



This is a repository copy of *JAK inhibition by methotrexate (and csDMARDs) may explain clinical efficacy as monotherapy and combination therapy.*

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/152772/>

Version: Published Version

Article:

Gremese, E., Alivernini, S., Toluoso, B. et al. (2 more authors) (2019) JAK inhibition by methotrexate (and csDMARDs) may explain clinical efficacy as monotherapy and combination therapy. *Journal of Leukocyte Biology*, 106 (5). pp. 1063-1068. ISSN 0741-5400

<https://doi.org/10.1002/jlb.5ru0519-145r>

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

MORE CELLS. LESS STRESS.

AUTOMATE YOUR CELL ISOLATION



**GIVING UP LONG-TERM
HABITS IS HARD.**

And using Ficoll® separation
feels like the only option.

But sometimes, you
have to face facts.

**The Sorterra™ cell isolation system
maximizes the number of functional cells.**


This automated microfluidics-enabled platform achieves
better purity and performance while increasing speed.

JUST HOW ADVANCED IS IT? GET THE FACTS!

DOWNLOAD OUR POSTER >

REVIEW

JAK inhibition by methotrexate (and csDMARDs) may explain clinical efficacy as monotherapy and combination therapy

Elisa Gremese^{1,2} | Stefano Alivernini^{1,2} | Barbara Tolusso¹ | Martin P. Zeidler³ | Gianfranco Ferraccioli² 

¹Division of Rheumatology, Fondazione Policlinico Universitario A. Gemelli–IRCCS, Rome, Italy

²Institute of Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy

³The Bateson Centre, Department of Biomedical Science, The University of Sheffield, Sheffield, S10 2TN, United Kingdom

Correspondence

Gianfranco Ferraccioli, Institute of Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy.

Email: gianfranco.ferraccioli@unicatt.it

Elisa Gremese, Institute of Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy.

Email: elisa.gremese@unicatt.it

Abstract

Methotrexate (MTX) is recognized as the anchor drug in the algorithm treating chronic arthritis (RA, psoriatic arthritis), as well as a steroid sparing agent in other inflammatory conditions (polymyalgia rheumatica, vasculitis, scleroderma). Its main mechanism of action has been related to the increase in extracellular adenosine, which leads to the effects of A_{2A} receptor in M1 macrophages that dampens TNF α and IL12 production and increases IL1Ra and TNFRp75. By acting on A_{2B} receptor on M2 macrophages it enhances IL10 synthesis and inhibits NF- κ B signaling. MTX has also been shown to exert JAK inhibition of JAK2 and JAK1 when tested in *Drosophila melanogaster* as a model of kinase activity and in human cell lines (nodular sclerosis Hodgkin's lymphoma and acute myeloid leukemia cell lines). These effects may explain why MTX leads to clinical effects similar to anti-TNF α biologics in monotherapy, but is less effective when compared to anti-IL6R in monotherapy, which acting upstream exerts major effects downstream on the JAK1-STAT3 pathway. The MTX effects on JAK1/JAK2 inhibition also allows to understand why the combination of MTX with Leflunomide, or JAK1/JAK3 inhibitor leads to better clinical outcomes than monotherapy, while the combination with JAK1/JAK2 or JAK1 specific inhibitors does not seem to exert additive clinical benefit.

KEYWORDS

adenosine, JAK inhibition, Methotrexate

1 | INTRODUCTION

Methotrexate (MTX) has become the anchor drug in RA and psoriatic arthritis (PsA) since 1962, when Black et al. showed that it was clinically effective in 21 patients with PsA¹ and Hoffmeister in 1972 published an abstract in which he showed that 11 of 29 patients with RA had major clinical improvement at 10–15 mg/weekly² and that the decrease of MTX dose led to 80% of disease relapse.

MTX is now the best first-line treatment in all the recommendations by the Scientific Societies and has a retention rate at 2 years of more than 60% both in RA and in PsA, associated with an

important clinical benefit.³ In clinical practice, once patients are considered to be incomplete responders (MTX-IR), MTX is used in combination with conventional synthetic disease modified antirheumatic drugs (cs-DMARDs),⁴ biologicals (b-DMARDs),⁵ or targeted synthetic DMARDs (ts-DMARDs).^{6,7}

Once biologics are compared directly with MTX in clinical trials, only tocilizumab (TCZ) and etanercept (to a lower degree, only for the ACR 20) appear to be superior to MTX, whereas ts-DMARDs as JAK inhibitors showed a higher efficacy than MTX monotherapy.^{8,9}

How can we explain the similar efficacy of MTX to anti-TNF α in monotherapy?

