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Mesenchymal stem cells combined with IFN-γ treatment versus mesenchymal stem cells monotherapy: safety and efficacy over five years extension follow-up

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Abstract

Background To report long-term safety and efficacy of mesenchymal stem cells (MSCs) in combination with and without IFN-γ in patients with rheumatoid arthritis (RA), using pooled data from two randomised clinical trials followed by long-term extension (LTE) study.

Methods Cumulative data from two phase 1/2 core trials and their LTE studies were analysed. Safety variables assessed included treatment-emergent adverse events (AEs), serious AEs (SAEs) and laboratory results. Efficacy assessments included ACR20/50/70 responses, Disease Activity Score 28 < 2.6 (remission) and ≤ 3.2 (LDA, low disease activity).

Results A total of 110 patients received MSCs monotherapy and MSCs combined with IFN-γ treatment. Event rates per 100 patient-years in MSCs monotherapy group and MSCs combined with IFN-γ treatment group, respectively, were 2.47 and 2.31 for SAEs. No increase in the rate of any AE was observed over five years. Clinical response rates remained stable during the LTE study. Initial improvements in LDA/remission observed at year one were sustained over five years of follow-up in both groups, while the MSCs combined with IFN-γ treatment group had higher ACR20 and LDA rates than the MSCs monotherapy group at both one year (100% versus 50.7%, $p < 0.001$; 39.3% versus 8.7%, $p < 0.001$) and five years (89.3% versus 44.9%, $p < 0.001$; 42.9% versus 8.7%, $p < 0.001$).

Conclusion The long-term safety and efficacy of MSCs with/without IFN-γ combination therapy remained stable. The efficacy of MSCs was maintained through at least five years. These findings support MSCs as a treatment option for patients with active RA.

Trial registration ChiCTR-ONC-16,008,770 (2016-07-03) and ChiCTR-INR-17,012,462 (2017-08-24).

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Highlights

- Mesenchymal stem cells (MSCs) have been shown to be effective in two phase 1/2 randomised clinical trials in adults with active rheumatoid arthritis (RA) who had an inadequate response to or were intolerant of conventional treatments, including csDMARDs, NSAIDs, bDMARDs, and steroids.
- This LTE study confirmed that MSCs monotherapy provides a sustained favorable safety and efficacy profile, and combination therapeutic strategy of MSCs plus IFN-γ greatly improve the clinical efficacy of MSC-based therapy in active RA.
- MSCs based cell-therapy is a new treatment option for non-responding or intolerant RA patients to conventional treatments. The long-term safety and efficacy profile was attributed to MSC-mediated immunoregulation. This should be considered in the future management of patients.

Keywords Rheumatoid arthritis, Mesenchymal stem cell, Interferon gamma, Long-term extension study

Background

Although the pathogenesis of rheumatoid arthritis (RA) is not yet fully understood, updated studies have suggested that various pro-inflammatory cytokines and immune cells play significant roles in its development and progression [1–3]. Despite great medical advances in RA therapeutic drugs, only a minority of patients can achieve disease remission and persistent remission off therapy [4].

Numerous clinical studies have employed allogeneic mesenchymal stem cells (MSCs), primarily derived from adult bone marrow, adipose tissue or umbilical cord (UC), for treating various clinical diseases [5]. The unique anti-inflammatory and immunosuppressive properties of MSCs have led to their widespread application in autoimmune diseases, garnering widespread recognition for their efficacy and short-term safety [6]. Over the past decade, MSCs transplantation (MSCT) has been attempted in systemic lupus erythematosus, Sjogren's syndrome, systemic sclerosis, graft-versus-host disease, and dermatomyositis/polymyositis, demonstrating satisfactory clinical safety [7–11]. Theoretically, long-term effects after MSCT may include potential immunosuppression, infection and tumorigenesis. However, to date, there have been no reports on the long-term safety and efficacy of MSCT for the treatment of patients with rheumatoid arthritis.

In the current study, MSCs monotherapy and MSCs combined with IFN-γ treatment were conducted in the two phase 1/2 randomised, double-blind 48-week studies. MSCs significantly improved the signs and symptoms of RA, and the physical function and health status of patients with moderately to severely active RA responded poorly to regular clinical treatments, including conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), biological DMARDs (bDMARDs) and steroids, or failed to tolerate the serious side effects of these drugs (ChiCTR-ONC-16008770 and ChiCTR-INR-17012462). In addition, MSCs in combination with IFN-γ demonstrated superiority to MSCs therapy alone [12, 13].

Patients who participated in the core double-blind randomised clinical trials (RCTs) could participate the following long-term extension (LTE) study after signing the informed consent form (ICF). This report includes long-term MSCs safety and efficacy data pooled from two phase 1/2 studies at single center and thus provides important information for future clinical practice.

Methods

Patient population

The core RCTs enrolled adults with active RA who had an inadequate response to or were intolerant of conventional treatments, including csDMARDs, NSAIDs, bDMARDs, and steroids. Patients who completed the 48-week follow-up period were eligible for continued observation in the long-term extension (LTE) study. Detailed inclusion and exclusion criteria for the core studies have been previously published [12, 13].

Study design

In the core RCTs, patients with an inadequate response to conventional treatments were randomized, depending on the study, to receive either a single intravenous dose of MSCs (1×10^6 cells/kg) or MSCs (1×10^6 cells/kg) combined with intramuscular IFN-γ (1 million IU). The source and preparation of MSCs, derived from a single donor, were consistent across all participants and have been previously described [12, 13]. Patients who completed the core RCTs and consented to participate in the LTE study were maintained on a standardized medication tapering protocol. Tapering protocols followed our published criteria, [13] requiring sustained DAS28 < 3.2 for ≥ 12 weeks prior to each step. If the status of a subject continued to improve, a withdrawal schedule was used to taper off the conventional drug treatment regimen in the following order: glucocorticoids, NSAIDs then DMARDs. All treatment modifications were approved by the rheumatologist in charge. The details of study design of the core RCTs are described in Supplemental material.

