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Optimization of Treatment Based on JAK-STAT Signaling Pathway: Comparison of the Efficacy of Iguratimod Combined Therapy and Biological Agents in the Reconstruction of Immune Function in RA Patients

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ABSTRACT

Rheumatoid arthritis (RA) involves aberrant JAK-STAT signaling. Adalimumab suppresses inflammation but offers limited immune reconstitution.

This retrospective study compared iguratimod+adalimumab versus adalimumab monotherapy in restoring immune function in RA patients.

A total of 112 RA patients treated between March 2022 and March 2025 were included and divided into a control group (adalimumab) and a study group (iguratimod + adalimumab). Clinical data of patients were collected before and after 24 weeks of treatment, including laboratory indicators [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP); T-lymphocyte subpopulations (CD4+, CD8+, CD4+ /CD8+ ratio)]; phosphorylated signal transducer and activator of transcription 3 (P-STAT3) levels; bone metabolism indicators osteocalcin N-terminal intermediate molecule (N-MID), total type I collagen amino-terminal prolongation peptide (T-P1NP), β-cross-linked C-telopeptide of type I collagen (β-CTX); pain assessment; American College of Rheumatology 50% improvement criteria (ACR50) response rate; Fugl-Meyer Motor Function Assessment Scale (FMAS) score of upper and lower limbs; and incidence of adverse reactions. After treatment, serum TNF-α, IL-6, CRP levels, CD8+ T lymphocytes, β-CTX, P-STAT3 levels, and pain assessment of patients in both groups were lower than those before treatment, and the study group was significantly lower; CD4+ T lymphocytes in the 2 groups, CD4+ /CD8+ ratio, N-MID, T-P1NP, limb function score upper and lower limb FMAS score, and ACR50 response rate after treatment were markedly higher in the study group.

In conclusion, iguratimod combined with adalimumab synergistically inhibits the JAK-STAT pathway, significantly improving immune reconstitution and inflammation control in RA, with superior efficacy to adalimumab alone.

Keywords: Adalimumab; Immune reconstitution; Iguratimod; Rheumatoid arthritis

INTRODUCTION

As a systemic autoimmune condition, rheumatoid arthritis (RA) is pathologically defined by persistent,

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symmetrical joint inflammation[1]; its pathological features include synovial hyperplasia, immune cell infiltration, and progressive articular degeneration, and the main pathological change is inflammatory synovitis. Severe cases can result in joint deformity and loss of function, and have a great impact on patients' daily life and work.[2] Globally, the incidence of RA is significantly higher in women than in men, and increases with age.[3,4] RA not only causes joint pain, deformity, and dysfunction, but also is often associated with systemic damage, which seriously affects the life quality and social function of the patient.[5] However, beyond inflammation control, a core therapeutic challenge lies in reversing the underlying immune dysfunction and restoring immune homeostasis, a process termed immune reconstitution. The clinical pathogenesis of RA has not been clearly defined and may be influenced by genetic and environmental factors. Clinical treatment of RA is mainly based on anti-inflammatory strategies and inhibition of arthropathy, with commonly used medications including glucocorticosteroids and antirheumatic drugs; however, the long-term use of such drugs may cause immune disorders and elevate blood pressure, leading to adverse reactions. Therefore, the development of novel therapeutic agents is particularly necessary. Although the application of biologics and small-molecule targeted drugs in recent years has significantly improved the therapeutic efficacy of RA, a subset of patients still exhibit poor response or resistance to the existing treatments, particularly with regard to achieving sustained immune reconstitution. This has prompted researchers to continuously explore new therapeutic targets and combination therapies.[6,7]

The pathological mechanism of RA patients involves aberrant immune activation, in which dysregulated JAK-STAT pathway activation is regarded as a pivotal mechanism. This pathway acts as a common downstream signaling pathway of various cytokines (eg, IL-6, IL-2, IL-7, IL-15), and plays a central regulatory role in the activation, proliferation, and differentiation of immune cells.[8–10] Studies have shown that the expression of JAK1, JAK3, and STAT3 is significantly elevated in synovial tissues of RA patients, and their activation is positively correlated with disease activity.[11,12] This sustained activation of the signaling pathway results in over-differentiation of T_H17 cells, secretion of proinflammatory cytokines, and increased osteoclast activity, resulting in synovial hyperplasia and bone erosion.[12] Adalimumab, an anti–tumor necrosis factor-α (TNF-α) monoclonal antibody, is effective in inhibiting inflammatory responses by neutralizing the biological activity of TNF-α. However, its direct modulation of the JAK-STAT pathway is limited, and its ability to promote immune reconstitution, such as normalizing T-cell subsets and restoring immune tolerance, remains suboptimal. This may partly explain the lack of efficacy of monotherapy in some patients.[13,14] Therefore, exploring combination therapeutic regimens that can synergistically inhibit the JAK-STAT pathway and foster immune reconstitution has become an important direction to optimizing RA treatment.

As a recently developed disease-modifying antirheumatic drug (DMARD), iguratimod demonstrates innovative immunomodulatory properties for RA management.[15] Unlike traditional JAK inhibitors, iguratimod mediates its anti-inflammatory and immunoregulatory actions via pleiotropic modulation of multiple molecular targets, comprising inhibition of STAT3 phosphorylation, reduction of proinflammatory cytokine production, and modulation of T_H17/Treg cell homeostasis.[16,17] These actions position it as a promising agent for promoting immune reconstitution. Studies have shown that iguratimod significantly reduces joint inflammation scores and bone destruction in a mouse model of collagen-induced arthritis (CIA), and the mechanism is closely relevant to the inhibition of JAK-STAT pathway activation.[18] Clinical observations have also shown that iguratimod monotherapy improves clinical symptoms and serological markers in RA patients, but its effect in combination with biologics (eg, adalimumab) on immune reconstitution has not been fully investigated.[19] Notably, recent studies have demonstrated that iguratimod inhibits STAT3 phosphorylation in human T cells, thereby modulating the T_H17/Treg balance and suppressing proinflammatory cytokine production.[20] Considering the central role of JAK-STAT transduction in RA disease pathogenesis alongside the clinical limitations of conventional therapies, through retrospective evaluation characterizes the combined therapeutic effects of iguratimod and adalimumab on immune reconstitution in RA, providing evidence for clinical protocol optimization.