Abbreviations: AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; b-DMARD, biological synthetic disease modified antirheumatic drugs; cs-DMARD, conventional synthetic disease modified antirheumatic drugs; DHFR, dihydrofolate reductase; ET, essential thrombocythemia; GPI, glucose-6-phosphate isomerase; IL6R, IL6 receptor; MTX, methotrexate; PsA, psoriatic arthritis; PV, polycythemia vera; TCZ, tocilizumab; ts-DMARDs, targeted synthetic DMARDs.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2019 The Authors. *Journal of Leukocyte Biology* published by Wiley Periodicals, Inc. on behalf of Society for Leukocyte Biology

1.1 | Intracellular and extracellular mechanisms of action of MTX

Being a chemotherapy agent, MTX acts as a folate antagonist that inhibits dihydrofolate reductase (DHFR) and ultimately purine synthesis, blocking highly replicative cells in the S phase. These effects can be bypassed and the clinical effects blocked by folinic acid supplementation acting on the folate pathway downstream of DHFR and relieving the inhibition of purine synthesis. However, at the lower doses used in RA, folinic acid reduces the side effects associated with MTX treatment without reverting its anti-inflammatory and immune-suppressive actions but maintaining its clinical efficacy, proving that other mechanisms of action are responsible for its DMARD activity. In addition, MTX blocks the 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC) that converts 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) to formyl-AICAR (FAICAR). FAICAR leads to enhanced release of adenine nucleotides into the extracellular space and converted to adenosine, which is thought to be the major driver of the pharmacologic action of MTX in RA.^{10,11} Adenosine inhibits oxygen radical by neutrophils, suppresses monocyte-derived cytokine/chemokine (among which TNF- α and IL12) production acting on A2_A, and through A2_B AR increases IL10 production and promotes M2 macrophage activation.¹²

These pharmacobiologic effects may explain why the combination therapy of MTX + anti-TNF α is more effective than MTX alone or anti-TNF alone.⁵ Moreover, rituximab (RTX) and abatacept (CTLA4-Ig) led to a better ACR 50 response or Boolean remission rate compared to MTX alone, yet it did not reach statistical significance,^{13,14} whereas in RA, only TCZ monotherapy showed to be clearly superior to MTX monotherapy.¹⁵

2 | MTX AND CYTOKINE SYNTHESIS

The histopathological analysis of synovial tissue inflammation in RA has shown that RA synovitis can be roughly divided into at least three major groups, myeloid, lymphoid, and fibroid pathotypes, respectively. If after MTX therapy the synovial inflammation still persists, the first subset responds better to TNF-I and the second to IL6-R inhibition.¹⁶ Why does not IL6 inhibition receives any additive effect by MTX?

In Jurkat cells stimulated with TNF, MTX suppressed NF- κ B activation by inhibiting I κ B α degradation and suppressing I κ B α phosphorylation, effects all determined by adenosine.¹⁷ MTX leads to the downregulation of TNF α and IL1 β and to an increase of IL1Ra and TNFRp75.¹⁸ It also inhibits NF- κ B activity in T cells, via BH4 depletion, and reduces activation of JNK, p53, and NF- κ B activity in fibroblast-like synoviocytes through the release of adenosine and adenosine receptor activation, normalizing in vivo elevated NF- κ B activity.¹⁹ In addition to these effects, in collagen-induced arthritis, Neurath et al. demonstrated that intraperitoneal MTX injection, prior to the development of arthritis, significantly suppressed IL15-induced synthesis in splenic mononuclear cells of TNF, indirectly. However, no significant effects were seen on IL6.²⁰ In the glucose-6-phosphate

isomerase-induced (GPI) model of arthritis, MTX inhibits GPI-induced arthritis, with a vanishing effect, showing its ability to significantly reduce IL6 and IL6 receptor (IL6R) expression. Nevertheless this effect was mirrored by the progressive decrease of SLC19A1 expression, which is an important folate carrier for MTX intracellular uptake (Fig. 1A). When SCL19A1 expression decreases, MTX does not exert its effects anymore. Therefore, IL6 is the major driver of MTX unresponsiveness in the GPI model of arthritis.²¹ These effects may explain the similar efficacy of MTX compared to TNF inhibitors used in monotherapy but when added to TNF-inhibitors (TNF-I) MTX because of the possible increase of IL1Ra and TNFRp75, can allow a further control of inflammation. The MOA of TNF-I is out of the scope of this review, yet it is important to consider that the biopharmacology may have practical translation into the clinics. If after MTX therapy the synovial inflammation still presents a lymphoid phenotype where a stronger IL6 inhibition is likely needed, MTX will exert little more effect.