Safety assessments

Safety assessments encompassed adverse events (AEs) and serious adverse events (SAEs), coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 27.1, along with laboratory tests, vital signs, physical examinations, and other clinically relevant parameters monitored throughout the study period.

Clinical response

Efficacy assessments included the Disease Activity Score 28 (DAS28) < 2.6 (indicating remission) and ≤ 3.2 (indicating low disease activity), [14] American College of Rheumatology (ACR) 20/50/70 responses, [15] and the Health Assessment Questionnaire-Disability Index (HAQ-DI). These assessments were conducted in compliance with the European Alliance of Associations for Rheumatology (EULAR) recommendations for reporting clinical trial extension studies in rheumatology [16].

Statistical analysis

The safety population comprised all subjects who received MSCs transplantation. Patients were analyzed according to treatment groups: (1) MSCs monotherapy and (2) MSCs combined with IFN-γ treatment. Baseline safety variables were defined as the most recent values recorded prior to MSCs transplantation. Adverse events (AEs) were summarized as the total number of events and exposure-adjusted event rates (ERs), calculated as the number of events per 100 patient-years (PYs) of observation. The total observation duration was defined as the time from the MSCs transplantation to the last recorded safety assessment.

Efficacy was evaluated in all randomised subjects receiving MSCs transplantation who completed ≥ 5 years of follow-up (Fig. 1). Discontinued patients or those not entering the LTE study phase were classified as

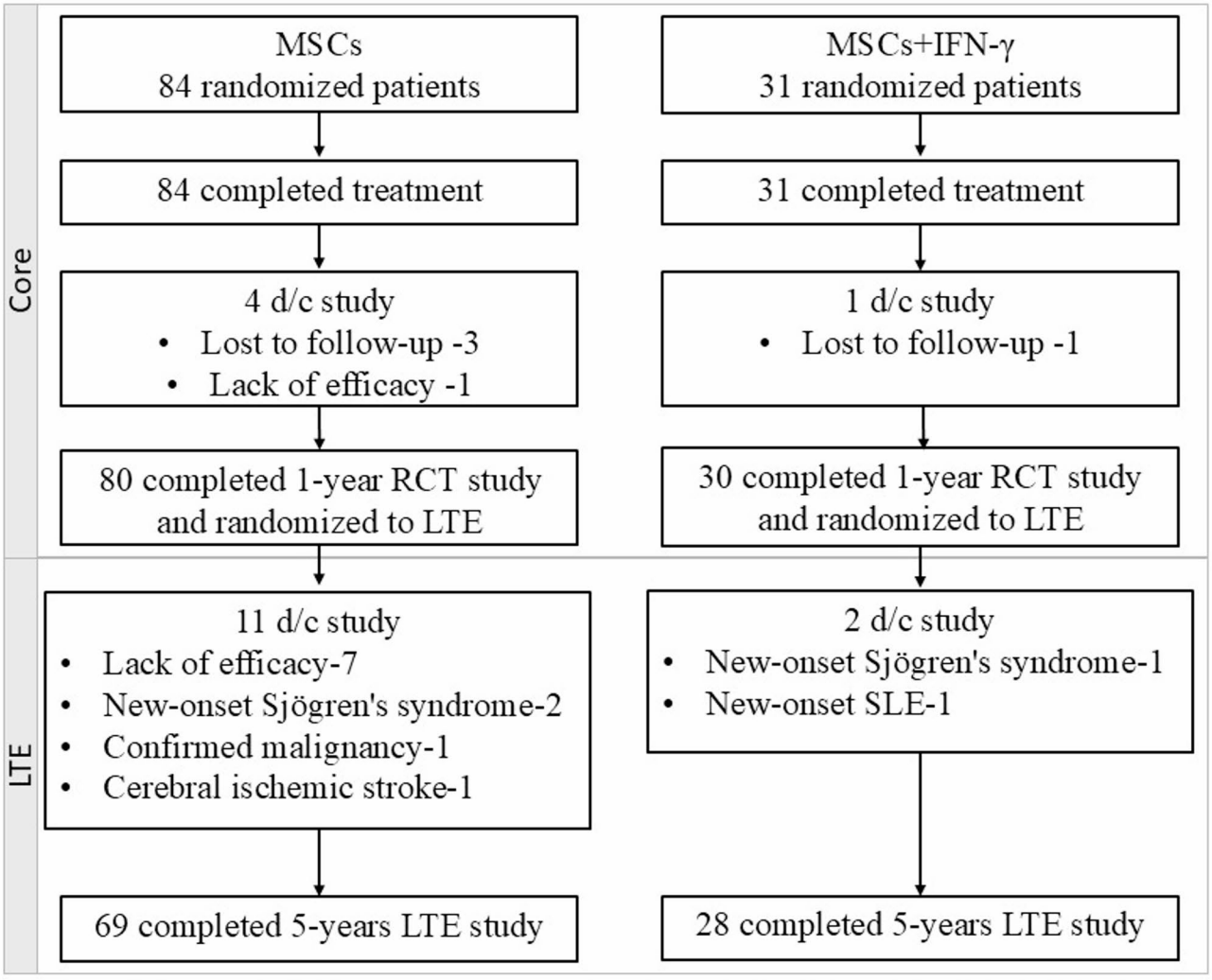

Fig. 1 Patient disposition. IFN-γ, interferon gamma; LTE, long-term extension; MSCs, mesenchymal stem cells; RCT, randomized controlled clinical trial; SLE, systemic lupus erythematosus