MATERIALS AND METHODS

Study Design and Participants

This clinical retrospective study was designed to

optimize treatment strategies based on JAK-STAT signaling pathway analysis, specifically evaluating of the efficacy of iguratimod combined with adalimumab and adalimumab in the reconstruction of immune function in patients with RA. A total of112 RA patients, enrolled between March 2022 and March 2025 were divided into a control group and a study group based on the intervention modality, with 56 patients in each group. Figure 1 illustrates the participant selection process, including screening, enrollment, group allocation, and follow-up, in accordance with the inclusion and exclusion criteria.

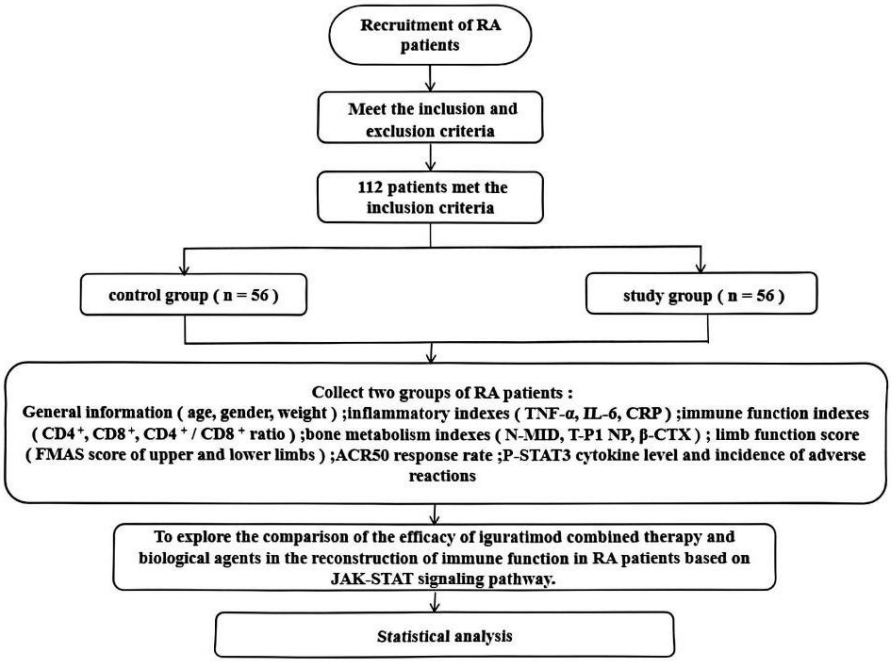

Figure 1. Design flow chart.

Inclusion and Exclusion Criteria

Inclusion criteria: (1) All patients fulfilled the 1987 and 2010 classification criteria for RA, eligible participants were adults (18–65 years) exhibiting active disease manifestations (DAS28-ESR score >3.2)[21]; (2) No recent treatment with any antirheumatic, anti-inflammatory, or immunosuppressive medication for at least 4 weeks prior to enrollment; (3) No prior use of biologic agents (including TNF-α inhibitors, IL-6 inhibitors, etc) or JAK inhibitors; (4) active disease phase at the time of enrollment. Exclusion criteria were as follows[21,22]: (1) Patients with other concomitant immune disorders; (2) Those who are intolerant to adalimumab injection or iguratimod tablets; (3) Those who have hematological disorders; (4) Those who have malignant diseases or a history of malignancy; (5) Recent clinically significant infections; (6) Prior use of any biologic or targeted synthetic DMARDs within the past 6 months; (7) Patients with uncontrolled major comorbidities (eg, cardiovascular, hepatic, or renal diseases) that could independently influence inflammatory or immune outcomes.

Research Methods

Both groups recieved glucocorticoids as basic treatment and diclofenac sodium for pain relief. The control group was treated with adalimumab (Vetter Pharma-Fertigung GmbH & Co KG; Registration No. S20160058) at a dose of 40 mg administered every other week for 24 weeks. The study group was treated with iguratimod (Centrum Pharmaceuticals Co Ltd, China National Pharmaceutical Licence No. H20110084 Specification: 25 mg/tablet) on the foundation of the control group, 1 tablet per session, twice daily, for 24 weeks. Glucocorticoid dosage and regimen were standardized and remained consistent between both groups throughout the study period.

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Baseline patient data (age, sex, weight, etc) were collected, along with pre- and posttreatment assessments of Disease Activity Score in 28 joints (DAS28-ESR), degrees of inflammatory factors TNF-α, interleukin-6 (IL-6), and C-reactive protein (CRP), immune function parameters ($CD4^+$ T lymphocytes counts, $CD8^+$ T lymphocytes counts, $CD4^+$ /$CD8^+$ ratio, bone metabolism markers (N-terminal osteocalcin mid-fragment (N-MID), total procollagen type 1 N-terminal propeptide (T-P1NP), and β-CrossLaps of type 1 collagen (β-CTX) indices), pain assessment, ACR50 response rate, the upper and lower extremity function Scores, lymphocyte counts, and incidence of adverse reactions.

The dosage of adalimumab (40 mg every other week) was selected based on established clinical guidelines for RA treatment, which demonstrate sustained TNF-α suppression and inflammatory control over 24 weeks. Iguratimod was administered at 25 mg twice daily, a regimen shown in prior pharmacokinetic and efficacy studies to achieve steady-state plasma concentrations within 4–6 weeks and maintain STAT3 pathway inhibition throughout the treatment period. The 24-week duration was chosen to allow sufficient time for immune reconstitution, as evidenced by T-cell subset rebalancing and bone turnover marker stabilization, which typically require at least 12–24 weeks to manifest in RA patients undergoing targeted immunomodulation.