MTX exerts its suppression on cytokines promoting the release of adenosine producing its modulatory effect on inflammatory signals through A2_A and A2_B receptors on macrophages leading to the suppression of proinflammatory cytokines release (i.e., TNF- α) and promotion of anti-inflammatory molecules (i.e., IL-10) (Fig. 1B).²² Moreover, MTX was demonstrated to repress the inflammatory signal in RA due to its inhibitory effect on another proinflammatory mediator, as High-mobility group box 1 (HMGB1). In particular, MTX interferes with HMGB1 and RAGE ligand, at molecular level, acting via JAK/STAT pathway with a repression of TNF- α overproduction.^{23,24}

3 | LEFLUNOMIDE, AURANOFIN, AND MTX: JAK-STAT INHIBITION

The first cs-DMARD showing a potential intracellular effect on transcription factors of the JAK/STAT pathway was Leflunomide (LFN). In 1998, Siemasko showed that in spleen cells from B10 E mice,²⁵ LFN diminished the tyrosine phosphorylation of JAK3 and STAT6 at concentrations of 100–200 μ M. Moreover, another cs-DMARD as Auranofin, markedly inhibited IL-6-induced phosphorylation of JAK1 at concentrations of 1.3 μ M and STAT3 in human Hep2 cell lines.²⁶ Auranofin inhibited constitutive and IL6-induced activation of JAK2 and phosphorylation of STAT3 in the range of 6 mg/day in humans. The most interesting results on csDMARDs were presented in 2015, when Thomas reported that MTX inhibited JAK/STAT pathway activity in *Drosophila melanogaster* used as a low complexity model system to screen the action of small molecules on JAK-STAT pathways.²⁷ When tested in human cells lines (HDLM2—Hodgkin's lymphoma derived, HEL—Acute myeloid leukemia expressing JAK2 V617F variant, a gain of function mutation associated with most human myeloproliferative neoplasms), MTX significantly reduced JAK1 phosphorylation and STAT1 and STAT5 phosphorylation at concentrations of 0.4–0.8 μ M in RA. When tested in the human Hodgkin's lymphoma-derived cell lines, MTX inhibited phosphorylation of STAT 1 and STAT5 but not of STAT3. Similarly, taken orally, 1 μ M ruxolitinib, a JAK1/JAK2 selective

