion of pro-inflammatory cytokines were suppressed, and the expansion of Tregs was induced [94]. These results indicated that MSCs inhibit the immune function of lymphocytes in vitro.

The ability of MSCs to regulate immunity and improve the symptoms of RA by regulating T cells in vivo has also been proved. Dan et al. have demonstrated that MSCs can slow down the progression of RA disease activity, including improving arthritis, delaying radiological progression, and inhibiting synovial hyperplasia in collagen-induced arthritis (CIA) rats via regulating T lymphocyte proliferation and apoptosis and Th17 and Treg cell ratios in spleen [95]. In addition, Tfh cells, which provide proliferative signals to B cells, have also been implicated in the immunosuppressive effects of MSCs. Liu et al. demonstrated that hUC-MSCs inhibited Tfh cell differentiation in RA patients partly via the production of IDO in vitro, and intravenous administration of hUC-MSCs in mice after the onset of CIA decreased the number of Tfh cells in the spleen and suppressed their capacity to support B lymphocyte differentiation in an ex vivo co-culture assay [96].

Yu et al. found that long-interval repeated intravenous administration of human UCB-MSCs in CIA mice improved symptoms of RA by increasing Tregs and significantly decreased the mRNA expression level and protein level of IL-1β and IL-6 but increased the mRNA expression level and protein level of IL-10 [97]. Furthermore, it has been reported that MSCs dampen RA progression through the induction of the balance between memory Th17 and Tregs [98]. In RA, MSCs can diminish the frequency of pathogenic memory Th17 cells and the production of pro-inflammatory cytokines such as IL-17, IL-22, and granulocyte-macrophage colony stimulating factor (GM-CSF) and promote their differentiation toward an anti-inflammatory phenotype. In parallel, MSCs might also increase the capacity of memory Treg cells to produce anti-inflammatory cytokines such as IL-10 or TGF-β1 and prolong their immunosuppressive capacity, maintaining their anti-inflammatory phenotype.

B cell There was the production of autoantibodies such as anti-citrullinated protein antibodies (ACPA) or RF in 80% of the patients. B cells play a key function in the production of these antibodies and cytokines, regulating the T cells and macrophages. Luz-Crawford et al. demonstrated that IL-1 receptor antagonists (IL1Ra) from MSCs inhibit B cell differentiation and joint inflammation progression [80]. Compared with wild-type MSCs, IL1Ra (-/-) MSCs did not efficiently support the survival of quiescent B lymphocytes and block their differentiation toward CD19(+) CD138(+) plasmablasts secreting IgG antibodies in vitro. In the CIA mouse model, IL1Ra (-/-) MSCs were unable to protect mice from arthritic progression and even worsened clinical signs, as shown by higher arthritic scores and incidence than control arthritic mice.

DCs The evidence points towards a significant role for DCs in disease maintenance and progression of RA. DCs are responsible for inducing inflammation by presenting antigens to autoreactive T cells with subsequent production of cytokines, which stimulate T-helper differentiation [99]. Shi et al. clarified the mechanism of MSCs-DCs crosstalk in RA treatment [100]. They found that in the CIA mouse model, alginate hydrogel-encapsulated MSCs induce a significantly higher expression of $CD39^+CD73^+$ on MSCs. These enzymes hydrolyze ATP to adenosine and activate $A_{2A/2B}$ receptors on immature DCs, further promoting the phenotypic transformation of DCs to tolerogenic dendritic cells (tolDCs) and regulating naïve T cells to Tregs. Therefore, encapsulated MSCs obviously alleviate the inflammatory response and prevent CIA progression. Besides, the three-dimensional (3D) co-culture of encapsulated MSCs with DCs demonstrates that MSCs can inhibit the maturation of DCs and the secretion of pro-inflammatory cytokines.

Macrophages In the synovium of RA, the synovium is infiltrated with large numbers of monocyte-derived macrophages, which actively contribute to joint inflammation, as well as proliferation of resident synovial macrophages in the lining layer, which contribute to proliferation of the synovial lining [17]. The imbalance in M1/M2 macrophage is considered to be associated with joint inflammation and damage. Shin et al. discovered that human UCB-MSCs (hUCB-MSCs) alleviate RA via directing macrophage polarization and block inflammasome activation [101]. hUCB-MSCs suppressed M1 macrophage proliferation and activated M2 macrophage production via TNF-α-mediated activation of cyclooxygenase (COX)-2 and TSG-6 [101]. Additionally, hUCB-MSCs downregulated nucleotide-binding domain, leucine-rich repeat pyrin 3 inflammasome-mediated IL-1β secretion, and caspase-1 production in macrophages through the IL-1β feedback loop in CIA mice [101]. Moreover, IL-1

receptor antagonist (IL-1Ra) is critical in the MSCs-mediated macrophage polarization [80]. In the CIA model, IL1RA (-/-) MSCs to induce M2 macrophage polarization was significantly reduced compared to normal MSCs, and the effect of improving joint inflammation was completely attenuated [80].

Neutrophils Accumulating evidence has shown the pivotal roles of neutrophils in the pathophysiology of RA by contributing to the initiation and perpetuation of immune dysregulation [102]. Neutrophils are abundant at the sites where autoimmune damage occurs, such as the SF of the affected joints and the pannus/cartilage interface [103]. Neutrophil extracellular traps (NETs) released by activated neutrophils are important for initiating and perpetuating synovial inflammation. NETs containing chromatin associated with granule enzymes, which provide a source of citrullinated autoantigens, leading to the initiation of arthritic inflammation [104]. In addition, NETs not only can induce fibroblast-like synoviocytes (FLS) inflammatory phenotype, resulting in the formation of pannus in chronic RA [105], but also can activate diverse innate and adaptive immune cells to augment inflammation in arthritic joints [106]. Zhao et al. demonstrate that infusion of gingival-derived MSCs (GMSCs) can ameliorate inflammatory arthritis mainly by suppressing neutrophil extracellular traps (NET) formation via the PGE2-protein kinase A (PKA)-extracellular signal-regulated kinase (ERK) signaling pathway [107]. They observed that adoptive transfer of GMSCs into the K/B × N serum transfer-induced arthritis (STIA) mice significantly ameliorated experimental arthritis and reduced neutrophil infiltration and NET formation. In vitro, co-culture of GMSCs with activated neutrophils inhibited the generation of NETs in neutrophils by secreted PGE2 to activate PKA, which ultimately inhibited the downstream ERK pathway that is essential for NET formation.