Observation Indicators

General Information

Age, sex, weight, body mass index (BMI), disease duration, diabetes mellitus, and smoking history of the patients were recorded. A total of 112 patients who fulfilled the inclusion exclusion criteria for this experiment were included. According to the intervention, the patients were categorized into a control group (n=56) and a study group (n=56).

Comparative Analysis of Pre- Versus Posttreatment Inflammatory Mediator Concentrations

Morning fasting venous blood samples were collected, dispensed into procoagulant tubes, and serum was separated and used to measure TNF-α and IL-6 levels via enzyme-linked immunosorbent assay (ELISA) using Human TNF-α ELISA Kit (Catalog No. ml064303; R&D Systems, China; sensitivity: <0.1 pg/mL) and Human IL-6 ELISA Kit (Catalog No. ml058097; R&D Systems, China; sensitivity: <0.1 pg/mL). For CRP measurement, serum samples were centrifuged directly into a fully automated biochemical analyzer (Roche Cobas c501, Roche Diagnostics).

Disease Activity Assessment

Disease activity was evaluated using the DAS28-ESR. The DAS28-ESR score was calculated based on the number of tender and swollen joints (out of 28), erythrocyte sedimentation rate (ESR), and patient's global health assessment measured on a visual analog scale (VAS). Scores were obtained at baseline and after 24 weeks of treatment.

Comparison of Immune Function Before and after Treatment

All blood samples were collected in the fasting state on the morning of the injection day. Venous blood was drawn into sodium heparin-coated tubes (0.2 mL). PBMC isolation was achieved through density gradient centrifugation using Ficoll-Hypaque. Isolated PBMCs were then stained with lymphocyte markers [CD3-FITC and CD4-APC], fixed with 4% paraformaldehyde for 15 minutes at room temperature in the dark, and subsequently washed in buffer containing 0.3% Tween. Then cells were resuspended in PBS and samples were immediately analyzed using a flow cytometer (FACSCanto II, BD Biosciences) to detect $CD4^+$ and $CD8^+$ T-lymphocyte percentages, and the $CD4^+/CD8^+$ ratio was calculated directly from the percentage values obtained by flow assay.[22]

Changes in P-STAT3 Levels

To mechanistically assess inhibition of the JAK-STAT pathway, serum levels of phosphorylated STAT3 (P-STAT3) were measured as a direct marker of pathway activation. This was performed using an enzyme immunoassay with the Human phosphorylated signal transducer and activator of transcription 3 ELISA kit (Catalog No. ml811023, R&D Systems, China; sensitivity: <0.1 pg/mL).

Comparison of Bone Metabolism Indexes before and after Treatment

Serum samples were collected and the levels of N-MID, T-P1NP, and β-CTX were measured using an automated immunoassay analyzer Cobas e601 (Roche Diagnostics, Germany), respectively.

Pain Assessment

Joint pain intensity was quantitatively evaluated

before and after intervention employing a standardized 0–10 numerical rating scale (NRS). This scale rates pain severity rather than using descriptive terms. The scale is split into 10 segments, and patients were asked to mark the number that best represented the severity of pain they had experienced in the past 24 hours. On this scale, 0 represents no pain; 1–3 indicates mild pain that does not disrupt sleep; 4–6 indicates moderate pain; 7–9 indicates severe pain that disrupts sleep; and 10 indicates extremely severe pain.[23]

Comparison of American College of Rheumatology 50% Response Criteria (ACR50) Response Rates

The number of swollen joints and joints with tenderness were assessed among 28 joints, and the ACR50 response rate was recorded using a standardized joint assessment recording form.

Comparison of Limb Function Values before and bfter Treatment between the Two Groups

The upper and lower extremity sections of the Fugl-Meyer Motor Function Rating Scale (FMAS) were used. Each item was rated on a scale of 0–2 (0=cannot be completed, 1=partially completed, and 2=completely completed), with total score ranging from 0–66.

Incidence of Adverse Reactions

Adverse events (AEs), including elevated aminotransferases, epigastric discomfort, oral ulcers, skin rashes, and lymphopenia (defined as lymphocyte count $<0.8 \times 10^9$/L) were recorded throughout the study. Lymphocyte counts were routinely monitored as part of immunotoxicity screening. AEs were actively solicited and documented at each clinical visit. Investigators documented all AEs, including their nature, time of onset, duration, severity, relationship to study drugs, and actions taken. The severity of AEs was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, ranging from Grade 1 (mild) to Grade 5 (death). Any Grade ≥3 AE or serious adverse event (SAE) required immediate reporting and was subjected to causality assessment by the study investigators.

Assessment of Joint Structural Damage

Radiographic evaluation of joint damage was performed using the Sharp/van der Heijde scoring method for hands and feet at baseline and after 24 weeks of treatment. X-rays were independently assessed by 2 experienced radiologists. The scoring system evaluates joint space narrowing and bone erosion, with higher scores indicating more severe structural damage.

Sample Size Calculation

Given the retrospective design of this study, a post hoc power analysis was conducted to confirm that the collected sample size was adequate to detect clinically meaningful differences. For the primary comparison of ACR50 response rates, we assumed a response rate of 60% in the control group (adalimumab monotherapy, based on historical data) and 80% in the combination group (a 20% absolute difference considered clinically significant). Using a χ^2 test for 2 proportions with α=.05 (2-tailed), our achieved sample size of 56 patients per group provided a statistical power of 84%. Additionally, for continuous outcome variables (eg, inflammatory markers), an effect size (Cohen *d*) of 0.5 could be detected with over 80% power with this sample size. Therefore, the available data from 112 patients (56 per group) were deemed sufficient for the planned statistical comparisons.