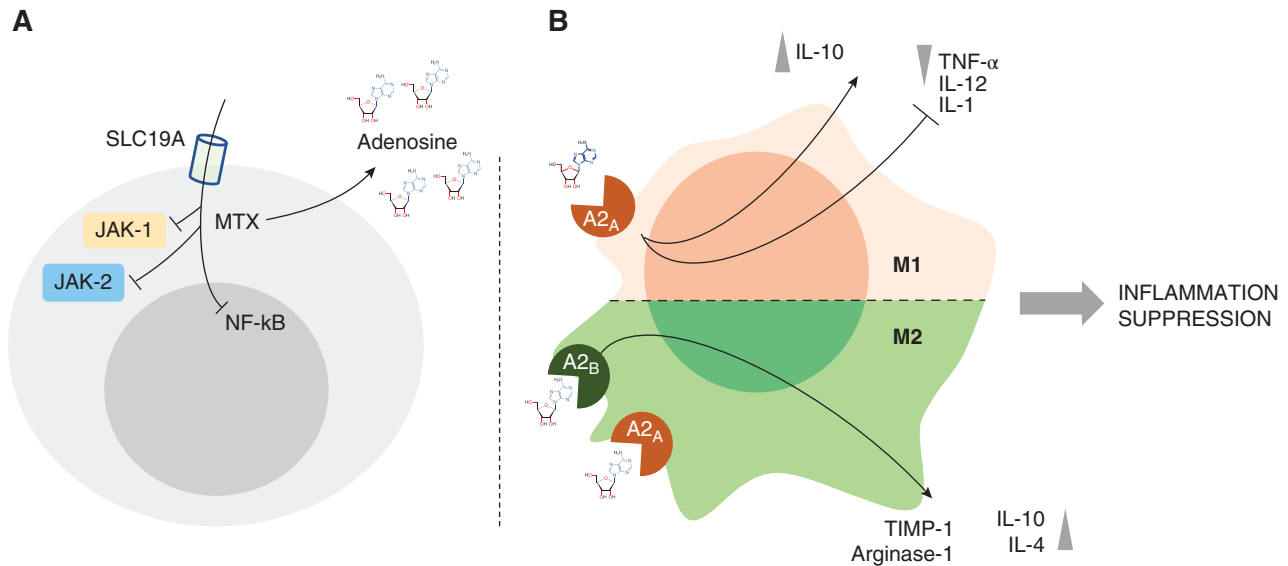


FIGURE 1 (A, B) MTX mechanisms of action and anti-inflammatory properties of adenosine on macrophages. (A) Intracellular uptake through SLC19A receptor, extracellular increase in Adenosine and repression of NF-kB activity. These effects and the inhibition of JAK-1/JAK-2 represent the autoimmune inflammation. (B) Schematic view of the anti-inflammatory activities of adenosine on M1 and M2 macrophages

inhibitor produced a deeper suppression of STAT5 phosphorylation. Interestingly, transgenic mice in which the JAK2 locus was replaced by the human JAK2 V617F allele, developing essential thrombocythemia (ET)- and polycythemia vera (PV)-like neoplasms showed a significant reduction of STAT3-STAT5 phosphorylation in splenocytes of homozygous mice treated with MTX,²⁸ while in silico modeling suggested that this inhibition may be the result of a direct binding of MTX to the JAK2 kinase domain ATP binding pocket. Accordingly, in two patients with myeloproliferative disorders as PV and ET, MTX at 10 mg weekly allowed the reduction of constitutional symptoms and the normalization of hematological values within 2 months and their maintenance.²⁹ Finally, molecular studies performed on RA patients and SLE patients demonstrated that low-dose MTX reduced in vivo JAK/STAT pathway activity,³⁰ supporting the hypothesis that MTX may act via inhibition of the JAK/STAT signaling.

4 | MTX, TNF-I, AND TOCILIZUMAB IN CLINICAL TRIALS

In all clinical trials, including TNF α inhibitors (i.e., infliximab, adalimumab, golimumab, certolizumab, and etanercept), the rate of ACR50 or DAS-remission achievement was not statistically different when comparing patients treated with monoclonals alone and MTX alone. On the other hand, the combination of b-DMARD and MTX, was associated with a significant reduction in joint damage compared to MTX alone and in MTX naive patients only.³¹ Considering mechanisms of action other than TNF inhibition, only TCZ proved to be clearly superior to MTX.⁹

Therefore, we hypothesize that MTX has similar efficacy to anti-TNF monotherapy through Adenosine and JAKs inhibition. However,

selective JAK inhibition does not directly suppress TNF synthesis by immune cells.³² Therefore, the additive role of MTX, when used in combination with TNF inhibitors, may be supported in vivo in RA by the MTX suppressive effects on IL1 β , IL3, IL5, IFN γ , and as expected from the JAK1 inhibition, also on IL6.³³

In clinical trials, TCZ was demonstrated to be superior to MTX, and to be more successful in RA patients who are incomplete responders to TNF inhibition.⁹ In fact, IL6 signaling through IL6-IL6R-gp130 binding, leads to JAK1/JAK2/STAT3 activation.³² Therefore, TCZ acting upstream of JAKs and blocking the interaction of IL6 with its receptors, can more strongly suppress the inflammatory signal and once MTX is added, acting downstream, limited additional effects are registered. These insights may allow to understand why TCZ appears to be the only b-DMARD superior to MTX alone.³⁴

5 | JAK INHIBITORS AND MTX

The inhibition of JAK-STAT phosphorylation has been tested in *Drosophila* as a cell model to screen small molecules, as well as in several immune cells, like splenocytes, T lymphocytes, and whole blood or directly on enzyme assays.³⁵⁻³⁸ When JAK inhibitors are tested, strong inhibition of JAK1 also inhibits JAK1/JAK3-dependent IL-15 signaling. When assessed in kinase assays using 1 mM ATP, none of the compounds are only JAK3 selective and all inhibit both JAK1/JAK3-dependent IL-15 signaling and JAK1/TYK2-dependent interferon α signaling.³⁹ This explains why at high concentrations compounds inhibiting JAK1-3 may also inhibit JAK2, thus, displaying a pan-JAK inhibition. JAK1-2-3-TYK2 inhibition and the effects on the inflammatory pathways have been comprehensively discussed in several

