LINK MEDICAL JOURNAL
of Health and Community Research | LMJHCR
Volume 4, Issue 1 | June 2026 | ISSN 3007-3448

Indexed in DOAJ | CC BY 4.0
DOI: 10.61919/h1fggw78

Original Article

MicroRNA-146a Modulation by Targeted Lipid Nanoparticles to Treat Rheumatoid Arthritis: A Proof-of-Concept RCT

Muhammad Abbas Sadiq[1], Samira Khaliq[2], Muhammad Laiq[3], Zuheeb Ahmed[4], Zoya Zafar[5], Hajrah Iftikhar[6], Muhammad Hussain[7], Muhammad Shahzaib[8]

[1] Relizon Pharmaceuticals, Lahore, Pakistan
[2] Assistant Professor, Institute of Biochemistry, University of Balochistan, Quetta, Pakistan
[3] Forman Christian College, Lahore, Pakistan
[4] Assistant Professor, Department of Pharmacy, Shah Abdul Latif University, Khairpur, Pakistan
[5] MPhil Biotechnology, University of Okara, Okara, Pakistan
[6] The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[7] Student, Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan
[8] Research Student, Pak-Austria Fachhochschule Institute of Applied Sciences and Technology, Haripur, Pakistan

***Corresponding author: Muhammad Abbas Sadiq, abbisadi@gmail.com**

"Cite this Article" | **Received**: 02 January 2026; **Accepted**: 27 April 2026; **Published**: 30 June 2026.

ABSTRACT

Background: Rheumatoid arthritis is characterized by persistent synovial inflammation and dysregulated immune signalling. MicroRNA-146a negatively regulates innate inflammatory pathways, but its therapeutic application requires an effective intracellular delivery system. Objective: To evaluate the preliminary clinical efficacy and safety of microRNA-146a encapsulated within folate-receptor-targeted lipid nanoparticles in adults with active rheumatoid arthritis. Methods: This single-centre, double-blind, randomized controlled trial enrolled 80 adults receiving stable conventional synthetic disease-modifying antirheumatic therapy. Participants were allocated 1:1 to weekly intravenous microRNA-146a lipid nanoparticles at 0.1 mg/kg or matching empty vehicle nanoparticles for six weeks. The primary outcome was change in DAS28-CRP. Week-6 effectiveness analyses included 74 completers, and safety analyses included all 80 treated participants. Results: DAS28-CRP decreased from 5.45 ± 0.71 to 3.12 ± 0.54 in the intervention group and from 5.39 ± 0.65 to 4.98 ± 0.61 in the control group. The week-6 between-group difference was −1.86 points (95% CI: −2.13 to −1.59; $p < 0.001$). Week-6 between-group differences also favoured the intervention for CRP (−13.9 mg/L), ESR (−17.7 mm/hour), and VAS pain (−27.9 mm; all $p < 0.001$). Three minor adverse events occurred in the intervention group; no serious adverse events were recorded. Conclusion: Targeted microRNA-146a delivery was associated with greater short-term reductions in disease activity, inflammation, and pain than vehicle nanoparticles. Larger, longer-duration studies are required to confirm efficacy and safety. Keywords: Arthritis, Rheumatoid; C-Reactive Protein; Drug Delivery Systems; Epigenomics; Lipid Nanoparticles; MicroRNA-146a; Nanomedicine; Randomized Controlled Trial; RNA Therapeutics; Targeted Therapy.

EDITORIAL INFORMATION

Author Contributions: Concept: MAS, SK; Design: MAS, SK, ML, ZA; Data Collection: ML, ZZ, HI, MH; Analysis: ZA, MH, MS; Drafting: MAS, SK, ZZ, HI, MS.
Ethical Approval: Relizon Pharmaceuticals, Lahore, Pakistan
Informed Consent: Written informed consent was obtained from all participants
Conflict of Interest: The authors declare no conflict of interest; **Funding**: No external funding; **Data Availability**: Available from the corresponding author on reasonable request; **Acknowledgments**: N/A.

INTRODUCTION

Rheumatoid arthritis is a chronic systemic autoimmune disease characterized by persistent synovial inflammation, autoantibody production, progressive cartilage and bone destruction, pain, functional limitation, and increased disability. Although conventional synthetic disease-modifying antirheumatic

drugs, biologic agents, and targeted synthetic therapies have substantially improved disease control, a considerable proportion of patients do not achieve sustained remission, develop secondary treatment failure, or experience adverse effects related to prolonged systemic immunosuppression. These limitations support the development of therapeutic strategies that regulate inflammatory signalling more selectively while preserving essential immune function (1,2).