Statistical Analyses

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All continuous data were tested for normal distribution using the Shapiro-Wilk test. Baseline characteristics are presented as mean ± standard deviation (mean ± SD) or counts (percentages), as appropriate. Within-group comparisons (pre- vs posttreatment) for continuous variables (eg, TNF-α, IL-6, CRP, T-lymphocyte subsets) were performed using paired Student *t* tests. Between-group comparisons (control vs study group) for continuous variables at each time point were performed using independent-samples *t* tests. Categorical variables (eg, ACR50 response rate, adverse event incidence) were expressed as percentages and compared between groups using the χ^2 test (or Fisher exact test). All tests were 2-tailed, and a *p* value <0.05 was considered statistically significant. For continuous variables, effect sizes (Cohen *d*) were calculated as the difference between group means divided by the pooled standard deviation, with interpretations: small ($d \approx 0.2$), medium ($d \approx 0.5$), and large ($d \geq 0.8$).

RESULTS

Comparison of General Information

The cohort comprised 112 eligible patients, who

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were allocated (n=56 per group) to either the control or experimental intervention arm. Demographic characteristics and baseline comparisons showed no significant differences between the two groups (p>0.05), indicating that the groups were comparable at the baseline (Table 1).

Comparison of Inflammatory Factor Levels before and after Treatment

As shown in Table 2, the TNF-α level in the study group was 18.25 ± 3.2 ng/L, which was significantly lower than that in the control group (24.36 ± 4.0 ng/L) after treatment (p<0.001), indicating that iguratimod may synergize with adalimumab to more effectively block the proinflammatory effects of TNF-α. The IL-6 reduction in the study group (60.4%) was markedly higher than that in the control group (46.4%), and the effect size (d=1.68) was extremely large; in addition, CRP decreased to 9.5 mg/L in the study group with an effect size (d=2.38) of very high clinical significance, suggesting that combination therapy achieves faster inflammatory control and may reduce the risk of cardiovascular events.

Comparison of Disease Activity (DAS28-ESR) before and after Treatment

As shown in Table 3, the baseline DAS28-ESR scores were comparable between the control group (5.42 ± 0.76) and the study group (5.38 ± 0.72) (p>0.05). After 24 weeks of treatment, both groups showed significant reductions in DAS28-ESR scores. However, the study group (2.85 ± 0.51) exhibited a significantly greater reduction compared with the control group (3.65 ± 0.63) (p<0.001), indicating superior disease activity control with combination therapy.

Comparion of Immune Function before and after Treatment

As shown in Table 4, the $CD4^+$ T lymphocytes cells in the study group increased from 21.36% to 35.30%, which was significantly higher than that in the control group (p<0.001). Conversely, the $CD8^+$ T lymphocyte in the study group decreased from 26.54% to 17.82%, which was significantly lower than that in the control group (p<0.001), demonstrating that combination therapy may reduce the abnormal activation of $CD8^+$ T lymphocytes and reduce their cytotoxic effects on joint tissues. Consequently, the CD4+/CD8+ ratio in the study group increased from 0.80 to 2.0, which was significantly better than that of the control group (p<0.001), indicating that the intervention more effectively restored the proinflammatory/anti-inflammatory cell balance, promoted recovery from immune dysregulation in RA patients, and accelerate the immune reconstruction.

Changes in P-STAT3 Levels

As shown in Table 5, P-STAT3 reduced to 0.85 ± 0.18 pg/mL in the study group and only to 1.40 ± 0.28 pg/mL in the control group, demonstrating statistically robust intergroup differences (p<0.001), suggesting that iguratimod could significantly block STAT3 phosphorylation.

Table 1. Comparison of general information

Norm	Control group (n=56)	Study group (n=56)	χ^2/t	p
Age, y, mean ± SD	52.3 ± 10.7	53.1 ± 9.8	t=0.42	0.674
Female, n (%)	42 (75.0%)	44 (78.6%)	χ^2=0.21	0.647
Weight, kg, mean ± SD	65.2 ± 12.4	63.8 ± 11.9	t=0.63	0.531
BMI, kg/m², mean ± SD	24.1 ± 3.5	23.7 ± 3.2	t=0.65	0.518
Duration of illness, y, mean ± SD	8.5 ± 4.2	9.0 ± 4.6	t=0.61	0.543
Diabetes, n (%)	10 (17.9%)	8 (14.3%)	χ^2=0.27	0.603
Smoking history, n (%)	15 (26.8%)	12 (21.4%)	χ^2=0.45	0.503

BMI: body mass index; SD: standard deviation.

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Table 2. Comparison of the levels of inflammatory factors before and after treatment in the 2 groups (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
TNF-α, pg/L	Pretreatment	56.28 ± 6.1	55.92 ± 5.8	0.32	[-1.82, 2.54]	0.06	0.751
	Posttreatment	24.36 ± 4.0	18.25 ± 3.2	8.92	[4.63, 7.59]	1.72	<0.001
IL-6, pg/mL	Pretreatment	32.1 ± 4.3	31.8 ± 4.1	0.38	[-1.21, 1.81]	0.07	0.704
	Posttreatment	17.2 ± 3.0	12.6 ± 2.5	8.67	[3.42, 5.78]	1.68	<0.001
CRP, mg/L	Pretreatment	36.0 ± 4.3	35.7 ± 4.0	0.39	[-1.42, 1.92]	0.07	0.696
	Posttreatment	14.0 ± 2.0	9.5 ± 1.8	12.31	[3.82, 5.18]	2.38	<0.001

CI: confidence interval; CRP: C-reactive protein; IL-6: interleukin-6; SD: standard deviation; TNF-α: tumor necrosis factor-α.

Table 3. Comparison of disease activity (DAS28-ESR) before and after treatment (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
DAS28-ESR	Pretreatment	5.42 ± 0.76	5.38 ± 0.72	0.28	[-0.24, 0.32]	0.05	0.777
	Posttreatment	3.65 ± 0.63	2.85 ± 0.51	7.39	[0.59, 1.01]	1.40	<0.001

CI: confidence interval; DAS28-ESR: Disease Activity Score in 28 joints-erythrocyte sedimentation rate; SD: standard deviation.

