EJR European Journal of Rheumatology

DOI: 10.5152/eurjrheum.2024.0086

Review

The Role and Mechanistic Effects of Methotrexate in the Treatment of Rheumatoid Arthritis

Yao Teng*, Lijuan Jiang*, Haifeng Yin*, Guo-Min Deng

Abstract

Methotrexate (MTX) remains a cornerstone in the management of rheumatoid arthritis (RA). Extensive basic pharmacological research has consistently demonstrated the efficacy of MTX in treating RA, while there is still insufficient understanding about the potential mechanisms. In this review, current knowledge on the mechanisms by which MTX acts in bone erosion and synovitis is summarized, and MTX-based combination therapies are discussed. Additionally, the bone-protective and anti-inflammatory roles of adenosine receptors are described, and biomarkers associated with MTX responders in RA are explored. This review sheds new light on future research directions for RA, as well as offers evidence-based recommendations to enhance the clinical utilization of MTX in RA treatment.

Keywords: Adenosine receptors; methotrexate; osteoporosis; rheumatoid arthritis; rheumatoid synovitis

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovitis and bone erosion, which may lead to irreversible disability and impose a significant financial burden on patients.[1]

The treatment goal for RA is to achieve disease remission or, at least, low disease activity for patients as quickly as possible, preventing disability, joint damage, and improving quality of life.[2] The treatment approaches for RA generally include disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs, and glucocorticoids.[3] Notably, methotrexate (MTX) as the first conventional DMARD holds a prominent position as a widely employed therapeutic agent in RA treatment due to its remarkable efficacy and cost-effectiveness.[4] Methotrexate therapy is correlated with a lower incidence of large joint replacement compared to treatment solely with biologic agents, suggesting its bone-protective characteristics in the management of RA.[5] Although MTX monotherapy serves as an effective initial treatment strategy, only approximately 30% of patients with RA manage to achieve remission or low disease activity through this monotherapy approach.[6] Notably, 1 study demonstrated that MTX achieved an evident clinical response by 12 weeks in RA treatment.[7] Thus, a significant number of patients necessitate MTX-based combination therapy in order to achieve this objective.

Mechanistically, MTX acts as a folate antagonist with anti-proliferative effects by reducing immune cell infiltration within the inflamed synovium in RA.[8] In addition, MTX exerts an anti-inflammatory role by promoting the release of adenosine, subsequently facilitating its binding to adenosine receptors (ARs) named A1R, A2aR, A2bR, and A3R, which have potential therapeutic implications of RA.[9]

Consequently, comprehensive exploration of the underlying mechanisms responsible for the therapeutic efficacy of MTX in RA is warranted. This review offers a summary of the anti-inflammatory and bone-protective roles of MTX in the treatment of RA and describes MTX-based therapies of RA.

Pathogenesis of Rheumatoid Arthritis

Mechanism of Methotrexate Reducing Synovitis in Rheumatoid Arthritis

RA is characterized by synovitis and bone erosion.[1] Extensive previous studies have substantiated the involvement of various immune cells in the pathogenesis of RA, including T cells, B cells, and monocytes/macrophages.[10] Methotrexate functions as a folate antagonist and may modulate the infiltration of these immune cells in the synovium of RA.[11]

ORCID iDs of the authors:
Y.T. XXX;
L.J. XXX;
H.Y. XXX;
G-M.D. XXX.

Cite this article as: Teng Y, Jiang L, Yin H, Deng G. The role and mechanistic effects of methotrexate in the treatment of rheumatoid arthritis. *Eur J Rheumatol.* 2025:12(4), -0086, 10.5152/eurjrheum.2025.24086.

Q1 Department of Rheumatology and Immunology, Union Hospital, , Huazhong University of Science and Technology Tongji College of Medicine, Wuhan, China

*These authors contributed equally to this work and share first authorship.Corresponding author: Guo-Min Deng
E-mail: gmdeng01@163.com

Received: September 13, 20245
Revision Requested: April 18, 2025
Last Revision Received: June 01, 2025
Accepted: June 02, 2025
Publication Date: December 31, 2025

Effects of Methotrexate on T Cells

RA is characterized by the abnormal activation of T helper (Th) cells, specifically imbalance between Th1 and Th2 cells, or Th17 and regulatory T (Treg) cell subsets.[12] Previous investigations have demonstrated that MTX can downregulate the ratio of Th1/Th2 cells by reducing the expression of the IL-12R and CXCR3 receptors.[13] Additionally, MTX has been shown to increase the population of Treg cells and induce a transition from Th1 to Th2 cells in RA rat models. Clinical studies have further supported that MTX ameliorates RA activity by normalizing the ratio of Th17 and Treg cells.[14] This piece of evidence demonstrates that MTX may suppress RA activity through restoring the balance between pro-inflammatory and anti-inflammatory T cell subsets.