Table 1 Demographics and other baseline characteristics

Characteristics	MSCs (*N* = 69)[#]	MSCs + IFN-γ (*N* = 28)[#]
Female no. (%)	59 (85.5)	24 (85.7)
Mean age (years)	46.7 ± 10.2	47.4 ± 10.8
Mean duration of RA (years)	4.02 ± 2.4	4.13 ± 2.6
DAS28-ESR	5.72 ± 0.56	5.70 ± 0.47
HAQ-DI	1.63 ± 0.21	1.60 ± 0.20
Erythrocyte sedimentation rate (mm/hr)	45.87 ± 9.39	46.59 ± 8.62
C-reactive protein level (mg/liter)	24.13 ± 5.83	23.43 ± 6.38
Positive for rheumatoid factor (%)	95.7	96.4
Positive for anti-CCP antibodies (%)	94.2	92.9
Medication history no. (%)		
DMARDs	69 (100)	28 (100)
bDMARDs	27 (39)	13 (46)
NSAIDs	69 (100)	28 (100)
Prednisone	59 (86)	25 (89)

HAQ-DI, the Health Assessment Questionnaire-Disability Index; DAS28-ESR, the Disease Activity Score for 28-joint counts based on the erythrocyte sedimentation rate; DMARDs, disease-modifying anti-rheumatic drugs; bDMARDs, biological DMARDs; NSAIDs, nonsteroidal anti-inflammatory drugs; CCP, cyclic citrullinated peptide. Value: Mean ± SD.

[#] Only data from patients who completed 5 years of follow-up were included here.

non-responders, while a completer analysis included only patients with data available at the analysis time point.

Statistical analyses were performed using the t-test for parametric data and the Mann-Whitney U test for non-parametric data. For comparisons involving more than two groups, one-way ANOVA followed by the Bonferroni test or two-way ANOVA was applied. Changes from baseline in DAS28-ESR, HAQ-DI, and other continuous endpoints were evaluated using a mixed-effect longitudinal model. The duration of clinical remission (DAS28 < 2.6), low disease activity (DAS28 ≤ 3.2), and ACR20/50/70 responses were assessed using Kaplan–Meier survival analysis. Disease activity clustering was analyzed with the trend χ^2 test. Medication regimens adjustments were analyzed with Fisher's exact test. All tests were two-sided, with a significance level set at $p < 0.05$. Analyses were conducted using SPSS V.27.0 (SPSS), and data are presented as mean ± SD.

Results

Patient disposition

A total of 110 RA patients were randomized to the core RCTs and enrolled in the LTE study, and 97 (88.2%) completed the five-year LTE study (Fig. 1). Overall, 13 (11.8% of those randomised initially) patients discontinued the study during the LTE study: 7 (6.4%) due to lack of efficacy, 6 (5.5%) due to AEs. Two patients who discontinued study from the MSCs combined with IFN-γ treatment group not only developed new-onset autoimmune disorders but also failed to achieve an ACR20 response. Among 97 patients who completed the 5-year follow-up, 69 patients received MSCs monotherapy, whereas 28 patients were treated with a combination of MSCs and IFN-γ. The maximum follow-up duration was seven years, with a mean follow-up period of six years (range: 5–7 years). Demographics and clinical characteristics were well-balanced across MSC treatment groups (Table 1) and between study completers and dropouts (Table S2).

Table 2 Summary of adverse events

	MSCs		MSCs + IFN-γ	
	N = 80		*N* = 30	
	PY = 445.15		PY = 172.89	
	Event	ER per 100 years	Event	ER per 100 years
At least one AE	161	36.17	62	35.86
Lymphocyte count decreased	71	15.95	27	15.62
Fever and chills	6	1.35	5	2.89
COVID-19	78	17.52	29	16.77
Hyperglycemia	3	0.67	1	0.58
Hyperlipidemia	2	0.45	0	0.00
Gastrointestinal disorders	1	0.22	0	0.00
SAE	11	2.47	4	2.31
Autoimmune disorders	2	0.45	2	1.16
SLE	0	0.00	1	0.58
Sjögren's syndrome	2	0.45	1	0.58
Infections	2	0.45	0	0.00
Pneumonia	1	0.22	0	0.00
Tuberculosis	1	0.22	0	0.00
Hypertension	1	0.22	0	0.00
Diabetes mellitus type 2	1	0.22	0	0.00
Malignancies	1	0.22	0	0.00
Cerebral infarction	2	0.45	0	0.00
Graft-versus-host disease	0	0.00	0	0.00
Death	0	0.00	0	0.00

AE, adverse event; ER, event rate; PY, patient-year; SAE, serious AE; SLE, systemic lupus erythematosus; COVID-19, Corona Virus Disease 2019.

Safety

Adverse events

A total of 223 adverse events (AEs) were recorded during the follow-up period and ER per 100 PY was 36.17 in MSCs monotherapy group and 35.86 in MSCs combined with IFN-γ treatment group (Table 2). Most frequently reported AEs were lymphocyte count decreased (ER was 15.95/100 PY in MSCs monotherapy group and 15.62/100 PY in MSCs combined with IFN-γ treatment group) and corona virus disease 2019 (COVID-19) (ER was 17.52/100 PY in MSCs monotherapy group and 16.77/100 PY in MSCs combined with IFN-γ treatment group). The majority of lymphocyte count decreased occurred within four weeks after treatment, no grade ≥ 3 decrease was observed, and resolved without interference

by the eighth week or 12th week. In addition, no significant abnormalities were detected via routine blood tests, liver and kidney function analysis, chest radiography, urine analysis, or electrocardiography (Supplemental Table S1).