In summary, MSCs alleviate RA by regulating multiple immune cells, such as T cells, B cells, DCs, macrophages and neutrophils, and multiple cytokine pathways in response to the imbalance of pro-inflammatory cytokines and anti-inflammatory cytokines in RA microenvironment to restore the immune tolerance of the RA patients. How to reduce the effect of RA inflammatory microenvironment on MSCs and maintain the anti-inflammatory capacity of MSCs is a question that must be explored.

Synovial homeostasis restoration by MSCs in RA

RA is characterized by the synovium transforming into a hyperplastic invasive tissue, which mediates destruction of cartilage and bone. FLS, which form the lining of the joint, have a major role in the initiation and perpetuation of destructive joint inflammation by producing pathogenic mediators such as matrix-degrading enzymes and RANKL [16]. RANKL promotes osteoclast differentiation and activation, leading to bone erosions. It has been reported that MSCs ameliorate the degree of RA bone erosion by inhibiting the decreas of RANKL in FLSs [108, 109]. Furthermore, studies have demonstrated that MSCs were able to decrease the expression of pro-inflammatory proteins in FLS, induce FLS apoptosis, inhibit FLS proliferation, and attenuate FLS invasiveness in vitro [110, 111]. In vitro, Zhao et al. demonstrated that IL-10 mediates the inhibitory effect of UC-MSCs on cadherin 11 (CDH11) expression by FLS from RA patients [112]. CDH11 can regulate the inflammatory response of synovial cells and promote synovial cell migration and cartilage erosion. Besides, Chiu et al. observed that IL-1β-stimulated hUC-MSCs adhering to FLS-RA occurred via lymphocyte function-associated antigen 1 (LFA-1)/LFA-1 ligand-intercellular adhesion molecule-1 interaction, and apoptosis of FLS-RA was induced via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor-death receptor (DR) 4, DR5 contact [113].

In summary, MSCs restore synovial homeostasis and inhibit bone and cartilage destruction by regulating FLS. How to utilize MSCs to make an impact at different stages of their life cycle and activation in FLS is a question that is worth exploring.

Bone metabolism and repair improvement by MSCs in RA

RA is a chronic inflammatory joint disease, which can cause cartilage and bone damage of joints. A major feature of RA is bone erosion, which develops as the disease progresses [114]. MSCs clearly have a prominent role during the process of bone metabolism in diseased conditions, whether direct or indirect, as they act as a source of progenitors for osteoblasts and osteoclasts.

Promotion of bone and cartilage regeneration

MSCs have the potential to differentiate into osteoblasts and chondrocytes. MSCs can differentiated into osteoblasts or osteocytes under osteoblast-conditioned medium including the inflammatory stimuli such as IL-1, and the addition of IL-6 and soluble IL-6 receptor to chondrogenic culture medium promoted chondrogenic differentiation of MSCs [115]. Gao et al. observed that BM-MSCs transplanted via tail vein improved bone destruction in CIA rats by inhibiting the expression of serum CXCL10 and CXCL10 and CXCR3 expression at the synovial membrane, regulating the RANKL/OPG ratio in the serum and synovial tissue, and directly differentiating into chondrocytes [116]. Moreover, they found that GFP-positive cells were observed in the cartilage tissue from day 11 until 42 days after GFP-MSCs transplantation. Anti-type II collagen/GFP double-positive cells were observed in the articular cartilage (especially

damaged cartilage) upon immunofluorescence staining of anti-type II collagen.

MSCs derived from inflammatory microenvironments demonstrated an inhibition in osteogenic differentiation potential and the capability of inducing apoptosis of osteoclasts and a differentiation switch from osteogenesis to adipogenesis with a frequent decline of self-renewal capacity [117]. Transplanted MSCs secrete various bioactive molecules, including growth factors, cytokines, and chemokines, which contribute to repairing damaged resident tissue MSCs [118], stimulate the proliferation and differentiation of osteoblasts, enhance bone matrix mineralization, and promote the recruitment of endothelial cells [119], as well as stimulate the proliferation and migration of endogenous chondrocytes, enhance extracellular matrix synthesis, and promote angiogenesis in the joints [116]. All of these contribute to bone healing and remodeling and cartilage regeneration.

In summary, MSCs promote bone and cartilage regeneration in the RA-inflamed joints by differentiating into osteoblasts and chondroblasts or paracrine activity to repaire damaged resident tissue. MSCs also stimulate the proliferation and migration of endogenous chondrocytes and osteoblasts. How to promote the differentiation of MSCs into bone and cartilage and enhance the regulation of MSCs to endogenous chondrocytes and osteoblasts in the RA inflammatory microenvironment are still challenges for the future.

Inhibition of bone and cartilage destruction

Articular bone erosions are a central clinical feature of rheumatoid arthritis. Bone erosion is a result of enhanced osteoclast differentiation and inhibition of osteoblast-mediated bone repair. The key ligands that promote osteoclast formation are M-CSF and RANKL. During the pathogenesis of RA, many cytokines, such as TNF, IL-1, and IL-6, promoted osteoclast formation via activating the RANKL-RANK signaling pathway [120]. Furthermore, Luo et al. observed that human gingival tissue-derived MSC (GMSC) inhibit osteoclast formation in vitro and in vivo partially via CD39-CD73-adenosine signals [109]. GMSC markedly suppressed human or mouse osteoclastogenesis and resulted in a dramatically decreased level of NF-κB p65/p50 in osteoclasts in vitro. Infusion of GMSC to CIA mice significantly attenuated the severity of arthritis, pathology scores, and frequency of osteoclasts, particularly bone erosion, as well as a decreased expression of RANKL in synovial tissues. Blockade of CD39/CD73 or adenosine receptors has significantly abrogated the suppressive ability of GMSC in vitro and the therapeutic effect of GMSC on bone erosion in CIA mice. Besides, Chang et al. demonstrated that AD-MSCs can inhibit RANKL-induced osteoclast genesis via CD39 signals and can inhibit osteoclast genesis without the involvement of Tregs [121]. AD-MSCs dramatically decreased the levels of NF-κB p65/p50 in osteoclasts in vitro and P65/50 and RANKL expression by synovial tissues in CIA mice.