Effects of Methotrexate on B Cells

Rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA) are autoantibodies characteristically found in RA.[15] Methotrexate has been reported to effectively suppress memory B cell function and augment the numbers of regulatory B cells in treatment of RA.[14,16] Methotrexate decreased ACPA titers in early RA.[17] In the CIA model, MTX treatment significantly reduced the severity of CIA by decreasing frequency of B cells.[18] These findings underscore that MTX exerts its anti-inflammatory effects through the suppression of B cell proliferation and differentiation, as well as the inhibition of pro-inflammatory cytokine secretion.

Main Points

- Methotrexate (MTX) achieves a notable clinical response after 3 months in treating rheumatoid arthritis (RA), and it holds a fundamental and central role in the management of RA.
- The objective of RA treatment is to achieve disease remission as soon as possible, indicating that MTX-based combination therapy is an essential approach for RA patients. However, the mechanism of MTX in the treatment of initial RA remains incompletely understood.
- Numerous RA patients may discontinue MTX treatment due to intolerance or the occurrence of unavoidable adverse effects. Research exploring potential biomarkers for MTX response assists doctors in more accurately assessing patients' treatment responses and tolerability, so that treatment plans can be adjusted promptly.

Effects of Methotrexate on Monocytes/Macrophages

Monocytes/macrophages have been shown to play an important role in the pathogenesis of RA.[19] Methotrexate can induce apoptosis of monocytic cell line in a time-dependent manner and upregulation of IL-1 and IL-6 in a dose-dependent manner.[20] In the CIA model, MTX drives macrophage polarization toward an anti-inflammatory M2 phenotype through epigenetic and metabolic reprogramming.[21] Clinical studies reported that MTX treatment decreased TNF-α levels in newly diagnosed untreated RA patients.[22] These findings suggest that MTX suppresses pro-inflammatory cytokine production and promotes a shift from pro-inflammatory M1 to anti-inflammatory M2 macrophages in the treatment of RA.

Mechanism of Methotrexate Ameliorating Bone Erosion in Rheumatoid Arthritis

Bone erosion is a characteristic feature of patients with RA, driven by inflammatory cells infiltrating the synovium. Macrophages stimulated by receptor activator of nuclear factor κB ligand (RANKL) can differentiate into mature osteoclasts, which are responsible for bone resorption.[23] Conversely, decoy receptor osteoprotegerin can reduce bone resorption.[24]

The mechanisms underlying how MTX prevents bone erosion in RA remain incompletely understood. Haynes et al[25] demonstrated that patients who received MTX for 6 months exhibited a higher OPG/RANKL ratio, which indicated a reduction in the risk of bone destruction. Moreover, clinical studies reported that MTX treatment was associated with reduced RANKL levels and inhibited bone injury in RA patients.[26] Mechanically, MTX significantly suppresses osteoclast formation by reducing RANKL-induced calcium influx, as well as the expression of NFATc1 and DC-STAMP, which are crucial for osteoclast differentiation.[27]

Mechanism of Methotrexate Via Adenosine Receptor Signaling in Rheumatoid Arthritis

MTX leads to an increased release of adenosine into the extracellular space, which subsequently regulates the function of immune cells by interacting with ARs expressed on neutrophils, macrophages, and T cells.[28] A previous study confirmed that MTX exerted its anti-inflammatory role by inducing adenosine release.[29] Subsequently, it was demonstrated that AR antagonists, theophylline and caffeine significantly reversed the anti-inflammatory effects of MTX in rat adjuvant arthritis model of RA,[30] which suggests adenosine extracellular accumulation of MTX played a key role in treating RA.

As shown in Figure 1, accumulating evidence indicates that adenosine is a potent regulator interacting with 4 AR subtypes, including A1R, A2aR, A2bR, and A3R.[31] A1R and A2aR are classified as high-affinity receptors, and are activated by physiological concentrations of extracellular adenosine, while A2bR and A3R activation generally require higher levels of adenosine.[8]

A1 receptor signaling mechanisms in cells of the immune system are not well-known. A1R can enhance chemotactic ability.[32] On the other side, A1R stimulation promoted multinucleated giant cell formation by decreasing ubiquitination and degradation of TRAF6.[33]

In a in vivo model of inflammation, MTX significantly increases adenosine release, resulting in a marked reduction in inflammation, which can be completely reversed by A2aR antagonists.[34] A2aR signaling can switch macrophages from an M1 phenotype to an M2-like phenotype, leading to an increase in the expression of the anti-inflammatory cytokine IL-10 and a decrease in the expression of IL-12 and TNF.[35] Blockade of A2aR significantly reduces Treg cells and enhances the response