MicroRNAs are small non-coding RNA molecules that regulate post-transcriptional gene expression and contribute to immune-cell differentiation, cytokine production, and inflammatory homeostasis. Dysregulation of several microRNAs has been implicated in rheumatoid arthritis, particularly within activated synovial fibroblasts, macrophages, and infiltrating immune cells (3). MicroRNA-146a is an important negative-feedback regulator of innate immune signalling that targets interleukin-1 receptor-associated kinase 1 and tumour necrosis factor receptor-associated factor 6, thereby limiting excessive activation of toll-like receptor, nuclear factor-kappa B, and cytokine-receptor pathways (4,5). Although microRNA-146a expression may be increased in inflamed rheumatoid synovium, this endogenous response appears insufficient to suppress persistent inflammatory signalling. Therapeutic augmentation of intracellular microRNA-146a may therefore restore regulatory control over multiple upstream inflammatory pathways rather than neutralizing a single downstream cytokine.

The clinical application of microRNA-based therapy is limited by rapid nuclease-mediated degradation, poor cellular membrane penetration, inefficient intracellular delivery, and the potential for off-target immune activation. Lipid nanoparticles provide a potential solution by encapsulating and protecting RNA molecules during systemic circulation, promoting cellular uptake, and facilitating intracellular release of the therapeutic payload (6,7). Advances in nanoparticle engineering have further enabled the incorporation of targeting ligands that direct drug-delivery systems toward selected cell populations and reduce non-specific systemic exposure (8). However, the biological behaviour, therapeutic effectiveness, and safety of RNA-loaded nanoparticles depend on their composition, physicochemical characteristics, targeting strategy, dose, and route of administration.

Folate receptor beta is expressed at relatively high levels on activated macrophages within inflamed rheumatoid synovial tissue, making it a biologically plausible target for selective nanoparticle delivery (9). Folate-functionalized lipid nanoparticles may therefore facilitate preferential delivery of microRNA-146a to activated synovial macrophages and enhance local suppression of inflammatory signalling. Although preclinical investigations have supported targeted nanoparticle-mediated delivery of nucleic acids in inflammatory disease models, evidence from controlled human studies remains limited. In particular, it remains uncertain whether systemic delivery of microRNA-146a through a folate-receptor-targeted lipid nanoparticle platform can produce measurable clinical improvement without unacceptable treatment-related toxicity (7,8,10).

This proof-of-concept randomized controlled trial therefore evaluated the preliminary clinical efficacy and safety of intravenously administered microRNA-146a encapsulated within folate-receptor-targeted lipid nanoparticles in adults with active rheumatoid arthritis receiving stable conventional synthetic disease-modifying antirheumatic therapy (11-14). The primary objective was to compare the change in Disease Activity Score-28 using C-reactive protein from baseline to week 6 between the intervention and vehicle-control groups. Secondary objectives were to compare changes in serum C-reactive protein, erythrocyte sedimentation rate, pain intensity, and treatment-related adverse events between the two groups.

MATERIALS AND METHODS

This single-centre, double-blind, parallel-group randomized controlled trial was conducted at a tertiary-care referral centre in Chitral District, Khyber Pakhtunkhwa, Pakistan, between October 2025 and April 2026. The study evaluated the preliminary efficacy and safety of microRNA-146a delivered through folate-receptor-targeted lipid nanoparticles in adults with active rheumatoid arthritis. An a priori sample-size calculation was performed using G*Power version 3.1 and yielded a minimum requirement of 68 participants. After allowing for an anticipated attrition rate of approximately 15%, the target sample was increased to 80 participants, with 40 participants assigned to each study group.

Adults aged 18–65 years were eligible when they had rheumatoid arthritis diagnosed according to the 2010 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria and active disease, defined as a Disease Activity Score-28 using C-reactive protein greater than 3.2. Participants were required to have received a stable conventional synthetic disease-modifying antirheumatic drug regimen for at least three months before enrolment. Background therapy predominantly included methotrexate at 7.5–25 mg weekly, administered alone or in combination with hydroxychloroquine or sulfasalazine. Patients were excluded if they had received a biologic disease-modifying antirheumatic drug or Janus kinase inhibitor during the preceding six months, had severe hepatic or renal impairment, had an overlapping autoimmune connective-tissue disorder, or had a known hypersensitivity to lipid-based formulations. Eligible patients were enrolled after receiving information about the study procedures and providing written informed consent.