Interestingly, in vitro culture of mouse CD11b (+) monocytes on MSC layers in the presence or absence of macrophage colony-stimulating factor (M-CSF) and RANKL prompted MSCs to independently support osteoclast development, and this effect was enhanced by M-CSF and RANKL. Co-culture of MSCs with osteoclasts in the presence of high concentrations of osteoclast-inducing factors, which reflected inflammatory pathology in vivo, prompted MSCs to exert an osteoclastogenic suppressive effect. MSCs have a dual effect on osteoclasts by stimulating or inhibiting osteoclastogenesis, depending on the inflammatory milieu, which might shed light on understanding the involvement of MSCs in the inflammatory diseases [122].

In summary, MSCs play a crucial role in inhibiting the destruction of bone and cartilage by reducing the formation of osteoclasts. How to enhance the tissue protection role of MSCs in the RA inflammatory microenvironment needs to be developed in the future.

Strategies to improve the therapeutic effect of MSCs for RA and their challenges in clinical application

Although the therapeutic effects of MSCs have already been confirmed. However, differences in migration and homing ability, graft survival, donor-MSC potency in the pathological microenvironment (e.g., hypoxia, oxidative stress, and inflammation), and cellular heterogeneity have led to vastly different therapeutic outcomes of transplanted MSCs. Several strategies have been attempted to optimize the potency and therapeutic benefits of MSCs, which roughly include bioengineering MSCs through genetic engineering and biomaterial strategies engineering [123], pre-activation of MSCs [124], co-administration strategies [125, 126], and cell-free therapy [127].

Bioengineering MSCs

Gene modification of MSCs

Gene modification of MSCs through viral or non-viral vectors to induce the overexpression of functional proteins and soluble factors that enable MSCs to increase stemness, differentiation, migration and homing capacity, immunoregulation, and other repair-related abilities in vitro and in vivo, as well as to resist hostile microenvironments and apoptosis. A series of genes related to the therapeutic abilities of MSCs are utilized to increase cell survival and therapeutic effect; among them, COX-2 silence and TGF-β3 overexpression in human BMSCs and gain of CXCR7 function in human BM-MSCs have demonstrated improved therapeutic effects in arthritis

animal models through promoting the osteogenic and chondrogenic differentiation of MSCs and MSC-mediated immunomodulation [128, 129]. Other genes, IL-10, silent information regulator 2 type 1, TGF-β1, and bone morphogenetic proteins-2, were overexpressed in human amniotic MSCs and showed attenuated CIA progression and suppressed Th17 cell activation in CIA mice, while increasing proteoglycan expression in cartilage and decreasing the infiltration of inflammatory cells and factors in the joint tissues [82, 130–132].

Besides, Zhao et al. constructed a strain of sTNFR2-Fc-expressing MSCs (sTNFR2-MSCs) that protect MSCs against apoptosis/autophagy induced by TNF-α, in addition to releasing sTNFR2-Fc neutralizing TNF-α to block the relevant immune-inflammation cascade, and thus effectively alleviate CIA mice via migrating to the affected area, protecting articular cartilage destruction [133]. Choi et al. generated human AD-MSCs highly expressing CTLA4-IgG (CTLA4Ig-hASCs) [134], which decreased T-bet and GATA binding protein 3 expression in the CIA mice splenocytes and increased the ratio of Treg and Th17 cells more significantly than wild AD-MSCs. In addition, Kim et al. epigenetically modified human MSCs (Epi-hMSCs) with hypomethylating agents or HDAC inhibitors and intervened in synovial fluid mononuclear cells (SFMCs) from RA patients [135]. Epi-hMSCs significantly reduced the levels of IL-17 and IFN-γ secreted by SFMCs, and they also had a greater immunosuppressive effect on T-cell proliferation, cytokine expression, and Th17 cell differentiation.

However, viral transfections have high transfection efficiencies but are associated with high production costs and a higher risk of chromosomal instability, insertional mutagenesis, and proto-oncogene activation despite the inherent high transfection efficiency [136]. Non-viral transfections present transient gene expression and low transfection efficiency. Meanwhile, all aspects of non-viral gene transfer must be optimized, such as the construction of efficient plasmids and improved transfection protocols. All those hinder its clinical application.

Biomaterial strategies for engineering MSC

Improve the adaptation of mesenchymal stem cells to their environments The therapy capability of MSCs is influenced by inflammatory microenvironments, so it is important to reduce the influence of inflammatory microenvironments on MSCs and promote osteogenic and chondrogenic differentiation of MSCs. Recent advances in biomaterials have contributed to elongating the effective durations in clinical treatment of MSCs by offering a scaffold for the adherence and survival of MSCs, as well as preserving the functional components MSCs secreted. Liu et al. indicated that encapsulated BM-MSCs with thermosensitive hydrogels provide a relatively stable space for adhesion and proliferation of BM-MSCs [137]. The combination therapy of microfracture and in situ transplantation of thermogel-encapsulated BM-MSCs in CIA rats obviously down-regulated the ratio of $CD4^+$ to $CD8^+$ T lymphocytes in peripheral blood, inhibited the proliferation of antigen-specific lymphocytes and local joint inflammatory conditions, and prevented articular cartilage damage. Lu et al. developed nanoparticles (NPs) modified MSCs via engineering MSCs by actively MSC-targeting VQ-CuS@MnO_2/Metformin NPs with superoxide dismutase- and catalase-like activity [138]. The NP-modified MSCs enhanced the biological properties of stem cells desired in stem cell therapy, including cell migration, anti-inflammation, chondrogenesis, improved survival under RA-associated oxidized stress, and relieved RA symptoms in CIA and adjuvant-induced arthritis (AIA) rat models through effectively inhibiting synovial inflammation and reducing cartilage erosion. In addition, Shin et al. designed inflammation-targeting MSCs conjugated with triamcinolone-loaded gold nanostars (Edu-MSCs-AuS-TA) to enhance the migration efficacy and anti-inflammatory activity of MSCs [139]. Edu-MSCs-AuS-TA significantly alleviate arthritis-associated pain and improve general locomotor activity. More importantly, Edu-MSCs-AuS-TA greatly promoted cartilage regeneration with repolarization of macrophages from the M1 to M2 phenotype and inhibited neutrophil recruitment, even for severe stages of the arthritis model.