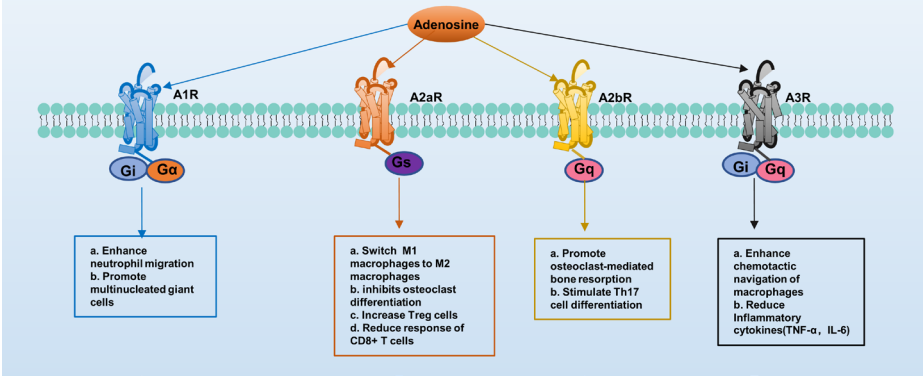

Figure 1. Schematic representation of the effects caused by 4 adenosine receptors activated by adenosine.

of CD8+ T cells.[36] In A2a-deficient mice, MTX failed to inhibit inflammation.[37] In addition, inflammatory cytokines such as TNFα can induce matrix metalloproteinase 3 (MMP3) production, which contributes to joint destruction in RA. Methotrexate treatment reduces TNFα-induced MMP-3 production in fibroblast-like synoviocytes via the A2aR signaling pathway.[38] Moreover, A2aR activation inhibits osteoclast differentiation. Adenosine released by MTX activates A2aR signaling, which also inhibit osteoclast formation in a mouse model of osteolysis caused by wear particles,[39] and it can suppress RANKL-driven osteoclastogenesis.[40] In the hypoxic microenvironments of RA, inhibition of A2bR prevents pathological bone resorption.[41] A2bR activation can stimulate T cells to differentiate into Th17 cells to enhance pro-inflammatory effects.[42] A3R is over-expressed in inflammatory cells and RA patients.[43] Moreover, targeting A3aR was identified as a novel anti-inflammatory strategy to inhibit inflammation in RA.[44]

Methotrexate exerts its anti-inflammatory and bone-protective roles by promoting adenosine release into the extracellular space in patients with RA; this process may be influenced by interactions with ARs (shown in Figure 2). The adenosine pathway, particularly through A2aR activation, serves as the central mechanism underlying MTX's therapeutic efficacy in RA, mediating both immunomodulation and joint protection. These findings not only explain MTX's clinical benefits but also reveal multiple opportunities for treatment optimization through receptor-targeted strategies. Future research should focus on translating these mechanistic insights into personalized therapeutic approaches, potentially revolutionizing RA management by combining MTX with selective AR modulators.

Methotrexate Monotherapy and Methotrexate-Based Combinations in the Treatment of Rheumatoid Arthritis

Methotrexate Monotherapy

Given its established efficacy and favorable safety profile, MTX should be considered an anchor drug in the initial treatment regimen for RA.[2] According to the 2021 American College of Rheumatology Guidelines, MTX monotherapy is recommended as first-line treatment over monotherapy with biologic or targeted synthetic DMARDs for DMARD-naïve patients with moderate-to-high disease activity.[45] Low-certainty evidence indicates the potential superiority of tocilizumab monotherapy over methotrexate monotherapy, while moderate-certainty evidence supports the superior efficacy of Janus Kinase (JAK) inhibitor monotherapy compared to MTX monotherapy.[46] Additionally, the recommendations specify that patients should maintain remission for at least 6 months before tapering MTX.[46]

A study conducted by Hetland et al[47] has demonstrated that the therapeutic efficacy of MTX monotherapy is comparable to MTX-based combination therapy (administered concurrently with certolizumab pegol, abatacept, or tocilizumab). On the contrary, Taylor et al[48] discovered that the use of MTX combined with baricitinib in the treatment of moderate to severe active RA has been shown to be superior to MTX monotherapy in terms of pain relief, duration of pain-free periods, and overall improvements in physical health. The 2 studies address distinct clinical scenarios: Hetland informs initial therapy in early RA, while Taylor guides treatment escalation in moderate to severe active RA. Methotrexate monotherapy is considered superior to combination therapy in mild-moderate RA.[47]