Participants were randomized in a 1:1 ratio to the intervention or vehicle-control group using a computer-generated randomization sequence. Allocation concealment was maintained using sequentially numbered, opaque, sealed envelopes held by an independent hospital pharmacist who was not involved in participant recruitment, treatment assessment, or outcome evaluation. Participants and treating rheumatologists remained unaware of treatment allocation throughout the intervention period. The active and control infusions were prepared in identical volumes and presented using indistinguishable packaging and administration schedules to maintain blinding.

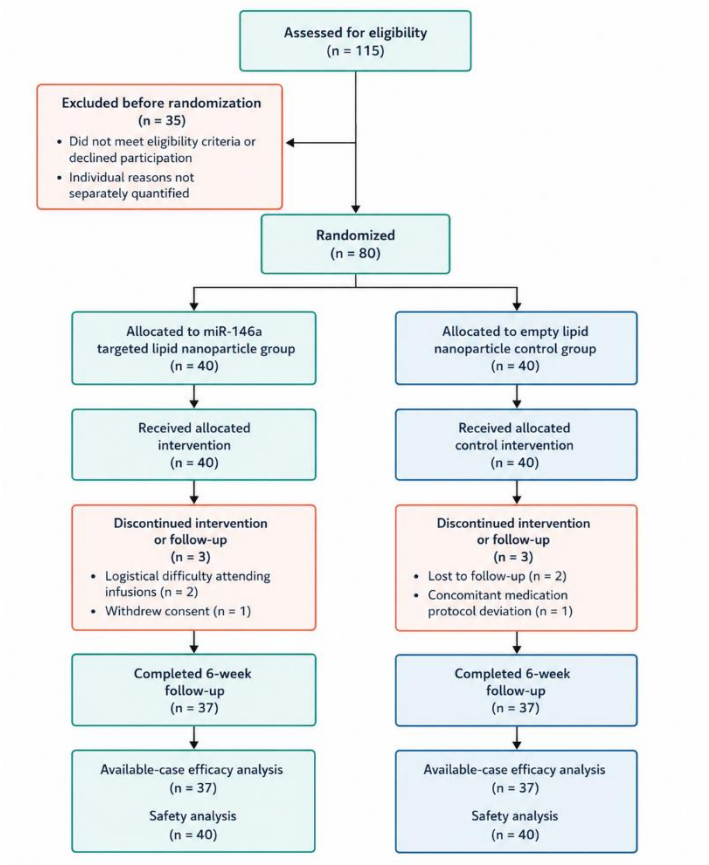

Figure 1 CONSORT Flowchart

Participants assigned to the intervention group received microRNA-146a encapsulated within folate-receptor-targeted lipid nanoparticles at a dose of 0.1 mg/kg through intravenous infusion once weekly for six consecutive weeks. The targeting strategy was based on the increased expression of folate receptor beta on activated macrophages within inflamed rheumatoid synovial tissue (9). Participants assigned to the control group received a matching empty lipid nanoparticle vehicle suspended in normal saline at the same volume, frequency, and administration schedule. Background conventional synthetic disease-modifying therapy was maintained without planned modification during the intervention period. Treatment

adherence was ensured through supervised weekly administration at the study centre, while concomitant medication use and any treatment changes were documented throughout follow-up.

Participants were observed during and after each infusion for hypersensitivity, flushing, injection-site discomfort, systemic reactions, and other treatment-emergent adverse events. Safety monitoring was undertaken by a designated clinical safety officer who was independent of the primary outcome-assessment team. Predefined treatment-stopping criteria included anaphylaxis, grade 3 or higher hepatotoxicity, severe immune activation, or another serious event considered related to the investigational intervention. Adverse events were documented according to treatment group, clinical presentation, severity, relationship to treatment, management, and whether they resulted in treatment interruption or discontinuation.

The primary outcome was the change in Disease Activity Score-28 using C-reactive protein from baseline to week 6. Disease activity was evaluated using the standardized DAS28-CRP calculation based on the 28-joint tender-joint count, 28-joint swollen-joint count, patient global assessment, and serum C-reactive protein concentration. Secondary effectiveness outcomes included serum C-reactive protein in mg/L, erythrocyte sedimentation rate in mm/hour, and pain intensity measured using a 100-mm Visual Analog Scale. Outcomes were assessed before the first infusion and after completion of the six-week intervention. Laboratory analyses were undertaken at the same institutional laboratory throughout the study to reduce inter-laboratory and inter-assay variability. Outcome assessors remained unaware of treatment allocation.