Serious adverse events

Serious adverse events (SAEs) were noted in eight (7.3%) patients with a rate of 2.47/100 PY with MSCs monotherapy group and 2.31/100 PY with MSCs combined with IFN-γ treatment group. The most commonly reported SAEs were autoimmune disorders and infections. The ER of autoimmune disorders were 0.45/100 PY and 1.16/100 PY in MSCs monotherapy group and MSCs combined with IFN-γ treatment group, respectively (Table 2), including new-onset systemic lupus erythematosus (SLE) and Sjögren's syndrome. Serious infections occurred only in MSCs monotherapy group with a rate of 0.45/100 PY (Table 2). Among the two cases of serious infections, one case of pneumonia was caused by COVID-19, and one case of tuberculosis developed in a patient treated with MSCs monotherapy with a negative T-cell spot of tuberculosis (T-SPOT.TB) test at baseline while comorbid rheumatoid arthritis-associated interstitial lung disease (RA-ILD), experienced worsening of interstitial lung disease following COVID-19 infection, required hospitalization, and was subsequently diagnosed with concurrent pulmonary tuberculosis. Simultaneously, this patient with RA-ILD was diagnosed with rectal cancer and underwent surgical treatment. No unusual types of malignancies or clustering were observed. Cerebral infarction occurred in one RA patient with elevated baseline blood pressure who was subsequently diagnosed with hypertension and later experienced two episodes of cerebral infarction. No patients developed graft-versus-host disease (GVHD) and death throughout the LTE study.

Clinical response

Disease activity

Efficacy assessments demonstrated that MSCs combined with IFN-γ is a more effective treatment than MSCs monotherapy for active RA with both clinical and functional improvements observed in many patients.

There was a significant difference in the clinical response rates between the two MSCs administration regimens. As expected, efficacy results in the completer population of MSCs combined with IFN-γ treatment were higher compared with MSCs monotherapy population (Table 3). In the RCT period, ACR20 response rates and DAS28 LDA (≤ 3.2) rates were higher in RA patients treated with MSCs combined with IFN-γ than in patients with MSCs monotherapy (100% vs. 50.7%, 39.3% vs. 8.7%, respectively), and the rates remained stable up to year five of the LTE study (89.3% vs. 44.9%, 42.9% vs. 8.7%, respectively) (Table 3). For the more stringent responses, namely ACR50/70 and DAS28 remission (< 2.6), patients received MSCs combined with IFN-γ also achieved a higher rate than control received MSCs monotherapy, although it had not statistically significant difference. Of note, even when using these most stringent assessment methods, at year three and year five of LTE study, a significant difference was also observed with DAS28 remission rates (25% vs. 1.4% and 25% vs. 2.9%, respectively) between the two MSCs administration regimens. The decreases of DAS28 and HAQ-DI scores also stabilized from year one of RCTs to year five of LTE study (Table 3).

Furthermore, analysis of the durations of ACR20/50/70, DAS28 remission, and DAS28 LDA revealed that, as of the last follow-up visit, clinical efficacy stabilized in both MSCs monotherapy and MSCs combined with IFN-γ treatment group (Fig. 2A–E), which demonstrated a remarkable result given the preceding treatment history of this pooled population.

The change in disease activity clusters of the two MSCs groups remained relatively stable throughout the LTE study. Compared with that in the MSCs monotherapy group, the improvement in disease activity in the MSCs

Table 3 Efficacy assessments

Variable	Year 1		Year 3		Year 5	
	MSCs (N=69)[#]	MSCs+IFN-γ (N=28)[#]	MSCs (N=69)	MSCs+IFN-γ (N=28)	MSCs (N=69)	MSCs+IFN-γ (N=28)
DAS28-ESR<2.6— % of patients (SD)	2.9 (16.8)	14.3 (35.0)	1.4 (12.0)	25.0 (43.3)*	2.9 (16.8)	25.0 (43.3)*
DAS28-ESR≤3.2— % of patients (SD)	8.7 (28.2)	39.3 (48.8)*	8.7 (28.2)	46.4 (49.9)*	8.7 (28.2)	42.9 (49.5)*
ACR 20 response — % of patients (SD)	50.7 (50.0)	100 (0)*	46.4 (49.9)	92.9 (25.8)*	44.9 (49.7)	89.3 (30.9)*
ACR 50 response — % of patients (SD)	21.7 (41.2)	39.3 (48.8)	20.3 (40.2)	35.7 (47.9)	18.8 (39.1)	32.1 (46.7)
ACR 70 response — % of patients (SD)	4.3 (20.4)	14.3 (35.0)	2.9 (16.8)	14.3 (35.0)	2.9 (16.8)	14.3 (35.0)
△DAS28-ESR (SD)	−1.34 (0.93)	−2.64 (0.48) *	−1.34 (0.95)	−2.66 (0.48)*	−1.38 (0.95)	−2.67 (0.48)*
△HAQ-DI (SD)	−0.39 (0.28)	−0.89 (0.26) *	−0.36 (0.27)	−0.91 (0.29)*	−0.35 (0.28)	−0.90 (0.29)*

* P<0.001 for the comparison with MSCs. ACR, American College of Rheumatology; EULAR, European League against Rheumatism and SD, standard deviation.

[#]Only data from patients who completed 5 years of follow-up were included here.