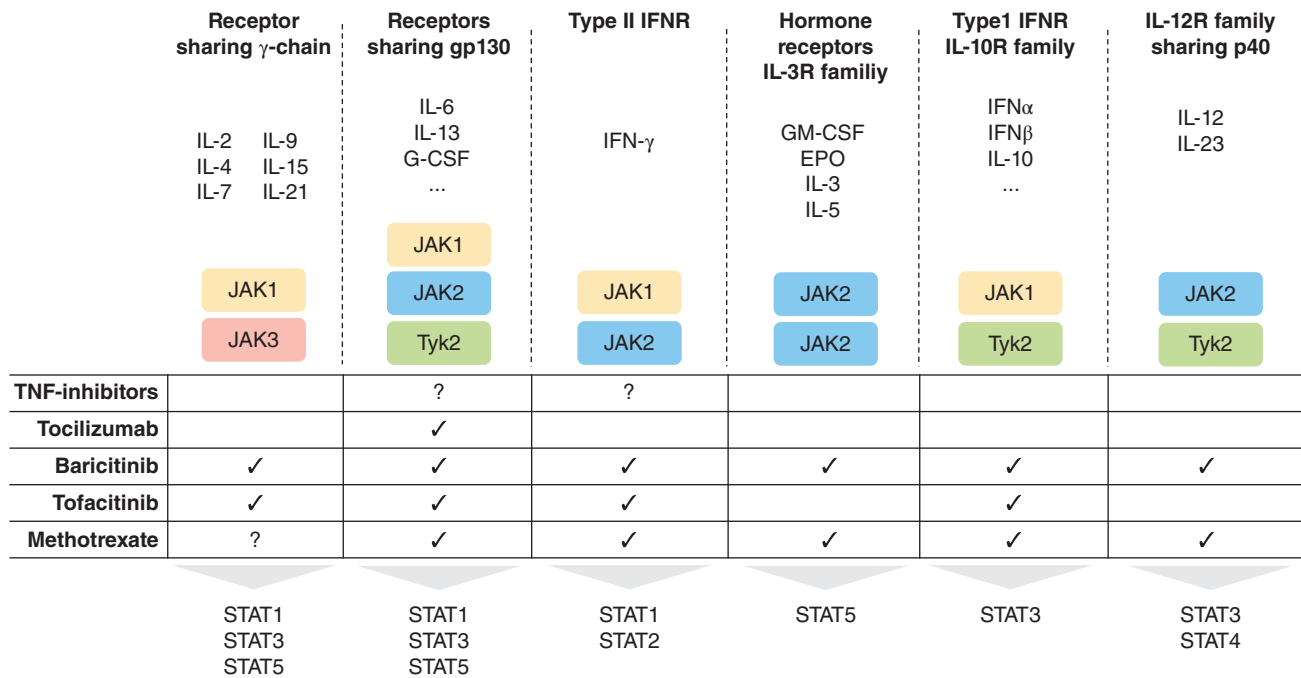


FIGURE 2 Schematic view of the effects on JAKs played by conventional synthetic (MTX), targeted synthetic (tofacitinib/baricitinib) and biological disease modifying anti-rheumatic drugs (TNF-inhibitor/tocilizumab). MTX: Methotrexate

reviews.^{39,40} JAK3 appears to be mainly involved in hematopoietic cells and has a crucial role in lymphocyte biology while JAK1,2 and TYK2 are expressed ubiquitously.³⁷ For this reason, JAK3 inhibition appeared to be a first rational target in rheumatic inflammatory diseases. The randomized clinical trial comparing tofacitinib and MTX showed a higher effectiveness of tofacitinib at 5 and 10 mg compared to MTX at 10–20 mg/week,⁶ suggesting that specific inhibition of JAK1/JAK3, by tofacitinib, appears to be superior to MTX, which acts by inhibiting JAK1/JAK2.