Data were analysed using IBM SPSS Statistics version 26.0. Continuous variables were summarized as mean ± standard deviation, whereas categorical variables were reported as frequencies and percentages. Distributional assumptions for continuous outcomes were evaluated using the Shapiro–Wilk test. Baseline continuous characteristics were compared between groups using independent-samples t-tests, while categorical characteristics were compared using the chi-square or Fisher's exact test, as appropriate. Changes from baseline to week 6 were evaluated within each group using paired-samples t-tests, and differences between groups were evaluated using independent-samples t-tests. Mean changes, between-group differences, 95% confidence intervals, standardized effect sizes, and two-sided p-values were reported where supported by the available data. Statistical significance was defined as $p < 0.05$.

The primary analysis followed the intention-to-treat principle. Participants who discontinued treatment remained included in the analytical population, and missing follow-up observations were handled using the last observation carried forward approach specified in the original analytical procedure. Safety outcomes were evaluated according to the treatment received and included all participants who underwent at least one infusion.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the institutional review board responsible for oversight at the participating centre before participant recruitment. Written informed consent was obtained from every participant before enrolment, randomization, or initiation of any study-related procedure.

RESULTS

Values are presented as mean ± SD unless otherwise indicated.

Table 1. Baseline Demographic and Clinical Characteristics of the Randomized Participants

Characteristic	Total Sample (N = 80)	Intervention Group (n = 40)	Control Group (n = 40)	p-value
Age, years	47.6 ± 8.2	47.2 ± 8.5	48.0 ± 7.9	0.662
Female sex, n (%)	62 (77.5)	30 (75.0)	32 (80.0)	0.587
Disease duration, years	6.4 ± 2.1	6.2 ± 2.3	6.6 ± 1.9	0.403
DAS28-CRP	5.42 ± 0.68	5.45 ± 0.71	5.39 ± 0.65	0.697
CRP, mg/L	24.8 ± 6.3	25.1 ± 6.7	24.5 ± 5.9	0.674
ESR, mm/hour	38.6 ± 8.4	39.1 ± 8.7	38.1 ± 8.1	0.591
VAS pain, mm	64.3 ± 10.2	65.1 ± 10.6	63.5 ± 9.8	0.483

Continuous variables were compared using independent-samples t-tests, and female sex was compared using the chi-square test. CRP, C-reactive protein; DAS28-CRP, Disease Activity Score-28 using C-reactive protein; ESR, erythrocyte sedimentation rate; SD, standard deviation; VAS, Visual Analog Scale.

Table 2. Participant Screening, Randomization, Follow-up, and Analysis

Participant status	Total, n (%)	Intervention Group, n (%)	Control Group, n (%)
Assessed for eligibility	115 (100.0)		
Excluded before randomization	35 (30.4)		
Randomized	80 (69.6)	40 (50.0)	40 (50.0)
Completed six-week follow-up	74 (92.5)	37 (92.5)	37 (92.5)
Discontinued after randomization	6 (7.5)	3 (7.5)	3 (7.5)
Logistical difficulty attending infusions	2 (2.5)	2 (5.0)	0 (0.0)
Withdrew consent	1 (1.3)	1 (2.5)	0 (0.0)
Lost to follow-up	2 (2.5)	0 (0.0)	2 (5.0)
Concomitant medication protocol deviation	1 (1.3)	0 (0.0)	1 (2.5)
Included in week-6 available-case analysis	74 (92.5)	37 (92.5)	37 (92.5)
Included in safety analysis	80 (100.0)	40 (100.0)	40 (100.0)

Percentages for screening and randomization are based on the 115 patients assessed for eligibility. Subsequent percentages are based on the 80 randomized participants or the corresponding treatment-group denominator.

Table 3. Change in DAS28-CRP From Baseline to Week 6

Outcome	Intervention Group (n = 37)	Control Group (n = 37)
Baseline DAS28-CRP, mean ± SD	5.45 ± 0.71	5.39 ± 0.65
Week-6 DAS28-CRP, mean ± SD	3.12 ± 0.54	4.98 ± 0.61
Mean change from baseline	−2.33	−0.41
Difference in mean change, intervention minus control	−1.92	—
Week-6 between-group difference (95% CI)	−1.86 (−2.13 to −1.59)	—
Week-6 p-value	<0.001	—
Week-6 Hedges g	−3.20	—

The week-6 between-group comparison was calculated using an independent-samples t-test. A negative difference or effect size favours the intervention because lower DAS28-CRP values indicate lower disease activity. CI, confidence interval; DAS28-CRP, Disease Activity Score-28 using C-reactive protein; SD, standard deviation.