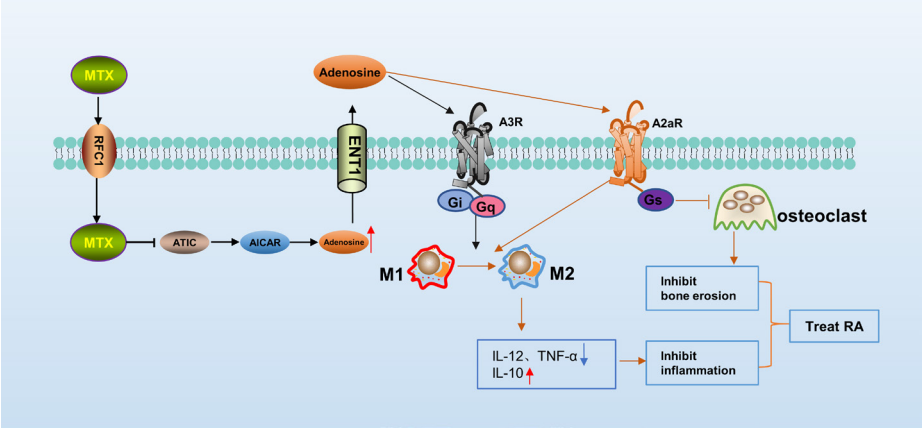

Figure 2. The adenosine signaling pathway of methotrexate (MTX) in treating rheumatoid arthritis (RA). Methotrexate is absorbed into cells via reduced folate carrier 1. Methotrexate inhibits the key enzyme 5-aminoimidazole-4-carboxamide ribonucleotide transformylase to increase the accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide, which transports adenosine out of the cell by equilibrative nucleoside transporter. The generated adenosine could activate the adenosine receptor. Consequently, M1 macrophages switch to M2 macrophages, and inflammatory cytokine expression is reduced. Moreover, A2aR activation inhibits osteoclast formation. Thereby, MTX plays a role in bone protection and anti-inflammatory effects in treating RA.

Methotrexate-Based Combinations

Given that the intricate pathophysiology of RA, clinical therapy involving first-line drug MTX does not always fulfill the therapeutic demands; thus, the use of MTX in combination with other drugs is recommended.

For patients with active RA who have had no or limited prior treatment with DMARDs, the study verified that administration of baricitinib in combination with MTX showed greater cumulative pain relief, reduced overall pain, and mild pain compared to monotherapy with MTX.[48] Furthermore, 30%-39% of these patients had received concomitant corticosteroids, which reflects the significance of corticosteroids in the treatment of early RA. For patients with active RA who are biologic-naïve and ACPA-positive, treatment with abatacept and MTX has demonstrated remarkable efficacy in improving clinical symptoms and inhibiting the progression of structural damage compared with treating MTX alone.[49] In addition, Verschueren et al[50] conducted an investigator-initiated, randomized, open-label, superiority trial in RA patients. The pragmatic clinical trial had a 2-year intervention period with a 1-year follow-up, enrolling over 300 diagnosed RA patients. The study found that combining MTX with either leflunomide or sulfasalazine showed no clinical advantage over MTX plus glucocorticoids, regardless of patients' risk stratification. Until now, the short-term use of MTX combined with glucocorticoids is the preferable strategy for early RA patients in the real word,[51] while glucocorticoids may cause osteoclast activation.[52] Notably, the previous study and other studies both demonstrate that the MTX-glucocorticoid combination therapy exerts protective effects against bone erosion in RA by functionally downregulating osteoclast activity.[53-55]

Collectively, MTX monotherapy remains the cornerstone for mild-moderate RA due to its balance of efficacy and safety, whereas

MTX-based combinations emerge as critical therapeutic strategies for managing high-risk populations or refractory cases. Treatment selection requires a multidimensional assessment of disease characteristics and patient factors, emphasizing the necessity for personalized approaches. Future research should clarify biomarker-guided strategies.

Adverse Effects of Methotrexate

Side effects accompanied by the clinical use of MTX in treating RA may hinder patients from continuing their treatment.[56] Long-term use of MTX frequently results in gastrointestinal, liver, lung, and renal toxicity complications. Besides, MTX can inhibit hematopoietic stem cell proliferation and differentiation, leading to bone marrow suppression manifested as anemia, leukopenia, and thrombocytopenia.[56] Approximately 30% of RA patients discontinue MTX therapy due to adverse effects.[57] To prevent toxicity, clinicians advise RA patients on MTX therapy to maintain plasma levels under 0.01 μmol/L through frequent monitoring.[58] In addition, MTX exhibits well-documented teratogenicity, necessitating a minimum 3-month pre-conception discontinuation period for both male and female patients. Its use is strictly contraindicated during pregnancy and lactation.