Table 4. Comparison of immune function between the 2 groups before and after treatment (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
$CD4^+$ T cells, %	Pretreatment	20.98 ± 4.0	21.36 ± 4.2	0.48	[-1.32, 2.08]	0.09	0.631
	Posttreatment	28.45 ± 3.5	35.30 ± 3.2	10.56	[5.62, 8.08]	2.07	<0.001
$CD8^+$ T cells, %	Pretreatment	27.12 ± 3.8	26.54 ± 4.0	0.78	[-0.82, 1.98]	0.15	0.436
	Posttreatment	20.16 ± 3.0	17.82 ± 3.2	4.25	[1.18, 3.50]	0.78	<0.001
$CD4^+$ T cells/$CD8^+$ T cells	Pretreatment	0.78 ± 0.05	0.80 ± 0.04	1.12	[-0.01, 0.05]	0.22	0.267
	Posttreatment	1.41 ± 0.20	2.0 ± 0.23	15.32	[0.51, 0.67]	2.76	<0.001

CD: cluster of differentiation; CI: confidence interval; SD: standard deviation.

Table 5. Changes in P-STAT3 levels (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
P-STAT3, pg/mL	Pretreatment	2.15 ± 0.35	2.08 ± 0.32	1.12	[-0.10, 0.24]	0.21	0.267
	Posttreatment	1.40 ± 0.28	0.85 ± 0.18	12.47	[0.42, 0.68]	2.34	<0.001

CI: confidence interval; P-STAT3: phosphorylated signal transducer and activator of transcription 3; SD: standard deviation.

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Schematic Representation of Immunological Pathways Affected by Iguratimod and Adalimumab

To visually summarize the mechanistic synergy between iguratimod and adalimumab in modulating RA-associated immune pathways, we present a schematic illustration (Figure 2). The diagram highlights how adalimumab blocks TNF-α upstream, while iguratimod inhibits downstream STAT3 phosphorylation and modulates the T_H17/Treg balance. This dual-action approach synergistically suppresses inflammatory cytokine production and osteoclast activation, while promoting immune reconstitution in RA patients.

Comparison of Bone Metabolism Indexes before and after Treatment

As shown in Table 6, N-MID increased from 11.89 ng/mL to 24.35 ng/mL in the study group, which was significantly higher than that of the control group (p<0.001); T-P1NP rose from 30.95 ng/mL to 55.28 ng/mL in the study group, demonstrating statistically superior efficacy relative to control conditions (p<0.001); meanwhile, β-CTX in the study group decreased from 0.95 pg/mL to 0.42 pg/mL, with a very large effect size, demonstrating statistically significant superiority to the control cohort (p<0.001). These findings indicate that the combined treatment reduced osteoclast differentiation and bone matrix degradation, while promoting bone tissue repair.

Pain Assessment

As presented in Table 7, pretreatment assessment revealed comparable baseline pain scores between groups (control group: 6.4 ± 1.1, study group: 6.3 ± 1.2) (p=0.653). After treatment, the pain score in the combination therapy group was significantly reduced to 2.2 ± 0.8, with substantially enhanced therapeutic outcomes relative to control group, which was 3.8 ± 1.0 (p<0.001). This result suggests that iguratimod combined with adalimumab is more effective in relieving pain symptoms in patients with RA, and that the underlying mechanism of the combination may be related to a synergistic reduction in the release of proinflammatory factors.

Comparison of ACR50 Response Rates

As presented in Table 8, the study group exhibited a clinically superior ACR50 response (82.1%) compared with the control group (58.9%) (p=0.005), indicating that combination therapy significantly improvedjoint swelling and tenderness, rapidly relieved symptoms (eg, morning stiffness and pain), and enhanced quality of life in patients with RA.

Comparison of Limb Function Scores before and after Treatment

As shown in Table 9, the FMAS score of upper limb in the study group rose from 37.1 to 64.5, with significantly enhanced therapeutic outcomes relative to the control groups (p<0.001). This demonstrates that the combined therapy effectively relieved relieved hand joint swelling and pain. Similarly, the lower limb FMAS score in the study group rose from 15.37 to 31.2, which was significantly higher than in the control group (p<0.001), indicating improvements inwalking speed, ability to climb stairs and squatting capacity. These findings suggest that the combined treatment improved motor function in RA patients.

Incidence of Adverse Reactions

As shown in Table 10, the study group demonstrated an increasing propensity for adverse event occurrence, with a marginally increased incidence of elevated aminotransferase levels, oral mucosal ulceration, and epigastric discomfort compared with the control, but none of the differences reached statistical significance (p>0.05). This result demonstrated that although the combination regimen showed advantages in terms of efficacy, it may increase the risk of certain adverse reactions, especially hepatotoxicity and mucosal reactions, which may be related to its enhanced immunomodulatory effect. No cases of clinically significant lymphopenia were observed in either group during the study period.

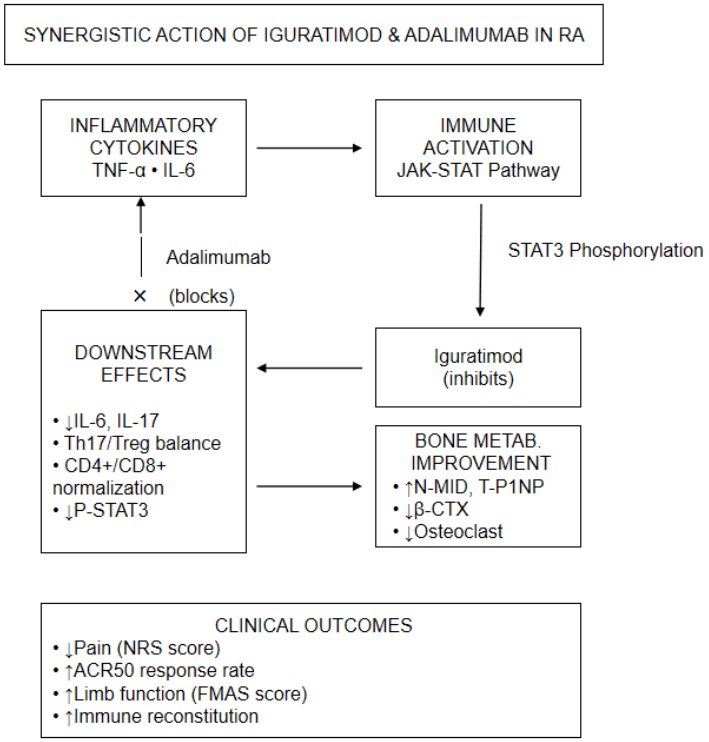

Figure 2. Schematic representation of the key immunological pathways modulated by iguratimod and adalimumab in RA.