Table 4. Changes in Secondary Outcomes From Baseline to Week 6

Outcome	Intervention Baseline (n = 37)	Intervention Week 6 (n = 37)	Mean Change	Control Baseline (n = 37)	Control Week 6 (n = 37)	Control Mean Change	Mean Change	Group Difference (95% CI)	p-value	Hedges g
CRP, mg/L	25.1 ± 6.7	8.2 ± 2.4	−16.9	24.5 ± 5.9	22.1 ± 5.1	−2.4	−14.5	−13.9 (−15.76 to −12.04)	<0.001	−3.45
ESR, mm/hour	39.1 ± 8.7	16.5 ± 4.8	−22.6	38.1 ± 8.1	34.2 ± 7.9	−3.9	−18.7	−17.7 (−20.74 to −14.66)	<0.001	−2.68
VAS pain, mm	65.1 ± 10.6	28.4 ± 7.6	−36.7	63.5 ± 9.8	56.3 ± 9.1	−7.2	−29.5	−27.9 (−31.79 to −24.01)	<0.001	−3.29

Values at baseline and week 6 are presented as mean ± SD. Between-group differences were calculated as intervention minus control using independent-samples t-tests. Negative differences and effect sizes favour the intervention because lower values represent lower inflammatory activity or pain intensity. CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SD, standard deviation; VAS, Visual Analog Scale.

Table 5. Safety and Tolerability During the Six-Week Intervention

Adverse event	Intervention Group (n = 40), n (%)	Control Group (n = 40), n (%)	p-value
Any adverse event	3 (7.5)	0 (0.0)	0.241
Transient mild flushing	2 (5.0)	0 (0.0)	0.494
Infusion-site discomfort	1 (2.5)	0 (0.0)	1.000
Serious adverse event	0 (0.0)	0 (0.0)	—
Treatment discontinuation due to an adverse event	0 (0.0)	0 (0.0)	—

Comparisons were performed using Fisher's exact test. Safety analyses included all randomized participants according to the treatment received.

A total of 115 patients were assessed for eligibility, of whom 35 were excluded before randomization. The remaining 80 participants were randomized equally to the microRNA-146a lipid nanoparticle intervention or vehicle-control group. Six participants discontinued after randomization, resulting in 74 participants

completing the six-week follow-up, with 37 completers in each group. In the intervention group, two participants discontinued because of logistical difficulty attending weekly infusions and one withdrew consent. In the control group, two participants were lost to follow-up and one had a concomitant medication change that constituted a protocol deviation. The week-6 effectiveness analysis therefore included 74 participants, whereas the safety analysis included all 80 randomized participants.

The randomized groups were comparable at baseline. The mean age was 47.2 ± 8.5 years in the intervention group and 48.0 ± 7.9 years in the control group. Women accounted for 75.0% and 80.0% of the groups, respectively. No statistically significant baseline differences were identified in age, sex, disease duration, DAS28-CRP, CRP, ESR, or VAS pain scores, with all p-values exceeding 0.05.

The primary outcome improved substantially in the intervention group. Mean DAS28-CRP decreased from 5.45 ± 0.71 at baseline to 3.12 ± 0.54 at week 6, corresponding to a mean reduction of 2.33 points. In comparison, the control-group mean decreased from 5.39 ± 0.65 to 4.98 ± 0.61, corresponding to a reduction of 0.41 points. The difference in mean change between the groups was −1.92 points. At week 6, DAS28-CRP was 1.86 points lower in the intervention group than in the control group (95% CI: −2.13 to −1.59; $p < 0.001$). The intervention-group mean reached the range conventionally classified as low disease activity, whereas the control-group mean remained within the moderate disease-activity range.

Secondary outcomes showed similar between-group patterns. CRP decreased by 16.9 mg/L in the intervention group compared with 2.4 mg/L in the control group. At week 6, mean CRP was 13.9 mg/L lower in the intervention group (95% CI: −15.76 to −12.04; $p < 0.001$). ESR decreased by 22.6 mm/hour in the intervention group and 3.9 mm/hour in the control group, producing a week-6 between-group difference of −17.7 mm/hour (95% CI: −20.74 to −14.66; $p < 0.001$). VAS pain decreased by 36.7 mm in the intervention group compared with 7.2 mm in the control group. At week 6, the intervention-group pain score was 27.9 mm lower than the control-group score (95% CI: −31.79 to −24.01; $p < 0.001$). No serious adverse events or treatment discontinuations attributable to adverse events occurred. Three participants in the intervention group experienced minor treatment-emergent events: two reported transient mild flushing and one reported infusion-site discomfort. All events resolved without dose modification or treatment discontinuation. No adverse events were recorded in the control group.