These toxic effects can be attributed to MTX's antifolate properties and anti-proliferative effects. Current guidelines recommend an initial oral methotrexate dose of 7.5-20 mg weekly for RA, requiring subsequent dose adjustment according to clinical efficacy and tolerability. Folic acid supplementation (avoided on MTX dosing days) prevents most toxicities by competing with MTX for cellular uptake transporters. Thus, healthcare providers should closely monitor and manage any side effects from MTX treatment in patients.

Biomarkers for Assessing Methotrexate Therapeutic Response of Rheumatoid Arthritis

Although MTX is an effective and economical treatment option in clinical practice, many patients may discontinue MTX treatment due to intolerance or unavoidable side effects. Thus, it is necessary to identify biomarkers that predict MTX response to avoid delays in non-responsive patients.

Metabolomics, the study of small-molecule metabolites in biological systems, has emerged as a valuable approach for identifying biomarkers predictive of MTX therapeutic response in RA.[59] By analyzing metabolic profiles in urine and serum, researchers have identified specific metabolites, such as nornicotine and N-methylisoleucine, that differ between MTX responders and non-responders.[60] These metabolic signatures offer a non-invasive method to monitor treatment efficacy and predict response early in the course of therapy. Otherwise, compelling evidence indicates that MTX treatment significantly reduces serum levels of soluble CD14 (sCD14) in RA patients who respond to the drug. This reduction correlates with clinical improvements, such as decreased disease activity scores.[61]

Despite the potential of metabolomics and sCD14, neither biomarker has undergone rigorous validation in large, multicenter trials. Most studies to date are small-scale or exploratory, limiting the generalizability of findings. Additionally, the specificity of these biomarkers may be confounded by comorbidities, necessitating testing in diverse patient populations. Future research should prioritize: Multimodal biomarker studies integrating metabolomics, proteomics, and genomics to enhance predictive accuracy. Real-world evidence generation through pragmatic trials in underrepresented populations. Mechanistic investigations to clarify causal pathways and therapeutic targets. Otherwise, future research should also focus on elucidating the mechanisms of patients who are intolerant to MTX.

Conclusion

This review comprehensively outlines the diverse mechanisms of methotrexate treatment in managing RA and the adverse effects associated with its clinical use. Despite significant progress in the understanding, the role of MTX in RA warrants further investigation, and only a few biomarkers for predicting methotrexate response have been reported. Thus, future investigations should focus on elucidating the mechanisms underlying MTX-based therapy, which may facilitate the discovery of novel and more efficacious therapeutic agents. Furthermore, identifying additional molecular targets within this pathway could provide new opportunities for RA management.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

: .

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G-M.D., Y.T.; Design – Y.T.; Supervision – G-M.D.; Resources – H.F.Y.; Materials – L.J.J.; Data Collection and/or Processing – Y.T., H.F.Y., L.J.J.; Analysis and/or Interpretation – Y.T.; Literature Search – Y.T., H.F.Y., L.J.J.; Writing Manuscript – Y.T., L.J.J.; Critical Review – Y.T., H.F.Y.Concept – G-M.D., YT..; Design – Y.T.; Supervision – G-M.D.; Resource – H.F.Y..; Materials – L.J.J..; Data Collection and/or Processing – Y.T., H.F.Y., L.J.J.; Analysis and/or Interpretation – Y.T..; Literature Search – Y.T., H.F.Y., L.J.J..; Writing – Y.T., I.J.J..; Critical Reviews – Y.T., H.F.Y..

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This work was supported by National Natural Science Foundation of China (G-MD, 82171786).