Table 6. Before and after treatment in the 2 groups bone metabolism indicators comparison (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
N-MID, ng/mL	Pretreatment	12.02 ± 1.36	11.89 ± 1.42	0.49	[-0.31, 0.57]	0.09	0.624
	Posttreatment	19.87 ± 2.8	24.35 ± 3.1	7.92	[3.28, 5.68]	1.53	<0.001
T-P1NP, ng/mL	Pretreatment	31.29 ± 4.1	30.95 ± 3.9	0.44	[-1.02, 1.70]	0.08	0.659
	Posttreatment	46.10 ± 5.3	55.28 ± 6.0	8.37	[6.92, 11.44]	1.62	<0.001
β-CTX, pg/mL	Pretreatment	0.96 ± 0.04	0.95 ± 0.05	1.15	[-0.01, 0.03]	0.22	0.253
	Posttreatment	0.56 ± 0.08	0.42 ± 0.06	10.24	[0.11, 0.17]	1.98	<0.001

β-CTX: β-CrossLaps of type 1 collagen; CI: confidence interval; N-MID: N-terminal osteocalcin mid-fragment; SD: standard deviation; T-P1NP: total procollagen type 1 N-terminal propeptide.

Table 7. Pain assessment (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
Pain assessment	Pretreatment	6.4 ± 1.1	6.3 ± 1.2	0.45	[-0.32, 0.52]	0.09	0.653
	Posttreatment	3.8 ± 1.0	2.2 ± 0.8	9.35	[1.25, 1.95]	1.78	<0.001

CI: confidence interval; SD: standard deviation.

Table 8. Comparison of ACR50 response rates

Norm	Time	Control group (n=56)	Study group (n=56)	χ^2	95% CI	Effect size	*p*
ACR50 response rate, %	Pretreatment	0	0	-	-	-	-
	Posttreatment	58.9% (33/56)	82.1% (46/56)	7.89	[1.25, 4.97]	0.37	0.005

ACR50: American College of Rheumatology 50% improvement criteria; CI: confidence interval.

Table 9. Comparison of limb function scores between the 2 groups before and after treatment (mean ± SD)

Norm	Time	Control group (n=56)	Study group (n=56)	*t*	95% CI	Effect size	*p*
Upper limb FMAS score, points	Pretreatment	36.85 ± 5.0	37.10 ± 5.2	0.26	[-1.62, 2.12]	0.05	0.796
	Posttreatment	58.20 ± 7.5	64.50 ± 8.2	4.21	[3.42, 9.18]	0.81	<0.001
Lower limb FMAS score, points	Pretreatment	15.20 ± 3.0	15.37 ± 3.2	0.28	[-0.82, 1.16]	0.05	0.778
	Posttreatment	27.80 ± 4.8	31.20 ± 5.2	3.55	[1.52, 5.28]	0.69	<0.001

CI: confidence interval; FMAS: Fugl-Meyer Motor Function Assessment Scale; SD: standard deviation.

Table 10. Incidence of adverse reactions

Norm	Control group (n=56)	Study group (n=56)	χ^2	*p*
Elevated aminotransferase	5 (8.9%)	9 (16.1%)	1.42	0.233
Epigastric discomfort	7 (12.5%)	11 (19.6%)	1.08	0.299
Canker sore	3 (5.4%)	8 (14.3%)	2.77	0.096
A rash	4 (7.1%)	6 (10.7%)	0.48	0.488

DISCUSSION

The present study demonstrates that the combination of iguratimod and adalimumab achieves superior clinical outcomes in RA patients compared with adalimumab monotherapy, as evidenced by enhanced inflammatory control, immune reconstitution, bone protection, and functional recovery. These benefits are mechanistically underpinned by synergistic inhibition of the JAK-STAT signaling pathway, specifically through marked suppression of STAT3 phosphorylation.

The observed reductions in serum IL-6 and TNF-α levels in the combination therapy group (all $p<0.001$) align closely with the intracellular inhibition of STAT3 phosphorylation (P-STAT3) demonstrated in this study. IL-6 and TNF-α are key upstream activators of the JAK-STAT pathway, particularly via STAT3 phosphorylation, which in turn amplifies the production of proinflammatory cytokines, forming a positive feedback loop in RA pathogenesis. The synergistic reduction in both cytokines and P-STAT3 suggests that iguratimod not only inhibits STAT3 directly but may also attenuate the cytokine-driven activation loop. This dual modulation, reducing cytokine levels while suppressing their downstream signaling, provides a mechanistic explanation for the enhanced anti-inflammatory and immunomodulatory effects of the combination therapy. Such alignment reinforces the hypothesis that targeting the JAK-STAT pathway at multiple nodes (ie, upstream cytokines and downstream transcription factors) offers a more comprehensive therapeutic strategy in RA.