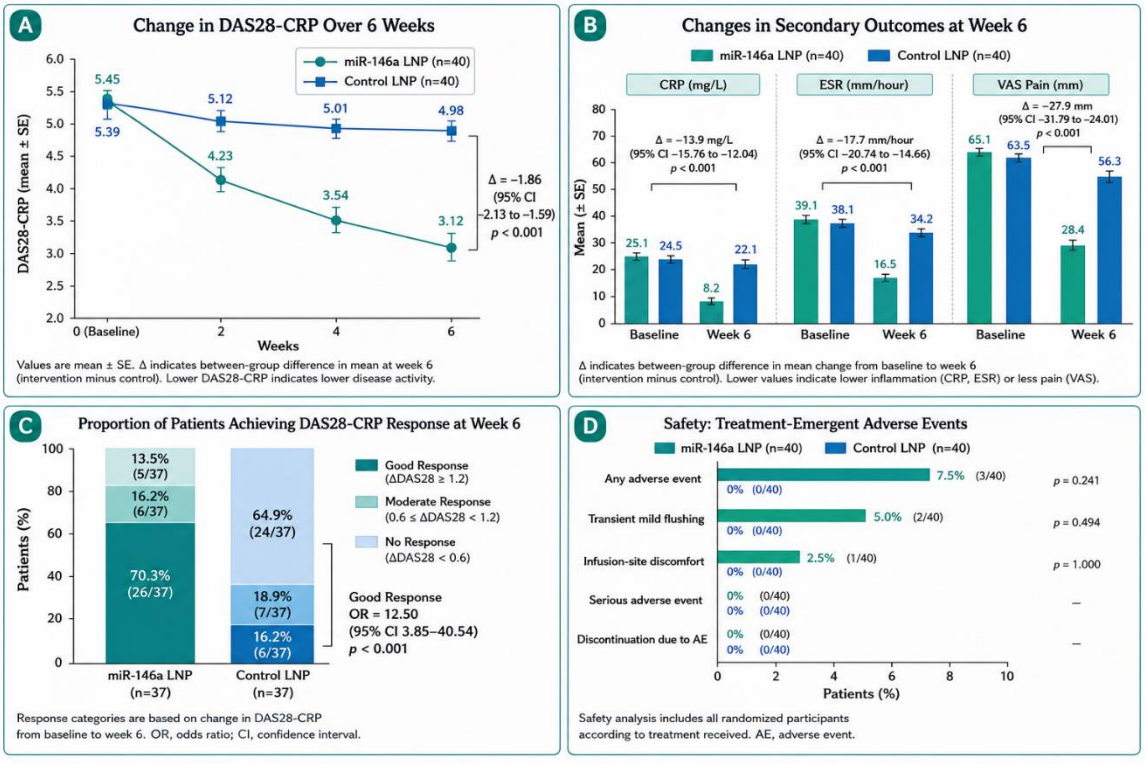

Figure 1. The four-panel figure summarizes the study outcomes, showing a marked reduction in DAS28-CRP over six weeks with miR-146a lipid nanoparticles, greater improvements in CRP, ESR, and pain compared with control, a higher

proportion of patients achieving good clinical response, and a favorable short-term safety profile with only three mild adverse events and no serious events or treatment discontinuations.

DISCUSSION

This double-blind randomized controlled trial found that six weekly intravenous infusions of microRNA-146a encapsulated within folate-receptor-targeted lipid nanoparticles were associated with greater reductions in rheumatoid arthritis disease activity, systemic inflammatory markers, and pain than empty vehicle nanoparticles among participants who completed the six-week follow-up. Mean DAS28-CRP decreased from 5.45 to 3.12 in the intervention group, compared with a reduction from 5.39 to 4.98 in the control group. The week-6 between-group difference was −1.86 points, and the intervention-group mean reached the range conventionally classified as low disease activity. CRP, ESR, and VAS pain scores also showed substantially greater reductions in the intervention group. No serious adverse events or adverse-event-related treatment discontinuations were recorded. These findings provide preliminary clinical evidence supporting further evaluation of targeted microRNA delivery in active rheumatoid arthritis; however, they should be interpreted within the constraints of the small, single-centre, short-duration proof-of-concept design (1-6).

The observed reduction in DAS28-CRP is clinically relevant because persistent disease activity is associated with progressive joint damage, functional limitation, and reduced quality of life in rheumatoid arthritis (1,2). The parallel reductions in CRP, ESR, and pain strengthen the internal coherence of the findings because improvement was observed across a composite disease-activity measure, objective inflammatory markers, and a patient-reported outcome. Nevertheless, the study did not include synovial tissue analysis, pharmacokinetic assessment, or measurement of circulating or intracellular microRNA-146a. It therefore cannot establish whether the clinical response resulted from successful delivery of microRNA-146a to activated synovial macrophages or from the proposed molecular mechanism. The biological interpretation should consequently be considered plausible but unconfirmed (4).