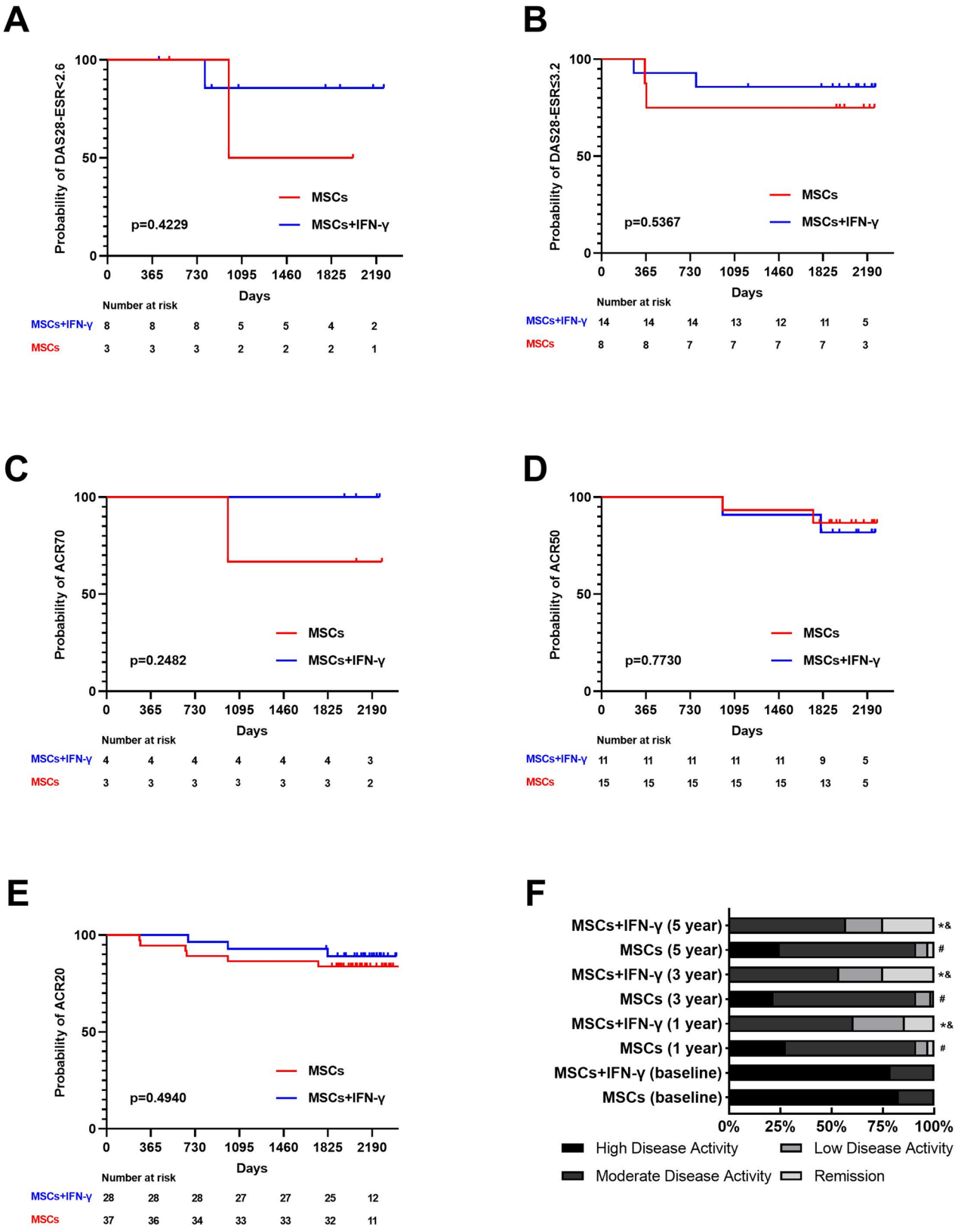

Fig. 2 Survival analysis of RA patients who achieved DAS28 remission, DAS28 LDA or ACR20/50/70 during follow-up. **A**–**E** Kaplan–Meier plot of RA patients who achieved DAS28 remission (DAS28 < 2.6), DAS28 LDA (DAS28 ≤ 3.2), ACR70, ACR50, and ACR20 in the MSCs + IFN-γ and MSCs group. **F** Cluster assessment of disease activity in the MSCs + IFN-γ and MSCs groups during the LTE study; * compared with MSCs group at same follow-up period; # compared with MSCs group at baseline; & compared with MSCs + IFN-γ group at baseline

combined with IFN-γ treatment group was much more obvious that most patients had moderate or low disease activity, and a few patients experienced remission (Fig. 2F).

Medication regimens

Consistent with the observed clinical efficacy, medication regimens adjustments revealed that the number of patients using biological DMARDs (bDMARDs) and prednisone decreased in both of MSCs groups while maintaining anchor therapy with csDMARDs during the LTE study (Table 4). Notably, compared with the MSCs monotherapy group, the MSCs combined with IFN-γ treatment group exhibited a greater reduction in prednisone use. Additionally, following the approval of targeted synthetic DMARDs (tsDMARDs) in China in 2019, some patients with suboptimal clinical responses or those pursuing clinical remission opted for tsDMARDs, although no significant differences were observed between the two groups. Notably, sustained drug-free remission was observed in one patient in the MSCs combined with IFN-γ treatment group, with remission achieved within the first follow-up year and maintained through the fifth year.

Discussion

This LTE study confirmed that MSCs monotherapy provides a sustained favorable safety and efficacy profile, and a combination therapeutic strategy of MSCs plus IFN-γ greatly improved the clinical efficacy of MSC-based therapy in patients with active RA. Over five years, patients experienced no increase in TEAEs or SAEs, and no cases of GVHD and death were reported. While the initial sample size ($n = 110$) was determined by power calculations accounting for expected attrition, and the final follow-up rate (88.2%, $n = 97$) is reasonable for long-term studies (mean dropout rates in RA clinical trials: 11%–39%), [17, 18] the modest effective sample size remains a key limitation. This may reduce statistical power to detect smaller clinically relevant effects or perform certain subgroup analyses. Critically, the sample size also impacts generalizability. Our findings, particularly those with small effect sizes or non-significant results, require cautious interpretation in broader populations and may be most applicable to patients meeting our inclusion criteria. Although our dropout analysis showed no significant baseline differences (Tabel S2, $p > 0.05$), future larger-scale studies are essential to confirm these findings and enhance generalizability.