Studies have shown that RA patients exibit abnormal humoral immunity. Following the onset of RA, immunological alterations may occur, with the main clinical features being abnormal activation or inactivation of the immune system, which are mainly manifested as cellular immune dysfunction and reduced activity of nonspecific cells.[24] T lymphocytes which primarily derive from bone marrow hematopoietic progenitor cells, play role in immune regulation, dysfunction of cellular immunity can contribute to the development of RA.[25] $CD4^+$ T lymphocytes are known to enhance immune responses, while CD8+ T lymphocytes possess strong killing ability. In RA , the suppressive T lymphocytes may be reduced and their activity diminished, leading to an imbalance of $CD4^+/CD8^+$ T lymphocytes cells, which leads to inflammatory damage.[26] This preliminary investigation revealed a significant reduction in $CD8^+$ T-cell prevalence within the study group compared with controls ($p<0.001$). The overactivation of $CD8^+$ T lymphocytes is associated with joint destruction in RA, and this result suggests that combination therapy may be more effective in preventing the abnormal proliferation of cytotoxic T cells. The $CD4^+/CD8^+$ T-lymphocyte ratio was markedly higher in the study group opposed to the control group after treatment ($p<0.001$). The restoration of this ratio suggests that combination therapy may be more effective in reestablishing immune homeostasis and reducing autoimmune attacks. The present study confirmed that iguratimod combined with adalimumab significantly improved T-cell subset distribution and optimized the $CD4^+/CD8^+$ T-lymphocyte ratio in RA patients, which may be more effective in reestablishing immune function. Emori et al found that a JAK inhibitor combined with a biologic markedly increased the $CD4^+/CD8^+$ T-lymphocyte ratio and promoted the proliferation of Treg cells in RA patients.[27] In addition, Samarpita et al also showed that iguratimod can reduce T_H17 cell differentiation by inhibiting the STAT pathway, thereby improving T-cell subset imbalance.[28] Together, these results support the findings of the present study that combination therapy offers significant advantages in immunomodulation.

The $CD4^+/CD8^+$ T-lymphocyte ratio serves as a surrogate marker for overall immune homeostasis, reflecting the balance between helper and cytotoxic T cells. In RA, a reduced $CD4^+/CD8^+$ ratio often indicates excessive $CD8^+$ cytotoxic activity and impaired $CD4^+$ regulatory function, which is closely linked to the T_H17/Treg imbalance. Specifically, a lower $CD4^+/CD8^+$ ratio correlates with an expanded T_H17 population and diminished Treg frequency, promoting a proinflammatory milieu that exacerbates synovitis and bone erosion. In the present study, the significant elevation of the $CD4^+/CD8^+$ ratio in the combination therapy group suggests not only a restoration of T-cell subset equilibrium but also an indirect rebalancing of T_H17/Treg dynamics, likely mediated through the dual inhibition of the JAK-STAT and TNF-α pathways. JAK-STAT inhibition preferentially suppresses T_H17 differentiation while preserving or enhancing Treg function, thereby contributing to immune reconstitution in RA.

Dysregulated JAK-STAT signal transduction, especially STAT3, plays a key role in the pathogenesis of RA, and its sustained phosphorylation promotes proinflammatory cytokine production and synovial cell proliferation.[34] In the present study, the inhibitory effect of iguratimod combined with adalimumab on the JAK-STAT pathway was primarily evaluated by measuring serum P-STAT3 levels, a direct indicator of STAT3 phosphorylation. Although other phosphorylated JAK/STAT isoforms were not measured, the observed reductions in downstream cytokines (IL-6, TNF-α) and improvements in T-cell subsets support functional JAK-STAT pathway modulation. Pretreatment assessment showed no intergroup disparity in P-STAT3 levels ($p=0.267$), whereas therapeutic intervention induced statistically significant suppression of STAT3 activation in the treatment arm, and the difference was highly statistically significant ($p<0.001$), suggesting a clinically meaningful effect This observation corroborates the findings of Palmroth et al, who reported that JAK inhibitors significantly inhibited STAT3 phosphorylation.[28] The present study further demonstrates that the inhibitory effect of iguratimod in combination with adalimumab on STAT3 was significantly stronger than that of adalimumab alone. The underlying mechanisms may include: 1) iguratimod can block STAT3 phosphorylation[29]; and 2) adalimumab indirectly reduces STAT3 activation by lowering the level of TNF-α.[30]

In recent years, JAK inhibitors such as tofacitinib have emerged as effective targeted therapies for RA, directly inhibiting JAK-STAT signaling and providing rapid symptom relief and functional improvement. Tofacitinib, a pan-JAK inhibitor, has demonstrated significant efficacy in reducing disease activity and

improving patient-reported outcomes in both monotherapy and combination regimens. However, its safety profile, particularly regarding increased risks of infections, cardiovascular events, and thromboembolism, remains a clinical concern. In contrast, iguratimod exhibits a more selective immunomodulatory profile, primarily through inhibition of STAT3 phosphorylation and modulation of the T_H17/Treg balance, without broad suppression of multiple JAK isoforms. Our study shows that combining iguratimod with adalimumab achieves comparable or superior immunomodulatory and anti-inflammatory effects to those reported for tofacitinib combinations, while maintaining a favorable safety profile with no significant increase in adverse events. This suggests that iguratimod may offer a complementary mechanism to biologics with a potentially milder side-effect burden, representing a viable alternative for patients in whom JAK inhibitors are contraindicated or poorly tolerated.