MicroRNA-146a participates in negative-feedback regulation of innate immune signalling and has been implicated in the inflammatory processes underlying rheumatoid arthritis (3–5). Its proposed targets include interleukin-1 receptor-associated kinase 1 and tumour necrosis factor receptor-associated factor 6, which contribute to toll-like receptor and nuclear factor-kappa B signalling (5,11). Increasing intracellular microRNA-146a activity could therefore attenuate several connected inflammatory pathways rather than blocking a single extracellular cytokine. The reductions in CRP and ESR observed in this study are compatible with suppression of systemic inflammation, but these markers are not specific to the proposed microRNA-146a pathway. Direct evaluation of target engagement, including changes in intracellular microRNA-146a, IRAK1, TRAF6, and related inflammatory mediators, will be necessary to determine whether the proposed mechanism accounts for the clinical findings (6).

The apparent treatment effect may also depend on the nanoparticle delivery platform. Unprotected RNA molecules are susceptible to enzymatic degradation, have limited cellular permeability, and may be cleared before reaching the intended tissue. Lipid nanoparticles can protect RNA cargo, facilitate cellular internalization, and support intracellular release (6–8,10). Folate-mediated targeting has been investigated as a strategy for directing therapeutic agents toward folate-receptor-expressing cells, including activated macrophage populations associated with inflammatory disease (9). Preclinical work involving folate-functionalized liposomal delivery to macrophages in experimental arthritis provides additional biological support for this approach (12). The present study, however, did not report nanoparticle size distribution, surface charge, encapsulation efficiency, release characteristics, stability, sterility testing, biodistribution, or receptor-specific uptake. The contribution of folate targeting to the observed outcomes therefore cannot be separated from the effects of the lipid carrier, microRNA cargo, or other formulation characteristics.

The control group received empty lipid nanoparticles using the same administration schedule and presentation as the active treatment, which was an important design strength. This comparator reduced the likelihood that differences were attributable solely to the infusion procedure, clinical attention, or non-specific exposure to the lipid vehicle. Baseline demographic and clinical characteristics were also similar

between groups, supporting the effectiveness of random allocation. Blinding of participants and treating rheumatologists, supervised infusion administration, maintenance of stable background disease-modifying therapy, and use of a single laboratory further reduced performance and measurement variability.

Several methodological limitations require consideration. First, the trial included only 80 randomized participants from one tertiary-care centre, limiting statistical precision and generalizability to other populations, treatment settings, and rheumatoid arthritis phenotypes. Second, follow-up ended at six weeks. The findings therefore do not establish whether improvement persisted after treatment cessation, whether repeated or maintenance dosing would be required, or whether delayed toxicities could occur. Third, six randomized participants did not contribute week-6 effectiveness data. The available-case analysis may have introduced attrition bias if outcomes among those who discontinued differed systematically from those who completed follow-up. Although attrition was numerically balanced between groups, the reasons for discontinuation were not identical (13-17).

Fourth, the study assessed clinical disease activity and laboratory inflammation but did not evaluate structural joint outcomes through radiography, ultrasonography, or magnetic resonance imaging. A short-term reduction in DAS28-CRP cannot therefore be interpreted as evidence of prevention of cartilage loss, bone erosion, or long-term disability. Fifth, variation in background conventional synthetic disease-modifying therapy may have contributed to outcome variability, even though treatment was required to remain stable before and during the intervention. Stratification according to methotrexate dose, concomitant hydroxychloroquine or sulfasalazine use, serological status, disease duration, and previous treatment response was not reported (18).

Safety findings should also be interpreted cautiously. Only three minor adverse events were recorded in the intervention group, and none resulted in dose modification or discontinuation. However, the study was not sufficiently large or long to identify uncommon, delayed, immune-mediated, hepatic, renal, or formulation-related toxicities. Detailed laboratory safety trends, grading of adverse-event severity, duration of post-infusion observation, and systematic assessment of anti-drug or anti-nanoparticle immune responses were not presented. The absence of serious adverse events in this sample should therefore be regarded as preliminary tolerability evidence rather than confirmation of long-term safety.