The therapeutic efficacy of MSCs is threatened by the accumulated reactive oxygen species (ROS) and poor oxygen supply. Zhao et al. developed a nanozyme-reinforced hydrogel as an H_2O_2-driven oxygenerator to reshape the hostile RA microenvironment and improve prosthetic interface osseointegration [140]. The nanozyme-reinforced hydrogel encapsulated with BMSCs alleviated the symptoms of RA, including suppression of local inflammatory cytokines and improvement of osseointegration. In addition, Koo et al. developed a nanovesicle system of MSCs containing ceria, which, as an antioxidant for scavenging ROS, has been used in RA treatment [141]. The system showed both the antioxidant properties of ceria and the immunomodulatory properties of MSCs and successfully treated and prevented rheumatoid arthritis by relieving the main symptoms and also by restoring the immune system through the induction of Treg in a mouse model of CIA.

Improve the homing and survival ability of mesenchymal stem cells Due to MSCs being significantly diluted in the blood after intravenous administration and remaining in the pulmonary vessels, fewer cells reached the treatment site. To improve their homing efficiency, Zhao et al. constructed a bioengineered composite scaffold for RA

management through integration of 3-dimensional (3D) printed porous metal scaffolds (3DPMS) and infliximab-based hydrogels [142]. The infliximab-based hydrogels can enhance AD-MSCs survival, engraftment, and function in aiding RA management, like reducing cartilage damage and improving repair effects. Zhu et al. proposed a novel ECM-inspired injectable hydrogel for AD-MSCs encapsulation and RA treatment [143]. AD-MSCs-laden hydrogel has tremendous therapeutic outcomes, including inflammation attenuation, cartilage protection, and bone mineral density promotion in CIA rat model. In addition, Shi et al. encapsulated MSCs in alginate hydrogel to improve cell survival and retention in situ, maximizing efficacy in vivo [100]. Encapsulated MSCs significantly decreased TNF-α and TFN-γ expression in DCs in vitro and promoted the phenotypic transformation of DCs to tolDCs and alleviated arthritis in the CIA mouse model.

However, the implantation of biomaterials could induce foreign-body responses in the host immune system, which can potentially result in fibrosis and failure of the implantation. Besides, the uneven distribution of oxygen and nutrients in the 3D spatial structure will also affect stem cells.

Pre-activation of MSCs

MSCs can be pre-activated to achieve the desired function and reverse their inactivation because they can recognize the stimuli in the microenvironment and remember them [90]. Different attempts have been made to improve the efficacy of MSCs by modifying the culture conditions, including changing the culture media with additives, such as inflammatory cytokines, growth factors or regenerative cytokines, bioactive compounds, the disease-associated effector cells or patient's serum, and other conditions, such as hypoxia, 3D culture, photostimulation, magnetoelectric stimulation, and heat shock [124].

Pre-activation of MSCs with cytokines

IL-1β stimulated MSCs not only increased macrophage polarization into M2 macrophages and enhanced apoptosis of M1 macrophages in vitro and rehabilitated the imbalance of the M1/M2 ratio and reduced inflammation in RA mice [144], but also induced apoptosis of fibroblast-like synoviocytes in CIA mice [113]. Soluble IL-6R-pretreated human BM-MSCs promoted differentiation of MSCs into chondroblasts and articular cartilage repair. AIA rats implanted with poly-lactic-co-glycolic acid and sIL-6R-treated MSCs showed similar knee joint imaging to sham rats using x-rays [145]. Epigallocatechin-3-gallate (EGCG) pretreated AD-MSCs synergistically enhanced the neuroprotective ability of AD-MSCs to repress the negative effects of RA on the brain by ameliorating inflammation and apoptosis in brains of RA and reactivating RA-induced repression of the PI3K/Akt survival pathway [146].

Pre-activation of MSCs with 3D culture

It is well known that the 3D method in MSC enhanced the therapeutic potential of MSCs. 3D spheroid culture systems, the simplest method for 3D culture, can benefit the therapeutic potential of MSCs through increasing stemness and facilitating differentiation into different cell lineages [147], enhancing proliferation, migration, and homing efficiency [148], promoting the secretion of therapeutic factors including immunomodulatory factors [149–151], and pro-angiogenic cytokines [152]. Furthermore, it was shown that both MSC spheroids and MSCs from spheroids more effectively suppress TNF production by LPS-stimulated peritoneal macrophages in vitro and inflammatory reactions in an in vivo mouse model of zymosan-induced peritonitis [149, 150]. Ueyama et al. demonstrated that AD-MSC spheroids injected intra-articularly into the knees of RA mouse models reduced intra-articular inflammation and facilitated regenerate damaged cartilage [153].

Pre-activation of MSCs with disease-associated effector cells or their released active substances

Now, disease-specific pre-activation that directs the use of disease-associated effector cells or their released active substances to pre-activate MSCs is another pre-activation method that can accurately target the main pathogenic factors in disease development and react and respond quickly in vivo to achieve more efficient therapeutic effects. Several studies have demonstrated that pre-activated MSCs with mast cells (MCs) and stroke serum significantly improved the therapeutic effects of MSCs [154, 155]. MCs are tissue-resident cells of the innate immunity and present in synovia, and their activation has been linked to the potentiation of inflammation in the course of RA [156]. Therefore, pre-activated MSCs with MCs may be an effective approach for RA treatment.

Notwithstanding, the application of the pre-activation of MSCs confronted several challenges: (1) choosing the best sources of MSCs. MSCs from different tissue sources respond differently to the same pretreatment; (2) choosing reasonable and effective MSC pre-activation methods. Each pretreatment targets improving a specific aspect of MSCs, and finding the best pre-activation way is very important; (3) developing standard platforms for evaluating the safety of pre-activated MSCs. The long-term effect of priming MSCs and the epigenetic modifications, immunogenicity, and tumorigenicity of primed and non-primed MSCs have not been evaluated; (4) developing the appropriate GMP standards for quality control of pre-activated MSC products. The

quality of cryopreserved primed-MSCs at different passages and selecting which one component of the secretome of MSCs as an indicator of both pre-activation and therapeutic efficacy of educated MSCs have not been determined. Therefore, based on the pathological characteristics of the disease and the type of MSCs used for treatment, the optimal pre-activation method is selected to promote the MSC proliferation, survival, and migration of MSCs; enhance MSC paracrine and immune regulatory abilities; inhibit MSC aging; and reverse and repair damaged MSCs.