Patients with RA are known to have an increased risk of infection, and therapy with immunosuppressive drugs may increase this risk [19]. The observed ER for serious infections (0.45/100 PY in MSCs monotherapy and 0/100 PY in MSCs combined with IFN-γ) was much lower than

Table 4 Medication regimens adjustments of DMARDs and steroids between MSCs and MSCs+IFN-γ group

Drug chane- no. (%)	Baseline			Year 1			Year 3			Year 5		
	MSCs (N=69)	MSCs+IFN-γ (N=28)	*p*	MSCs (N=69)	MSCs+IFN-γ (N=28)	*p*	MSCs (N=69)	MSCs+IFN-γ (N=28)	*p*	MSCs (N=69)	MSCs+IFN-γ (N=28)	*p*
csDMARDs	69 (100)	28 (100)	>0.999	69 (100)	26 (93)	0.081	69 (100)	26 (93)	0.081	69 (100)	26 (93)	0.081
tsDMARDs	0	0	>0.999	0	0	>0.999	2 (3)	1 (4)	>0.999	13 (19)	1 (4)	0.061
bDMARDs	27 (39)	10 (36)	0.820	13 (19)	1 (4)	0.061	11 (16)	1 (4)	0.170	12 (17)	2 (7)	0.338
Prednisone	57 (83)	24 (86)	>0.999	36 (52)	2 (7)	**<0.001**	34 (49)	2 (7)	**<0.001**	23 (33)	2 (7)	**0.009**

DMARDs, disease-modifying anti-rheumatic drugs; csDMARDs, conventional synthetic DMARDs; tsDMARDs, targeted synthetic DMARDs; bDMARDs, biological DMARDs

that for other biological or targeted synthetic DMARDs (b/tsDMARDs), such as tocilizumab (3.5–4.9/100 PY), sarilumab (3.7/100 PY in combination with csDMARDs) and olokizumab (2.34–2.95/100 PY) [20–22]. There was no increase in the rate, severity or change in the nature of infections with time. While direct comparisons are limited by the absence of a concurrent active comparator, systematic benchmarking against historical data reveals MSC therapy's distinct value: (1) SAE rates (2.3–2.5/100 PY) represent a 5–6-fold reduction vs. conventional therapies; [23] (2) 5-year ACR20 rates (45–89%) exceed DMARDs efficacy at 30 weeks (31–59%); [24] (3) MSCs offer a critical option for bDMARD-refractory patients or those at high infection risk. These findings suggest that MSCs transplantation does not appear to increase the risk of infection compared with other immunosuppressive drugs and that MSCs modulate the immune system rather than directly suppress it [25–27]. In other words, MSCs selectively suppress pathologically overactivated inflammatory responses without compromising normal immune function [28].

A decrease in lymphocyte count is the most common laboratory change observed with MSCs transplantation. ERs of lymphocyte count decreased of MSCs monotherapy was comparable with MSCs combined with IFN-γ treatment. However, no serious laboratory abnormalities occurred throughout the LET study. The observed decrease in lymphocyte counts aligns with the in vitro findings that MSCs inhibit lymphocyte proliferation [29]. Concurrent with the reduction in lymphocyte numbers, our previous research also revealed a reversal in the Treg/Th17 ratio, further corroborating the immunomodulatory function of MSCs [13].

During the course of this LTE study, the COVID-19 pandemic occurred, and as a result, the majority of patients contracted COVID-19. Among them, one patient required hospitalization due to progression to COVID-19 pneumonia. Another patient affected by COVID-19 who had pre-existing ILD at baseline, experienced a worsening of ILD following COVID-19 infection. During hospitalization, the patient was also diagnosed with tuberculosis, highlighting the susceptibility of RA-ILD patients to concurrent respiratory infections [30]. While COVID-19 infections constituted frequent AEs (17.52/100 PY), re-analysis excluding these events showed stable non-COVID SAE rates (2.25–2.31/100 PY). The low incidence of severe COVID-19 (1 pneumonia SAE vs. 18–29% in general RA cohorts) suggests MSC immunomodulation may mitigate pandemic-related risks [31]. Hybrid follow-up and universal vaccination further reduced bias.

In addition to a lack of efficacy, the diagnosis of new autoimmune disorders emerged as another significant reason for patients discontinuing participation in the LTE study. A total of four patients were diagnosed with new-onset autoimmune disorders, including three patients with Sjögren's syndrome and one patient with SLE. RA patients are known to be at potential risk of developing other autoimmune diseases, among which Sjögren's syndrome is the most frequently observed. The prevalence of Sjögren's syndrome in RA patients ranges from 10% to 30%, which is higher than that reported in the general population [32]. In contrast to the relatively high prevalence of Sjögren's syndrome, the prevalence of SLE in RA patients is exceedingly rare, which is termed "rhupus syndrome" and ranges from 0.01% to 2% [33]. Moreover, it is noteworthy that RA patients who developed new autoimmune disorders during the follow-up period did not exhibit significant clinical responses after receiving either MSCs monotherapy or MSCs combined with IFN-γ treatment. While the immunostimulatory properties of IFN-γ provide a plausible biological basis for the observed numerical imbalance, our analyses revealed no evidence linking adverse events to baseline autoantibody status, demonstrated a very low absolute risk, and identified no safety signal in prior IFN-γ receptor agonist trials [34, 35] Furthermore, counter-regulation by mesenchymal stromal cells (MSCs) is a likely contributing factor. These findings suggest that MSCs transplantation for RA may have limited expected efficacy in patients with potential comorbid autoimmune disorders, but the underlying reasons for this finding remain to be explored.