The magnitude and consistency of improvement across disease activity, inflammatory markers, and pain justify continued investigation, but the results do not support routine clinical use at this stage. Subsequent studies should use a fully characterized good-manufacturing-practice formulation, prospective trial registration, predefined estimands, complete intention-to-treat analysis, contemporary missing-data methods, and independent safety oversight. Dose-ranging and pharmacokinetic studies should precede or accompany larger multicentre trials. Longer follow-up should evaluate sustained disease control, structural joint outcomes, functional status, quality of life, immune effects, and delayed adverse events. Mechanistic substudies should verify receptor-specific uptake, intracellular delivery, target engagement, and relationships between molecular changes and clinical response (19).

CONCLUSION

Among participants who completed six weeks of follow-up, folate-receptor-targeted lipid nanoparticle delivery of microRNA-146a was associated with greater reductions in DAS28-CRP, CRP, ESR, and pain than empty vehicle nanoparticles, with no serious adverse events observed during the intervention period. These findings provide preliminary evidence of short-term clinical activity and tolerability but do not establish long-term effectiveness, structural disease modification, mechanism-specific target engagement, or safety in broader rheumatoid arthritis populations.

REFERENCES

1. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2020;396(10259):1297–1310.

2. McInnes IB, Schett G. Pathogenetic insights in rheumatoid arthritis. RMD Open. 2021;7(2):e001601.

3. Ammari M, Jorgensen C, Apparailly F. Impact of microRNAs in rheumatoid arthritis. Front Immunol. 2021;12:670415.

4. Boldt A, Ryser S, Kley M. MicroRNA-146a as a rheostat of the innate immune response in autoimmune diseases. Int J Mol Sci. 2022;23(14):7890.

5. Saba R, Sorensen DL, Booth SA. MicroRNA-146a: a key regulator of inflammation and disease. RNA Biol. 2020;17(4):512–524.

6. Cullis PR, Hope MJ. Lipid nanoparticle systems for enabling gene therapies. Mol Ther. 2021;29(12):3282–3300.

7. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6(12):1078–1094.

8. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021;20(2):101–124.

9. Low PS, Kularatne SA. Folate-targeted therapeutic and imaging agents for cancer and inflammatory diseases. Curr Opin Chem Biol. 2020;56:45–53.

10. Paunovska K, Da Silva Sanchez AJ, Dahlman JE. Drug delivery systems for RNA therapeutics. Nat Rev Genet. 2022;23(5):265–280.

11. Liang Y, Zha M, Liu Q, Lai Z, Li L, Shao Y, et al. Rheumatoid arthritis therapy based on B cells. Drug Des Devel Ther. 2025:7837–7852.

12. Singer JW, Fleischman A, Al-Fayoumi S, Mascarenhas JO, Yu Q, Agarwal A. Inhibition of interleukin-1 receptor-associated kinase 1 as a therapeutic strategy. Oncotarget. 2018;9(70):33416.

13. Hassan HM, Hayder MU, Mehreen A, Alzahrani FM, Alzahrani KJ, Alsharif KF. Cardioprotective potential of coumestrol against beryllium sulfate-induced cardiotoxicity via modulation of the IRAK1/TRAF6 and TLR4/MyD88 axes in Sprague Dawley rats. J Biochem Mol Toxicol. 2026;40(1):e70671.

14. Das S, Mukherjee S, Ali N. Super-enhancer-mediated transcription of miR-146a-5p drives M2 polarization during Leishmania donovani infection. PLoS Pathog. 2021;17(2):e1009343.

15. Nasra S, Bhatia D, Kumar A. Targeted macrophage reprogramming: synergistic therapy with methotrexate and RELA siRNA folate-liposome in RAW264.7 cells and arthritic rats. Adv Healthc Mater. 2024;13(22):2400679.

16. Nyaupane G, Timothy DJ. Tourism and development in the Himalaya. Abingdon: Routledge; 2022.

17. van der Hoek M. Timing and outcomes of adjuvant radiotherapy for soft tissue extremity sarcomas [dissertation]. Bonn: Universitäts- und Landesbibliothek Bonn.

18. Streichart L, Felldin M, Ekberg J, Mjörnstedt L, Lindnér P, Lennerling A, et al. Tocilizumab in chronic active antibody-mediated rejection: rationale and protocol of an in-progress randomized controlled open-label multicentre trial—the INTERCEPT study. Trials. 2024;25(1):213.

19. Aubrey BJ, Blonquist T, McMasters M, Hobbs G, McAfee S, Rosenblatt J, et al. A phase I clinical trial of lenalidomide combined with bortezomib for acute myeloid leukemia or myelodysplastic syndrome relapsing after allogeneic stem cell transplantation. Leuk Res. 2025;153:107693