Co-administration strategies

Since MSC is susceptible to receiving the influence of the pathological microenvironment of RA patients, like inflammatory and oxidative stress, the combined use of co-administrative assistant substances that improve the pathological microenvironment of patients during MSC treatment can help to improve the therapeutic effect of MSC.

Co-administration with immune cells

At present, new immunotherapy by combining immune cells and MSC has been developed. Li et al. developed new immunotherapy by tolerogenic DC (Tol-DCs) and MSCs and discovered that administration of RelB gene-silenced Tol-DC and MSC in CIA mice improved clinical symptoms, decreased clinical scores, and attenuated joint damage, which was associated with suppression of CII-specific T cell responses, polarization of Th and inhibition of proinflammatory cytokines, and reduced cartilage degeneration [126]. Lim et al. demonstrated that a combination of MSCs and Tr1 cells prevented the development of destructive arthritis compared to single-cell therapy through increased type II collagen (CII)-specific $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells and inhibition of CII-specific $CD4^+$ $IL\text{-}17^+$ T cells [125]. Tr1 cells produce high levels of IL-10-dependent IFN-β, and production of IFN-β and IL-10 in Tr1 cells synergistically induces IDO in MSCs through the STAT1 pathway. These studies indicated that co-administration of MSCs and immune cells is a novel therapeutic modality for RA.

Co-administration with cytokines

The immunomodulatory functions of MSCs are not constitutive; instead, they are induced by inflammatory cytokines, such as IFN-γ, in the presence of one (or more) other cytokine(s), including TNF, IL-1α, or IL-1β, in the inflammatory microenvironment [88, 157]. Xu et al. demonstrated that the combination of hUC-MSCs transplantation with IFN-γ treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis [68]. In the murine studies, wild-type MSC transplantation significantly improved the clinical severity of CIA, while IFN-$\gamma R^{-/-}$ MSC transplantation aggravated synovitis and joint and cartilage damage. A phase 1/2 randomized controlled trial study demonstrated that MSC + IFN-γ combination therapy significantly increased the efficacy (good or moderate EULAR response) and ACR20 response rates at the third month of follow-up. In addition, Haikal et al. demonstrated that combined BM-MSC and IL-4 treatment reduced joint inflammation, synovial cellularity, levels of pro-inflammatory cytokines, vascularization, and bony destruction and improved biochemical markers in CIA model [158].

Co-administration with antioxidants

The survival rate of MSCs after transplantation and the affected efficacy are threatened by oxidative stress microenvironments and ROS production at RA patients' inflamed joint sites [159]. The combined use of antioxidants in MSC therapy neutralized the oxidative microenvironments and improved the anti-stress ability of MSCs [159]. Abd-Elhalem et al. confirmed that combined therapy of BM-MSCs with hesperidin (HSD) promotes RA therapy [160]. HSD is a prominent flavanone found in citrus fruits that has a broad range of biological effects, including anti-inflammatory and antioxidant capabilities [161]. In the AIA rat' model, BM-MSCs combined with HSD significantly decreased IFN-γ and increased TGF-β levels in spleen tissue and improved arthritis severity compared with the treatment of MSCs alone [160].

Co-administration with traditional Chinese medicine

In recent years, traditional Chinese medicine or its extracts, including Tripterygium wilfordii [162], paeoniflorin and total glucosides of paeony [163], and dendrobium huoshanense stem polysaccharide [164], in the treatment of RA has made remarkable achievements. Its mechanism of treating RA mainly involves these aspects: anti-inflammation, anti-oxidation, immune regulation, pro-apoptosis, inhibition of angiogenesis, inhibition of osteoclastogenesis, and inhibition of FLS proliferation, migration, and invasion [165]. Qi et al. have shown that Cervus and Cucumis peptide (LG) combined with UC-MSC synergistic treatment can significantly improve the curative effect of RA patients, including improved clinical curative effect and reduced side effects [166]. Also, an in-vitro cell study indicated that LG could enhance the immune regulatory function of UC-MSC by promoting the secretion of HGF, PGE2, and TSG-6 anti-inflammatory factors in a concentration-dependent manner. Ahmed et al. have shown that curcumin combined with BM-MSCs is effective at reducing inflammation in Freund's Adjuvant-induced Arthritis by restoring the high serum PGE2 and IL-17 levels and lowering the IL-13 level to normal levels and increasing the gene expression of COX-1 and IL-6 and decreasing IL-4, while also having

beneficial effects on the ankle joint, thymus, and spleen [167]. In addition, curcumin and BM-MSCs have protective effects on the testes and ovaries via their anti-inflammatory and immunomodulatory potentials as well as oxidative stress modulatory effects [168]. Therefore, co-administration strategies are a convenient way to apply for clinical use because they do not require additional preparatory steps.

Like pre-activation strategies, co-administration therapy strategies also face issues such as choosing cell sources, choosing combination substances to be used in combination, and developing safety and quality rating standards.

Cell-free therapy based on MSCs

EVs are important paracrine factors secreted by MSCs. EVs are membrane-bound vesicles that include apoptotic bodies (50–4000 nm), microvesicles (MPs) (100–1000 nm), and exosomes (Exos) (40–100 nm) [169]. MSC-derived EVs (MSC-EVs) display immunoregulatory functions similar to the parent MSCs. Both human UC-MSCs and the EVs secreted by them inhibited the proliferation of T cells, promoted T cell apoptosis, decreased RORγ levels, increased Foxp3 levels, and regulated the balance of Treg/Th17 cells in in vitro and in vivo experiments, resulting in delayed radiological progression and synovial hyperplasia inhibition [95].