References

1. Luo P, Gao FQ, Sun W, et al. Activatable fluorescent probes for imaging and diagnosis of rheumatoid arthritis. *Mil Med Res*. 2023;10(1):31. [CrossRef]
2. Smolen JS, Landewé RBM, Bergstra SA, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. *Ann Rheum Dis*. 2023;82(1):3-18. [CrossRef]
3. Zhang Q, Liu J, Zhang M, et al. Apoptosis induction of fibroblast-like synoviocytes is an important molecular-mechanism for herbal medicine along with its active components in treating rheumatoid arthritis. *Biomolecules*. 2019;9(12):795. [CrossRef]
4. Lindström U, di Giuseppe D, Exarchou S, et al. Methotrexate treatment in early psoriatic arthritis in comparison to rheumatoid arthritis: an observational nationwide study. *RMD Open*. 2023;9(2):e002883. [CrossRef]
5. Asai S, Kojima T, Oguchi T, et al. Effects of concomitant methotrexate on large joint replacement in patients with rheumatoid arthritis treated with tumor necrosis factor inhibitors: a multicenter retrospective cohort study in Japan. *Arthritis Care Res*. 2015;67(10):1363-1370. [CrossRef]
6. van Vollenhoven R, Takeuchi T, Pangan AL, et al. Efficacy and safety of upadacitinib monotherapy in methotrexate-naive patients with moderately-to-severely active rheumatoid arthritis (SELECT-EARLY): a multicenter, multi-country, randomized, double-blind, active comparator-controlled trial. *Arthritis Rheumatol*. 2020;72(10):1607-1620. [CrossRef]
7. Andersen PA, West SG, O'Dell JR, Via CS, Claypool RG, Kotzin BL. Weekly pulse methotrexate in rheumatoid arthritis. Clinical and immunologic effects in a randomized, double-blind study. *Ann Intern Med*. 1985;103(4):489-496. [CrossRef]
8. Cronstein BN, Sitkovsky M. Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. *Nat Rev Rheumatol*. 2017;13(1):41-51. [CrossRef]
9. Noack M, Miossec P. Effects of methotrexate alone or combined with arthritis-related biotherapies in an in vitro co-culture model with