The incidence of malignancy is similar to that of the general population and lower than that of bDMARDs, such as sarilumab (0.6–0.7/100 PY) and tocilizumab (1.4 and 0.7/100 PY depending on dosage) [20, 21] Another long-term observational study of MSCs transplantation also demonstrated a good safety profile in SLE patients, without an increased risk of malignancy [7].

The overall treatment response from any of the efficacy variables, including the disease activity score and ACR response, were attained early and maintained throughout the 5-year period. In contrast to other therapies that require repeated and frequent administration, a single dose of MSCs is sufficient to achieve significant clinical improvement in the majority of responsive patients within three to six months [13]. Furthermore, these improvements in the signs and symptoms of rheumatoid arthritis occurred early and were sustained over the 5-year follow-up period. Although the therapeutic efficacy of MSCs combined with IFN-γ treatment is superior to that of MSCs monotherapy, both MSCs regimens demonstrated durable clinical benefits, with the majority of patients maintaining their therapeutic response over the 5-year follow-up period. While MSC + IFN-γ demonstrates robust efficacy for moderate RA (evidenced by ACR20/LDA/DAS28 remission), achieving deep responses (ACR50/70) in severe disease may

require optimized dosing strategies. Additionally, this study revealed a significant reduction in the proportion of clinically responsive patients continuing to use prednisone and bDMRADs. However, it is important to note that DMARDs, as anchor therapy for RA, must still be maintained to ensure sustained therapeutic efficacy.

This study has several limitations. First, single-center enrollment may limit generalizability, although our cohort demographics align closely with national rheumatoid arthritis (RA) registry data [36]. Second, open-label LTE phase risks assessment bias, mitigated by blinded endpoint adjudication and objective biomarkers. Third, the high retention rate (88.2%) may preferentially retain treatment responders, but sensitivity analyses confirmed the robustness of the primary findings against this potential bias. Fourth, the absence of a placebo control, inherent to LTE extensions, was contextualized through comparison with active comparator groups from the core RCT phase and validated historical benchmarks. Fifth, systematic evaluation of radiographic progression was not performed; while stable HAQ-DI scores and reduced DAS28 correlate with lower erosion risk, [37, 38] future MSC trials will incorporate modified total sharp score (mTSS) assessments. These limitations do not invalidate the primary conclusion that MSC + IFN-γ induces durable remission, but highlight needs for multi-center replication and predictive biomarker integration.

Conclusions

MSCs have shown favorable safety and efficacy profiles for five years and continue to provide significant clinical benefit in the patient population evaluated in this study.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04772-x.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

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

Author contributions

YY, XH, SGZ and XX conceptualized the study. XH, YY, MY, ZL and WX developed the methodology and performed data curation. YY and XH carried out the formal analysis. YY, XH and MY conducted the investigation. YY and XX provided the resources. YY and XH wrote the original draft of the manuscript. SGZ and XX reviewed and edited the manuscript. XX and YY acquired the funding and supervised the study.

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Data availability

The data supporting the findings of this study are stored in the State Key Laboratory of Trauma and Chemical Poisoning repository. The study's data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Daping Hospital, Army Military Medical University of the Chinese People's Liberation Army (Approval ID: YIYANLUNSHEN (2016) NO. 007) on March 6, 2016, under the title "Safety and Efficacy Study of Umbilical Cord-Derived Mesenchymal Stem Cells for Rheumatoid Arthritis (RA)" and (Approval ID: YIYANLUNSHEN (2016) NO.016) on September 25, 2016, under the title "Safety and Efficacy Study of Umbilical Cord-Derived Mesenchymal Stem Cells combined with recombinant human gamma interferon (IFN-γ) in the treatment of Rheumatoid Arthritis (RA)".

Consent for publication

All enrolled patients have provided written informed consent for participation in the study and the use of samples.

Competing interest

The authors declare that there is no conflict of interest.

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References

1. Mcinnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365(23):2205–19.
2. Bi X, Guo XH, Mo BY, et al. LncRNA picsar promotes cell proliferation, migration and invasion of fibroblast-like synoviocytes by sponging miRNA-4701-5p in rheumatoid arthritis. EBioMedicine. 2019;50:408–20.
3. Zou Y, Xu S, Xiao Y, et al. Long noncoding RNA LERFS negatively regulates rheumatoid synovial aggression and proliferation. J Clin Invest. 2018;128(10):4510–24.
4. Brown P, Pratt AG, Hyrich KL. Therapeutic advances in rheumatoid arthritis. BMJ. 2024;384:e070856.
5. Rodriguez-Fuentes DE, Fernandez-Garza LE, Samia-Meza JA, et al. Mesenchymal stem cells current clinical applications: a systematic review. Arch Med Res. 2021;52(1):93–101.
6. Gao F, Chiu SM, Motan DA, et al. Mesenchymal stem cells and immunomodulation current status and future prospects. Cell Death Dis. 2016;7(1):e2062.
7. Wang D, Zhang H, Liang J, et al. A long-term follow-up study of allogeneic mesenchymal stem/stromal cell transplantation in patients with drug-resistant systemic lupus erythematosus. Stem Cell Reports. 2018;10(3):933–41.
8. Farge D, Loisel S, Lansiaux P, et al. Mesenchymal stromal cells for systemic sclerosis treatment. Autoimmun Rev. 2021;20(3):102755.