Accumulating evidence shows that small extracellular vesicles derived from MSCs have therapeutic effects on RA by restoring macrophage balance and regulating the balance of Treg/Th17 cells [170, 171]. Another study demonstrated that MSC-derived microparticles and Exos exert similar immunosuppressive functions by decreasing T and B lymphocyte proliferation and inducing Treg cell populations independently of MSC priming in vitro, and Exos were more efficient in suppressing clinical signs of inflammation in CIA models, which was associated with fewer plasmablasts and more B regulatory cell-like cells in lymph nodes [172]. In a model of porcine synovitis induced by bovine serum albumin demonstrated that the intra-articular injection of BM-MSC-EVs into pigs had an anti-inflammatory effect, with a reduced number of synovial lymphocytes and down-regulated level of TNF-α transcription [173]. Gingival MSCs derived Exos (GMSC-Exo) not only ameliorated inflammation and bone erosion of the metatarsophalangeal joint in CIA mice via inhibiting the IL-17RA-Act1-TRAF6-NF-κB signal pathway [174], but also effectively inhibited the invasive destructive properties of RA synovial fibroblasts as well as the potential for these cells to migrate to secondary locations and attack the cartilage in a chimeric model of RA [175]. Several studies have indicated that MSC-Exos contain miRNAs such as miRNA-148a [176], miR-205-5p [177], miR-223 [178], miRNA-320a [179], miR-378a-5p [180] and miR-451a [181] ameliorate rheumatoid arthritis. In addition, Exos produced by MSCs could be modified to improve their therapeutic effects on RA. MSC-derived Exos with overexpressed miRNA-124a [182], miR-146a/miR-155 [183], and miR-150-5p [184] have been proven to inhibit the proliferation and migration of RA-FLSs. Exos derived from fibrinogen-like protein 1-overexpressing inhibited the cell viability while increasing the cell apoptosis in RA-FLSs and suppressing the inflammation score, joint destruction, and inflammatory response in the RA rat model [185].

Moreover, EVs derived from preconditioned MSCs show enhanced therapeutic effects on RA. Kay et al. evaluated the therapeutic potential of EVs isolated from MSCs cultured normoxically (21% O_2, 5% CO_2), hypoxically (2% O_2, 5% CO_2) or with a pro-inflammatory cytokine cocktail in an AIA model, and they found all EV treatments reduced knee-joint swelling, while only normoxic and pro-inflammatory primed EVs improved histopathological outcomes [186]. EVs from IFN-β-primed BM-MSCs, compared to EVs from unlicensed BM-MSCs, more efficiently inhibit RA-FLSs migration and expression of RA-FLSs-related surface markers [187]. RA disease serum primed AD-MSCs resulted in a higher production of Exos, which ameliorated cartilage damage in an RA model by enhancing TGF-β1 production, inducing Th2 and M2 polarization, and lowering proinflammatory cytokines TNF-α, KC, and IL-12p70 in the host [188]. 3D-primed UC-MSC secretome has a significantly higher therapeutic potential than 2D-primed UC-MSC secretome and even UC-MSCs in an AIA model. A proteomic analysis was performed on both media conditioned by UC-MSC monolayer (CM2D) and 3D cultures (CM3D). CM3D was characterized by a prevailing expression of anti-inflammatory cytokines such as IL-10 and LIF, along with trophic factors involved in different mechanisms leading to tissue regeneration, such as PDGF-BB, FGF-2, I-309, SCF, and GM-CSF; CM2D presented relatively higher levels of IL-6, MCP-1, and IL-21 [189].

EVs, as natural nanomaterials, due to good biocompatibility, accurate targeting, low toxicity, and immunogenicity, act as a new generation of drug delivery systems, selectively delivering therapeutic drugs to the site of inflammation through interactions between their surface antibody or modified ligand with cell surface receptors. MSC-derived Exos loaded with curcumin not only improved the stability of free drugs but also effectively inhibited the proliferation and inflammatory response of synovial fibroblasts by reducing the levels of anti-apoptotic proteins IAP1 and IAP2 and inflammatory factors such as IL-6 and TNF-α [190]. The fine surface editing of MSC-Exos by the metabolic glycoengineering of AD-MSCs systemically administered into CIA mouse effectively accumulated in the inflamed joints and promoted

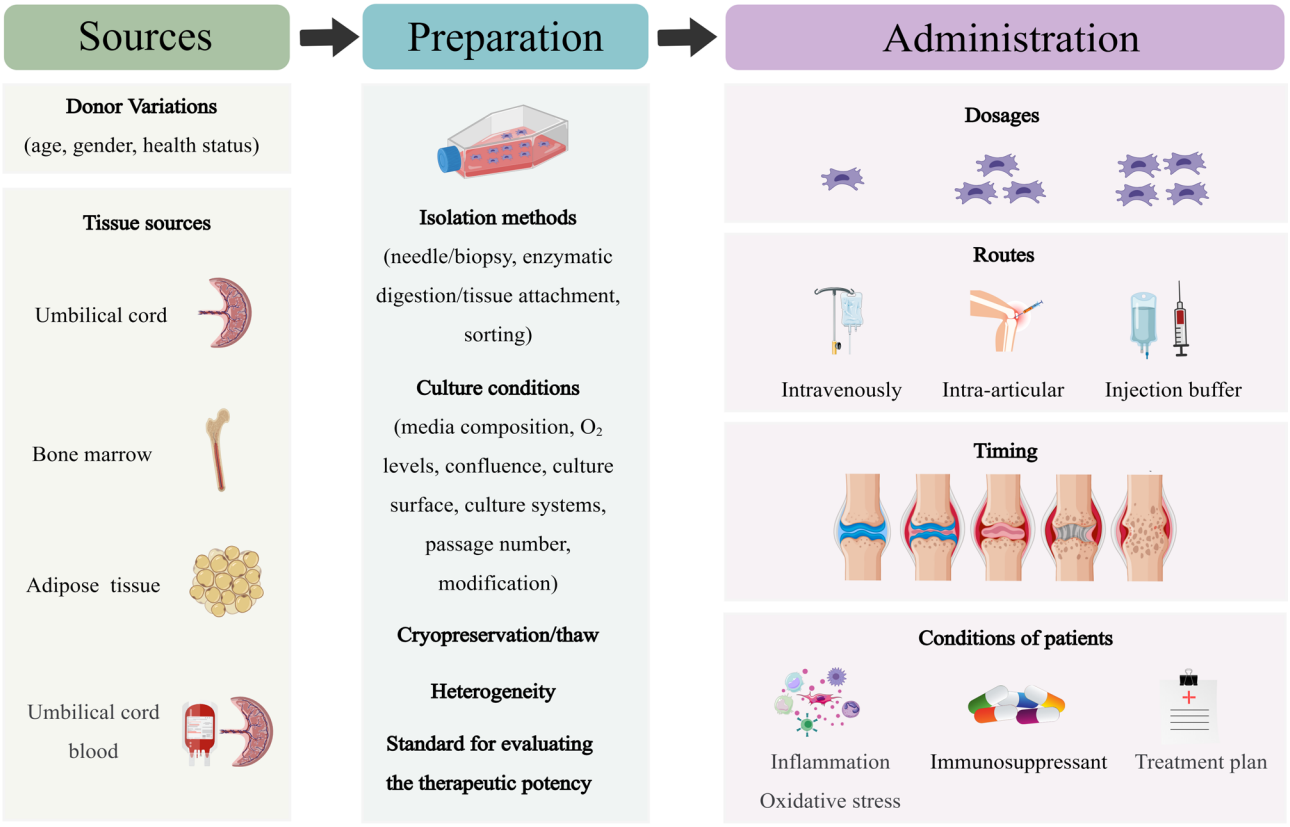

Fig. 6 The parameters need to be standardized precisely in clinical application of MSCs in RA. Created with MedPeer (medpeer.cn)