immune cells and synoviocytes. *Front Immunol*. 2019;10:2992. [CrossRef]
10. Fearon U, Hanlon MM, Floudas A, Veale DJ. Cellular metabolic adaptations in rheumatoid arthritis and their therapeutic implications. *Nat Rev Rheumatol*. 2022;18(7):398-414. [CrossRef]
11. Lopez-Olivo MA, Siddhanamatha HR, Shea B, Tugwell P, Wells GA, Suarez-Almazor ME. Methotrexate for treating rheumatoid arthritis. *Cochrane Database Syst Rev*. 2014;2014(6):CD000957. [CrossRef]
12. Suga K, Suto A, Tanaka S, et al. TAp63, a methotrexate target in CD4+ T cells, suppresses Foxp3 expression and exacerbates autoimmune arthritis. *JCI Insight*. 2023;8(10):e164778. [CrossRef]
13. Herman S, Zurgil N, Langevitz P, Ehrenfeld M, Deutsch M. Methotrexate selectively modulates TH1/TH2 balance in active rheumatoid arthritis patients. *Clin Exp Rheumatol*. 2008;26(2):317-323.
14. Nived P, Pettersson Å, Jönsson G, et al. Methotrexate reduces circulating Th17 cells and impairs plasmablast and memory B cell expansions following pneumococcal conjugate immunization in RA patients. *Sci Rep*. 2021;11(1):9199. [CrossRef]
15. Singh A, Behl T, Sehgal A, et al. Mechanistic insights into the role of B cells in rheumatoid arthritis. *Int Immunopharmacol*. 2021;99:108078. [CrossRef]
16. Fortea-Gordo P, Villalba A, Nuño L, et al. Circulating CD19+CD24hiCD38hi regulatory B cells as biomarkers of response to methotrexate in early rheumatoid arthritis. *Rheumatol (Oxf Engl)*. 2020;59(10):3081-3091. [CrossRef]
17. Jansen DTSL, Emery P, Smolen JS, et al. Conversion to seronegative status after abatacept treatment in patients with early and poor prognostic rheumatoid arthritis is associated with better radiographic outcomes and sustained remission: post hoc analysis of the AGREE study. *RMD Open*. 2018;4(1):e000564. [CrossRef]
18. Fan J, Luo J, Yan C, et al. Methotrexate, combined with cyclophosphamide attenuates murine collagen induced arthritis by modulating the expression level of Breg and DCs. *Mol Immunol*. 2017;90:106-117. [CrossRef]
19. Cutolo M, Campitiello R, Gotelli E, Soldano S. The role of M1/M2 macrophage polarization in rheumatoid arthritis synovitis. *Front Immunol*. 2022;13:867260. [CrossRef]
20. Olsen NJ, Spurlock CF, 3rd, Aune TM. Methotrexate induces production of IL-1 and IL-6 in the monocytic cell line U937. *Arthritis Res Ther*. 2014;16(1):R17. [CrossRef]
21. Xue G, Jiang H, Song Z, et al. Dual targeting biomimetic carrier-free nanosystems for photochemotherapy of rheumatoid arthritis via macrophage apoptosis and re-polarization. *Adv Sci (Weinh)*. 2025;12(10):e2406877. [CrossRef]
22. Lama M, Sarkar R, Ghosh B. Serum cytokine profiles in patients with rheumatoid arthritis before and after treatment with methotrexate. *J Interferon Cytokine Res*. 2023;43(8):344-350. [CrossRef]
23. Komatsu N, Takayanagi H. Mechanisms of joint destruction in rheumatoid arthritis - immune cell-fibroblast-bone interactions. *Nat Rev Rheumatol*. 2022;18(7):415-429. [CrossRef]
24. Kong YY, Feige U, Sarosi I, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature*. 1999;402(6759):304-309. [CrossRef]
25. Haynes DR, Crotti TN, Loric M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. *Rheumatol (Oxf Engl)*. 2001;40(6):623-630. [CrossRef]
26. Hensvold AH, Joshua V, Li W, et al. Serum RANKL levels associate with anti- citrullinated protein antibodies in early untreated rheumatoid arthritis and are modulated following methotrexate. *Arthritis Res Ther*. 2015;17(1):239. [CrossRef]
27. Kanagawa H, Masuyama R, Morita M, et al. Methotrexate inhibits osteoclastogenesis by decreasing RANKL-induced calcium influx into osteoclast progenitors. *J Bone Miner Metab*. 2016;34(5):526-531. [CrossRef]
28. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. *Nat Rev Rheumatol*. 2020;16(3):145-154. [CrossRef]
29. Cronstein BN, Eberle MA, Gruber HE, Levin RI. Methotrexate inhibits neutrophil function by stimulating adenosine release from connective tissue cells. *Proc Natl Acad Sci U S A*. 1991;88(6):2441-2445. [CrossRef]
30. Montesinos MC, Yap JS, Desai A, Posadas I, McCrary CT, Cronstein BN. Reversal of the antiinflammatory effects of methotrexate by the nonselective adenosine receptor antagonists theophylline and caffeine: evidence that the antiinflammatory effects of methotrexate are mediated via multiple adenosine receptors in rat adjuvant arthritis. *Arthritis Rheum*. 2000;43(3):656-663. [CrossRef]
31. Haskó G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discov*. 2008;7(9):759-770. [CrossRef]
32. Xu X, Zheng S, Xiong Y, et al. Adenosine effectively restores endotoxin-induced inhibition of human neutrophil chemotaxis via A1 receptor-p38 pathway. *Inflamm Res*. 2017;66(4):353-364. [CrossRef]
33. He W, Cronstein BN. Adenosine A1 receptor regulates osteoclast formation by altering TRAF6/TAK1 signaling. *Purinergic Signal*. 2012;8(2):327-337. [CrossRef]
34. Cronstein BN, Naime D, Ostad E. The antiinflammatory mechanism of methotrexate. Increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. *J Clin Invest*. 1993;92(6):2675-2682. [CrossRef]
35. Ferrante CJ, Pinhal-Enfield G, Elson G, et al. The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4Rα) signaling. *Inflammation*. 2013;36(4):921-931. [CrossRef]
36. Ma SR, Deng WW, Liu JF, et al. Blockade of adenosine A2a receptor enhances CD8(+) T cells response and decreases regulatory T cells in head and neck squamous cell carcinoma. *Mol Cancer*. 2017;16(1):99. [CrossRef]
37. Bedoui Y, Guillot X, Sélambarom J, et al. Methotrexate an old drug with new tricks. *Int J Mol Sci*. 2019;20(20):5023. [CrossRef]
38. Konishi H, Kanou SE, Yukimatsu R, et al. Adenosine inhibits TNFα-induced MMP-3 production in MH7A rheumatoid arthritis synoviocytes via A(2A) receptor signaling. *Sci Rep*. 2022;12(1):6033. [CrossRef]
39. Mediero A, Perez-Aso M, Wilder T, Cronstein BN. Brief report: methotrexate prevents wear particle-induced inflammatory osteolysis in mice via activation of adenosine A2a receptor. *Arthritis Rheumatol (Hoboken, NJ)*. 2015;67(3):849-855. [CrossRef]
40. Xiong L, Jung JU, Guo HH, et al. Osteoblastic Lrp4 promotes osteoclastogenesis by regulating ATP release and adenosine-A(2A)R signaling. *J Cell Biol*. 2017;216(3):761-778. [CrossRef]
41. Knowles HJ. The adenosine A(2B) receptor drives osteoclast-mediated bone resorption in hypoxic microenvironments. *Cells*. 2019;8(6):624. [CrossRef]
42. Wilson JM, Kurtz CC, Black SG, et al. The A2B adenosine receptor promotes Th17 differentiation via stimulation of dendritic cell IL-6. *J Immunol*. 2011;186(12):6746-6752. [CrossRef]
43. Fishman P, Cohen S. The A3 adenosine receptor (A3AR): therapeutic target and predictive biological marker in rheumatoid arthritis. *Clin Rheumatol*. 2016;35(9):2359-2362. [CrossRef]
44. Ochaion A, Bar-Yehuda S, Cohen S, et al. The anti-inflammatory target A(3) adenosine receptor is over-expressed in rheumatoid arthritis, psoriasis and Crohn's disease. *Cell Immunol*. 2009;258(2):115-122. [CrossRef]
45. Fraenkel L, Bathon JM, England BR, et al. 2021 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol*. 2021;73(7):1108-1123. [CrossRef]
46. Burmester GR, Rigby WF, van Vollenhoven RF, et al. Tocilizumab in early progressive rheumatoid arthritis: FUNCTION, a randomised controlled trial. *Ann Rheum Dis*. 2016;75(6):1081-1091. [CrossRef]
47. Hetland ML, Haavardsholm EA, Rudin A, et al. Active conventional treatment and three different biological treatments in early rheumatoid arthritis: phase IV investigator initiated, randomised, observer blinded clinical trial. *Bmj*. 2020;371:m4328. [CrossRef]
48. Taylor PC, Alten R, Álvaro Gracia JM, et al. Achieving pain control in early rheumatoid arthritis with baricitinib monotherapy or in combination with methotrexate versus methotrexate monotherapy. *RMD Open*. 2022;8(1):e001994. [CrossRef]