RA patients are often accompanied by significant bone metabolism abnormalities, in which pathological hyperactivation of the JAK-STAT signaling pathway is not only involved in the inflammatory response but also affects the balance between bone formation and resorption by regulating the RANKL/OPG system.[12] In this study, we analyzed the effect of iguratimod coupled with adalimumab on bone metabolism in RA patients by detecting the changes of serum bone metabolism markers (N-MID, T-PINP, and β-CTX). Pretreatment analysis revealed comparable bone metabolism indices across both study groups ($p>0.05$). However, the levels of the bone formation markers N-MID and postintervention T-PINP levels demonstrated markedly higher values in the study group relative to controls (both However, postintervention levels of the bone formation markers N-MID and T-P1NP were significantly higher in the study group compared with the control group ($p<0.001$); meanwhile, the bone resorption marker β-CTX was significantly lower in the study group than in the control group ($p<0.001$). These results are consistent with the findings of Yari et al, who reprted that JAK inhibitors can reduce osteoclast activity by inhibiting STAT3 phosphorylation while promoting osteoblast differentiation.[31]

Pain management in RA is one of the core goals of treatment, and its underlying mechanisms involve inflammation-mediated joint damage and peripheral nerve sensitization due to activation of the JAK-STAT signaling pathway. The greater pain reduction in the combination group likely reflects not only improved control of peripheral inflammation (via TNF-α and IL-6 suppression) but also central modulation of pain signaling. STAT3 activation in spinal glial cells is known to amplify nociceptive transmission; thus, iguratimod-mediated STAT3 inhibition may attenuate this central sensitization component, contributing to the superior analgesic effect observed, which is also agrees with Simon et al's proposed idea of inhibiting the neurotransmission of pain signaling.[32]

In the therapeutic evaluation of RA, the ACR50 response is an important indicator of clinical efficacy, which is relatively close to the degree of inhibition of the JAK-STAT signaling pathway.[33] In this study, the study group exhibited significantly greater ACR50 achievement rate ($p=0.005$), corroborating Wajid and colleagues' findings regarding the therapeutic synergy between JAK inhibitors and biologics.[34] Iguratimod may enhance the anti-inflammatory effect of adalimumab through: 1) coordinated inhibition of proinflammatory cytokine biosynthesis (notably IL-6 and TNF-α)[35]; and 2) more effective inhibition of synovial hyperplasia.[36]

RA patients often present with significant joint dysfunction, and the pathological mechanisms are closely related to chronic synovitis and joint destruction mediated by the JAK-STAT signaling pathway.[12] In this study, we evaluated the effect of iguratimod combined with adalimumab on the improvement of mobility function in RA patients by FMAS. Pretreatment assessment revealed comparable FMAS scores for both upper and lower extremities across study cohorts (both $p>0.05$). After treatment, the study group demonstrated markedly elevated upper limb FMAS scores compared with the control group ($p<0.001$), the study group exhibited significantly improved lower limb FMAS performance compared with controls ($p<0.001$). These results are consistant with the findings of Kiełbowski et al who reported that JAK inhibitors improve joint function by rapidly suppressing the inflammatory response.[33] The present study confirms the synergistic effect of iguratimod combined with adalimumab in improving motor function in RA patients by a mechanism that may involve: 1) more effective control of synovitis through dual inhibition of the STAT and TNF-α pathways[37]; and 2) reduction of structural destruction of the joints, thereby preserving motor function.[38] This finding has substantial clinical value, particularly for RA patients with high disease activity and significant functional impairment.

JAK-STAT signaling pathway inhibitors have demonstrated significant efficacy in the treatment of RA; however, their safety-_particularly the risk when combined with biologics-still needs to be fully evaluated. Our analysis revealed comparable adverse event rates between the study group and the control group (p>0.05), and neither cohort exhibited serious treatment-emergent adverse effects.

The sustained modulation of the JAK-STAT pathway through combined therapy may hold significant long-term clinical implications for RA management. First, by simultaneously targeting upstream TNF-α and downstream STAT3 phosphorylation, this dual approach could lead to more durable disease control and delay the progression of structural joint damage, thereby preserving physical function over extended periods. Second, the observed restoration of the $CD4^+/CD8^+$ T-cell balance and reduction in bone resorption markers (eg, β-CTX) suggest a potential for long-term immune homeostasis and bone protection, possibly reducing the incidence of osteoporosis and fragility fractures in RA patients. However, the long-term safety profile of such combination regimens requires careful monitoring, particularly regarding infection risk, hepatic tolerance, and mucosal integrity. Future longitudinal studies with extended follow-up are warranted to confirm whether the early immunomodulatory and osteoprotective benefits translate into sustained clinical remission, improved quality of life, and reduced disability over years of treatment.

Despite the promising findings, this study has several limitations inherent to its retrospective design. First, the nonrandomized allocation of treatment regimens may introduce selection bias, as patients were assigned based on clinical decisions rather than random assignment. Second, unmeasured confounding factors, such as variations in disease duration prior to treatment, concomitant medications, or lifestyle factors, could have influenced the outcomes. Third, the relatively small sample size and single-center setting may limit the generalizability of the results. Fourth, the absence of a placebo control or blinding procedures could lead to assessment bias. Fifth, the retrospective nature precluded the blinding of evaluators for clinical outcome assessments such as ACR50 and FMAS scores, which may have introduced observer bias. Although glucocorticoid dosages and regimens were standardized and maintained consistently between groups as part of the study protocol, the dosage was not individually titrated or adjusted based on a prespecified algorithm, which could represent a confounding variable. Disease activity assessment using DAS28-ESR followed standard procedures; however, its components (eg, patient global assessment) remain subjective and could be influenced by the lack of blinding. Finally, the observational nature of the study precludes definitive causal inferences regarding the synergistic effects of iguratimod and adalimumab. Future prospective, randomized, multicenter trials with larger cohorts and longer follow-up periods are warranted to validate these preliminary findings and to better establish the therapeutic synergy and long-term safety profile of this combination regimen.

Our findings position iguratimod-adalimumab combination therapy as a potentially strategic option for RA management, particularly in TNF-inhibitor inadequate responders or patients with high inflammatory burden and bone erosion risk. By simultaneously targeting upstream (TNF-α) and downstream (JAK-STAT) pathways, this dual-mechanism approach achieved superior multidomain improvement over monotherapy. This evidence may inform future refinements of treat-to-target guidelines, suggesting that combined targeted therapy could be considered when rapid immune reconstitution and osteoprotection are priorities. However, further validation in prospective trials is needed before formal guideline integration.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of The Second Hospital of Lanzhou University

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

AI ASSISTANCE DISCLOSURE

No AI tools were used in the preparation of this manuscript.

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