the polarization of macrophages from M1 to M2 in their inflamed sites and decreased peripheral proinflammatory cells (M1 macrophages, activated synovial fibroblasts) [191].

Currently, to maintain the biological activity of Exos and allow a controlled release, these paracrine factors can be encapsulated in biomaterials. Silk fibroin hydrogel encapsulated with olfactory ecto-MSC-derived Exos (Exos@SFMA) was photo-crosslinked in situ to yield a long-lasting therapeutic effect on modulating the immune microenvironment in RA [192]. This in situ hydrogel system exhibited flexible mechanical properties and excellent biocompatibility for protecting tissue surfaces in joints. The exosomes released from Exos@SFMA successfully inhibited Tfh cell polarization by expressing PD-L1 to down-regulate the phosphatidylinositol 3-kinase/AKT pathway in T cells. Exos@SFMA effectively relieved synovial inflammation and joint destruction by significantly reducing Tfh cell response and further suppressing the differentiation of germinal center B cells into plasma cells.

Compared with MSCs, the EVs derived from them are more targeted, smaller in size, and can cross the blood–brain barrier to better exert immune regulation and anti-inflammatory functions. However, there are still many challenges in the clinical application of MSC-EVs: (1) providing therapeutic doses of EVs to target sites. The circulatory half-life of EVs is short, and systemic injection of exosomes could be rapidly cleared by blood circulation; (2) different storage conditions affect the activity of EVs. After repeated freezing and thawing at − 80 °C, EVs partially aggregated or fused, reducing the total number of EVs and active substances; (3) the heterogeneity of EVs. The process of EVs secreted by MSCs and modification of EVs have a huge impact on their therapeutic capacity. Therefore, exploring the optimal dosage to be administered based on the disease's own characteristics is crucial for the application of EVs.

Standards need to be developed for clinical applications

Over the past decades, MSC-based research and therapy have made tremendous advancements due to their advantages, including immune evasion, diverse tissue sources for harvesting, ease of isolation, rapid expansion, and function of tissue repair and immune regulation. The slow progress in MSC product development may be due to a lack of some precise standards. As shown in Fig. 6, there are many key parameters influencing the efficacy of MSCs, including cell quality, dosages, routes, and condition of patients, that must be standardized to promote the clinical application of MSCs.

Cell quality

It is necessary to ensure the quality of MSC products by selecting appropriate cell sources, optimizing the cell preparation process including isolation methods, culture conditions (media composition, O_2 levels, confluence, culture surface and culture systems, passage number, modification), cryopreservation and thawing, and developing standard MSC quality assays. All of these affect the viability, heterogeneity, and biological efficacy of MSCs. Multiple reports have analyzed the impact of cell source and preparation process on cell quality and heterogeneity [193–195]. Taken together, it is necessary to determine what therapeutic role MSCs need to play based on the pathologic characteristics of the disease and then select the appropriate medium and culture system to reduce MSC heterogeneity and ensure MSC viability and biological potency. MSCs need to be alive and carry through lung clearance to maximize their therapeutic role.

In RA, from the registered clinical trial data, MSCs derived from UC, BM, AD, and UCB have been used, with UC-MSCs being the most commonly used (Fig. 3C). This is probably because UC-MSCs are relatively easy to obtain without ethical constraints, can be obtained in large numbers, and have a high capacity for proliferation and differentiation. In addition, allogeneic transplantation is more than autologous transplantation (Fig. 3D). Therefore, UC-MSCs transplantation seems to be the most suitable option the treatment of RA.

Routes of administration

In registered clinical trials, the routes of administration selected for MSC transplantation are IV and IA injection, primarily the IV (Fig. 3E). IV injection is most often used in therapy because of its ease of administration, low invasiveness, and reproducibility, but most of the infused MSCs accumulate in the lung and are cleared by the immune system [196]. It reported that only about 28% of MSCs survived after one day [197], and fewer than 1% of MSCs persist more than a week [196]. IA injection can deliver MSCs faster and avoid off-target effects. However, the oxidative stress microenvironments and excessive ROS, which mainly derived from macrophages and neutrophils at RA patients' inflamed joint sites, significantly reduced the survival rate of MSCs [159]. Therefore, it is difficult to determine which method of administration is optimal and needs to be based on the patient's comprehensive condition.

Dosages of administration

In the clinical research and application of MSC, cell dose may be one of the most absurd and least scientific links, with the number of MSCs used per patient ranging from four thousand to hundreds of millions of MSCs. MSC transplantation is performed according to patient body weight in most registered clinical trials ($1–4x10^6$/kg), some administer MSCs according to the quantity of cells (totally $2.5x10^7–5x10^8$ cells), some have not posted the data (Fig. 3F). Based on the published data, 3 clinical trials designed different doses of administration regimens (NCT01663116, NCT02221258, NCT01851070). However, they concluded that there was no evidence of dose-related toxicity over the dose range and time period studied but did not draw valid conclusions about the relationship between dose and efficacy. The results of NCT01663116 showed clinical benefit achieved in patients with RA treated with intravenous AD-MSCs tends either to wane or fluctuate after 3 months of cell administration [61].