49. Matsubara T, Inoue H, Nakajima T, et al. Abatacept in combination with methotrexate in Japanese biologic-naive patients with active rheumatoid arthritis: a randomised placebo-controlled phase IV study. *RMD Open*. 2018;4(2):e000813. [CrossRef]
50. Verschueren P, De Cock D, Corluy L, et al. Effectiveness of methotrexate with step-down glucocorticoid remission induction (COBRA Slim) versus other intensive treatment strategies for early rheumatoid arthritis in a treat-to-target approach: 1-year results of CareRA, a randomised pragmatic open-label superiority trial. *Ann Rheum Dis*. 2017;76(3):511-520. [CrossRef]
51. Giollo A, Fuzzi E, Doria A. Methotrexate in early rheumatoid arthritis: is the anchor drug still holding? *Autoimmun Rev*. 2022;21(4):103031. [CrossRef]
52. Jiang L, Qiu W, Wang X, et al. Immunoglobulin G inhibits glucocorticoid-induced osteoporosis through occupation of FcγRI. *iScience*. 2023;26(10):107749. [CrossRef]
53. Perpétuo IP, Caetano-Lopes J, Rodrigues AM, et al. Methotrexate and low-dose prednisolone downregulate osteoclast function by decreasing receptor activator of nuclear factor-κβ expression in monocytes from patients with early rheumatoid arthritis. *RMD Open*. 2017;3(1):e000365. [CrossRef]
54. Teng Y, Yin H, Feng R, et al. Methotrexate inhibits glucocorticoids-induced osteoclastogenesis via activating IFN-γR/STAT1 pathway in the treatment of rheumatoid arthritis. *RMD Open*. 2024;10(4):e004886. [CrossRef]
55. Delteil A, Lambert C, Pereira B, et al. Glucocorticoid trajectories over 2 years in patients with rheumatoid arthritis in a real-life setting. *RMD Open*. 2023;9(4):e003366. [CrossRef]
56. West J, Ogston S, Foerster J. Safety and efficacy of methotrexate in psoriasis: a meta-analysis of published trials. *PLoS One*. 2016;11(5):e0153740. [CrossRef]
57. Alarcón GS, Tracy IC, Blackburn WD, Jr. Methotrexate in rheumatoid arthritis. Toxic effects as the major factor in limiting long-term treatment. *Arthritis Rheum*. 1989;32(6):671-676. [CrossRef]
58. Khera S, Kapoor R, Pramanik SK. Solitary serum methotrexate level 36 hours post high-dose methotrexate: a safe, efficacious, and cost-effective strategy to monitor methotrexate toxicities in childhood leukemia in resource-limited centers. *Pediatr Blood Cancer*. 2020;67(7):e28387. [CrossRef]
59. Gosselt HR, Muller IB, Jansen G, et al. Identification of metabolic biomarkers in relation to methotrexate response in early rheumatoid arthritis. *J Pers Med*. 2020;10(4):271. [CrossRef]
60. Medcalf MR, Bantis LE, Shi P, et al. Plasma metabolomic profiling as a tool to identify predictive biomarkers of methotrexate efficacy in rheumatoid arthritis. *Semin Arthritis Rheum*. 2022;56:152056. [CrossRef]
61. Fuentelsaz-Romero S, Barrio-Alonso C, García Campos R, et al. The macrophage reprogramming ability of antifolates reveals soluble CD14 as a potential biomarker for methotrexate response in rheumatoid arthritis. *Front Immunol*. 2021;12:776879. [CrossRef]