9. Introna M, Rambaldi A. Mesenchymal stromal cells for prevention and treatment of graft-versus-host disease: successes and hurdles. Curr Opin Organ Transplant. 2015;20(1):72–8.
10. Wang D, Zhang H, Cao M, et al. Efficacy of allogeneic mesenchymal stem cell transplantation in patients with drug-resistant polymyositis and dermatomyositis. Ann Rheum Dis. 2011;70(7):1285–8.
11. Xu J, Wang D, Liu D, et al. Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome. Blood. 2012;120(15):3142–51.
12. Yang Y, He X, Zhao R, et al. Serum IFN-gamma levels predict the therapeutic effect of mesenchymal stem cell transplantation in active rheumatoid arthritis. J Translational Med. 2018;16(1):165.
13. He X, Yang Y, Yao M, et al. Combination of human umbilical cord mesenchymal stem (stromal) cell transplantation with IFN-gamma treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis. Ann Rheumatic Dis. 2020;79(10):1298–304.
14. Van Riel PL, Renskers L. The disease activity score (DAS) and the disease activity score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol. 2016;34(5 Suppl 101):S40-4.
15. Felson DT, Anderson JJ, Boers M, et al. American college of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum. 1995;38(6):727–35.
16. Buch MH, Silva-Fernandez L, Carmona L, et al. Development of EULAR recommendations for the reporting of clinical trial extension studies in rheumatology. Ann Rheum Dis. 2015;74(6):963–9.
17. Felson DT, Anderson JJ, Meenan RF. The comparative efficacy and toxicity of second-line drugs in rheumatoid arthritis. Results of two metaanalyses. Arthritis Rheum. 1990;33(10):1449–61.
18. Ibrahim F, Tom BD, Scott DL, et al. A systematic review of randomised controlled trials in rheumatoid arthritis: the reporting and handling of missing data in composite outcomes. Trials. 2016;17(1):272.
19. Doran MF, Crowson CS, Pond GR, et al. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum. 2002;46(9):2287–93.
20. Schiff MH, Kremer JM, Jahreis A, et al. Integrated safety in Tocilizumab clinical trials. Arthritis Res Ther. 2011;13(5):R141.
21. Fleischmann R, Genovese MC, Lin Y, et al. Long-term safety of sarilumab in rheumatoid arthritis: an integrated analysis with up to 7 years' follow-up. Rheumatol. 2020;59(2):292–302.
22. Feist E, Fleischmann RM, Fatenejad S, et al. Olokizumab plus methotrexate: safety and efficacy over 106 weeks of treatment. Ann Rheum Dis. 2024;83(11):1454–64.
23. Van Vollenhoven RF, Emery P, Bingham CO 3. Long-term safety of rituximab in rheumatoid arthritis: 9.5-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients. Ann Rheum Dis. 2013;72(9):1496–502., *et al.*
24. Wiens A, Correr CJ, Venson R, et al. A meta-analysis of the efficacy and safety of using Infliximab for the treatment of rheumatoid arthritis. Clin Rheumatol. 2009;28(12):1365–73.
25. Huang F, Liu ZM, Zheng SG. Updates on GMSCs treatment for autoimmune diseases. Curr Stem Cell Res Ther. 2018;13(5):345–9.
26. Chen F, Chen N, Xia C, et al. Mesenchymal stem cell therapy in kidney diseases: potential and challenges. Cell Transplant. 2023;32:9636897231164251.
27. Chow L, Johnson V, Coy J, et al. Mechanisms of immune suppression utilized by canine adipose and bone marrow-derived mesenchymal stem cells. Stem Cells Dev. 2017;26(5):374–89.
28. Huai Q, Zhu C, Zhang X, et al. Mesenchymal stromal/stem cells and their extracellular vesicles in liver diseases: insights on their immunomodulatory roles and clinical applications. Cell Biosci. 2023;13(1):162.
29. Ramasamy R, Tong CK, Seow HF, et al. The immunosuppressive effects of human bone marrow-derived mesenchymal stem cells target T cell proliferation but not its effector function. Cell Immunol. 2008;251(2):131–6.
30. Mena-Vazquez N, Redondo-Rodriguez R, Rojas-Gimenez M, et al. Rate of severe and fatal infections in a cohort of patients with interstitial lung disease associated with rheumatoid arthritis: a multicenter prospective study. Front Immunol. 2024;15:1341321.
31. Jin L, Gan J, Li X, et al. Rheumatoid arthritis and COVID-19 outcomes: a systematic review and meta-analysis. BMC Rheumatol. 2024;8(1):61.
32. Harrold LR, Shan Y, Rebello S, et al. Prevalence of Sjogren's syndrome associated with rheumatoid arthritis in the USA: an observational study from the Corrona registry. Clin Rheumatol. 2020;39(6):1899–905.
33. Kondo H, Watanabe R, Okazaki S, et al. Coexistence of rheumatoid arthritis and systemic lupus erythematosus is still rare in the biologic era: report of seven cases and literature review. Mod Rheumatol. 2020;30(6):1082–3.
34. Cannon GW, Pincus SH, Emkey RD, et al. Double-blind trial of recombinant gamma-interferon versus placebo in the treatment of rheumatoid arthritis 1989. Arthr Rheum. 2008;58(2):79–88.
35. Veys EM, Menkes CJ, Emery P. A randomized, double-blind study comparing twenty-four-week treatment with recombinant interferon-gamma versus placebo in the treatment of rheumatoid arthritis. Arthritis Rheum. 1997;40(1):62–8.
36. Yu C, Li M, Duan X, et al. Chinese registry of rheumatoid arthritis (CREDIT): I. Introduction and prevalence of remission in Chinese patients with rheumatoid arthritis. Clin Exp Rheumatol. 2018;36(5):836–40.
37. Wells G, Becker JC, Teng J, et al. Validation of the 28-joint disease activity score (DAS28) and European league against rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. Ann Rheum Dis. 2009;68(6):954–60.
38. Nakajima A, Aoki Y, Terayama K, et al. Health assessment questionnaire-disability index (HAQ-DI) score at the start of biological disease-modifying antirheumatic drug (bDMARD) therapy is associated with radiographic progression of large joint damage in patients with rheumatoid arthritis. Mod Rheumatol. 2017;27(6):967–72.

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