Most registered clinical trials transplant MSCs in one dose, while others transplant MSCs in several doses, ranging from two to five doses (Fig. 3G). Among them, only one (NCT01547091) has finished and posted the date. But the results of NCT01547091 have no conclusion about the effect of single and multiple dosing on efficacy. In an animal model, Yu et al. declared that long-interval repeated intravenous administration of hUCB-MSCs (every 2 weeks for three times) has therapeutic effects by improving symptoms of RA in a CIA mouse model in a dose-dependent manner [97]. However, An et al. declared that frequent injections of high-dose UC-MSCs (5×10^6 cells per week for 3 weeks) slightly aggravated synovitis and muscle cachexia in the murine CIA model and should therefore be avoided in the treatment of arthritis. Based on these studies, several injections with long intervals and repeated administrations are probably beneficial for improving the therapeutic effect of MSCs.

Patient condition

One thing that makes cell-based drugs very different from traditional chemical drugs is that cells are alive, whereas chemical drugs are dead. MSCs, as living cells, enter the organism and are bound to interact with the microenvironment within the organism. The patient's inflammatory state and changes in the tissue microenvironment, such as hypoxia and extracellular matrix state, are important factors that influence the efficacy of MSCs.

The properties of MSCs could be affected by their microenvironment to a great extent, including migration, differentiation, anti-oxidative stress, and immunoregulation. Xu's team found interferon (IFN)-γ is a key factor in determining the efficacy of MSC transplantation in the treatment of RA. In 2018, they proposed that high serum IFN-γ levels are a potent biomarker for predicting the therapeutic effect of MSC transplantation in active RA [65]. In 2020, they used murine studies and clinical studies to demonstrate that IFN-γ is a key factor in determining the efficacy of MSC transplantation in the treatment of RA and that an MSC

plus IFN-γ combination therapeutic strategy can greatly improve the clinical efficacy of MSC-based therapy in RA patients [68]. In murine studies, wild-type mouse BM-MSC transplantation significantly ameliorated the severity of CIA mice, including that of joint synovitis and articular and cartilage destruction, with a high level of endogenous IFN-γ, but these therapeutic effects were not observed with IFN-γR-/- MSC transplantation. In a phase 1/2 randomized controlled study, the 3-month follow-up results showed that the efficacy and ACR20 response rate of MSC transplantation plus IFN-γ combination therapy was 93.3%, while that of MSC transplantation was 53.3%. MSC plus IFN-γ combination therapy significantly improved clinical symptoms and disease activities, including DAS28-ESR, the Health Assessment Questionnaire-Disability Index, ESR and CRP values, and RF level, compared with MSC transplantation monotherapy. In addition, the MSC plus IFN-γ combination therapy rapidly increased the regulatory T cell (Treg)/T helper type (Th)17 cell ratio in RA patients, which indicated an enhancing activity of Tregs and benefited the treatment of RA. Their study clarified the key role of circulating IFN-γ and improved the clinical efficacy of MSC transplantation in RA patients. It is hoped that the pro-inflammatory and anti-inflammatory ability of MSCs can be regulated in the future to achieve better and more accurate therapeutic effects. Therefore, how to regulate and evaluate the immunomodulatory ability of MSCs is an important issue that needs attention when studying the immunomodulatory mechanism of MSCs.

Besides, the oxidative stress microenvironments and excessive ROS production, which mainly derived from macrophages and neutrophils in RA patients' synovial cavity, significantly reduced the survival rate of MSCs after transplantation and affected the efficacy [159]. It has been reported that the combined use of antioxidants, such as HSD in MSC therapy, promotes RA therapy [160]. Several studies have proved that bioactive compounds that acted as excellent anti-inflammatory and antioxidant agents ameliorated arthritis severity, such as dehydrozingerone, xanthorrhizol, and ligustrazine [198–200]. Interestingly, they are part of Chinese traditional medicine ingredients. Therefore, combined use of anti-inflammatory and antioxidant agents, especially traditional Chinese medicine, to ameliorate the inflammatory and oxidative stress environment in RA patients seems to be an effective way to improve the therapeutic effect of RA.

In addition, the patient's immune microenvironment and immunomodulatory plasticity of MSCs were also affected by the immunosuppressive agent. Prochymal, an allogeneic BM-MSC product for GvHD treatment, has no therapeutic efficacy in GvHD patients when combined with steroids [201]. Dexamethasone has been shown to affect the immunoregulatory capacity of MSCs by inhibiting the expression of inducible nitric oxide synthase and IDO [202]. These studies suggest that MSC treatment should be avoided in combination with immunosuppressive agents.

In general, combining the comprehensive conditions of patients and the therapeutic role of MSCs, we can assess patients' suitability for MSC therapy before MSC transplantation. At the same time, we can employ appropriate solutions to eliminate the influence of patients themselves on MSC treatment. For example, grouping patients based on their disease severity/stage of development, narrowing down the indications, and accurately recruiting patients who can benefit from MSC therapy. Alternatively, pre-treat patients to modify the tissue microenvironment in the patient's body to improve the potency and responsiveness of MSCs. Alternatively, the "natural properties" of MSCs can be enhanced through bioengineering solutions, such as engineering and pre-activation.

Conclusion

Involvement in the immunopathogenesis of RA is not a single point but a complex network. Blocking individual immune cells, signaling pathways, and cytokines may not always achieve the desired results, or many targeted therapies may help to break through the therapeutic dilemma, that is, regulate the immune balance in the body by inhibiting multiple targets of cytokines, signaling pathways, and immune cells. Thus, a challenging goal is to modulate the autoimmune system, induce remission of inflammation with permanent immune tolerance, prevent ongoing deterioration of joint structures, and repair existing damage.

MSCs are the most promising candidates for RA therapy due to their many advantages. Some satisfactory outcomes in RA have been observed in clinical trials, and multiple strategies have been proposed to enhance the therapeutic effects of MSCs, including bioengineering, pre-activation, co-administration strategies, and cell-free therapy. Further studies are needed to determine the efficacy and safety of these strategies to decide whether they can be used in the clinic. Besides, various parameters need to be optimized from cells to recipients, including the quality of MSCs, the routes and dosages for MSC administration, and patient condition. Patient condition, which is much easier to ignore, is an important factor that affects the outcome of MSCs. Therefore, more work needs to be done in the future to advance the development of MSC therapy.

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The authors declare that they have not use AI-generated work in this manuscript.

Author contributions
Y-F. G. and N. Z. screened the literature, designed the table and drafted the manuscript; C-H. H. revised the manuscript and supervised this study. All authors read and approved the final manuscript.

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Data availability
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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