When used in combination, MTX plus tofacitinib showed similar clinical effect to MTX plus adalimumab,⁴¹ suggesting that the specific inhibition led by tofacitinib combined with JAK1/JAK2 inhibition by MTX may have additive effects. The same results did not occur with baricitinib, which acts on JAK1/JAK2. In fact, clinical trial showed that the addition of MTX to baricitinib does not result in significantly better clinical benefits, already obtained by baricitinib alone, which can rescue MTX incomplete responders owing to its specific inhibition, though the combination led to a better control of erosions.⁴² Finally, given the different JAK targets being inhibited, it can be hypothesized that the combination of baricitinib plus LFN might be of clinical interest considering that LFN inhibits JAK3 and this may explain also the additive effect of LFN when used in combination with MTX.⁴³ The same should occur with Upadacitinib and Filgotinib.^{44,45} When examining the side effects associated to JAK inhibitors, the major one is represented by the occurrence of Herpes Zoster infection.⁴⁶ Despite discordant results have been described in the literature,⁴⁷ yet most recent data suggest that Zoster infection may be a side effect of MTX therapy.⁴⁸ All these data suggest that MTX may indeed display JAK1/JAK2 inhibition in vivo.

6 | PERSPECTIVES

MTX is a fundamental part of the therapeutic strategy in several inflammatory and autoimmune diseases. Its efficacy is recognized as a fundamental first step approach in several inflammatory diseases. Its effects on the purinergic pathways through adenosine modulation and on NF- κ B signal together with emerging insights into its action on JAK1/JAK2 pathways (Fig. 2) suggest that it will certainly remain the anchor drug of excellence in the clinics.

DISCLOSURES

EG and GF conceived and structured the review; EG, SA, BT, MPZ and GF performed research and wrote the manuscript; EG, SA, BT, MPZ and GF read and approved the final version of the manuscript. The authors declare no conflicts of interest.

ORCID

Gianfranco Ferraccioli  <https://orcid.org/0000-0002-6884-4301>

REFERENCES

- Black RL, O'Brien WM, Van Scott EJ, et al. Methotrexate therapy in psoriatic arthritis. *JAMA*. 1964;189:743-747.
- Hoffmeister RT. Methotrexate in rheumatoid arthritis. *Arthritis Rheumatol*. 1972;15:114.
- O'Dell JR, Haire CE, Erikson N, et al. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. *N Engl J Med*. 1996;334:1287-1291.

4. Ferraccioli GF, Gremese E, Tomietto P, et al. Analysis of improvements, full responses, remission and toxicity in rheumatoid patients treated with step-up combination therapy (methotrexate, cyclosporin A, sulphasalazine) or monotherapy for three years. *Rheumatology*. 2002;41:892-898.
5. Breedveld FC, Weisman MH, Kavanaugh AF, et al. The PREMIER study: a multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum*. 2006;54:26-37.
6. Van Vollenhoven RF, Fleischmann R, Cohen S, et al. Tofacitinib or adalimumab versus placebo in rheumatoid arthritis. *N Engl J Med*. 2012;367:508-519.
7. Taylor PC, Keystone EC, van der Heijde D, et al. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N Engl J Med*. 2017;376:852-862.
8. Nam JL, Takase-Minegishi K, Ramiro S, et al. Efficacy of biological disease-modifying antirheumatic drugs: a systematic literature review informing the 2016 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann Rheum Dis*. 2017;76:1113-1136.
9. Tarp S, Furst DE, Dossing A, et al. Defining the optimal biological monotherapy in rheumatoid arthritis: a systematic review and meta-analysis of randomised trials. *Semin Arthritis Rheum*. 2017;46:699-708.
10. Haskó G, Bruce Cronstein B. Regulation of inflammation by Adenosine. *Frontiers Immunol*. 2013;4:85.
11. Friedman B, Cronstein B. Methotrexate mechanism in treatment of rheumatoid arthritis. *Joint Bone Spine*. 2019;86:301-307.
12. Koscsó B, Csóka B, Kokal E, et al. Adenosine augments IL-10-induced STAT3 signaling in M2c macrophages. *J Leukoc Biol*. 2013;94:1309-1315.
13. Edwards JCW, Szczepanski L, Szechiński J, et al. Efficacy of B-cell-targeted therapy with Rituximab in patients with rheumatoid arthritis. *N Engl J Med*. 2004;350:2572-2581.
14. Emery P, Burmester G, Bykerk V, et al. Evaluating drug-free remission with abatacept in early rheumatoid arthritis: results from the phase 3b, multicentre, randomised, active-controlled AVERT nstudy of 24 months, with a 12-month, double-blind treatment period. *Ann Rheum Dis*. 2015;74:19-26.
15. Emery P, Pope JE, Kruger K, et al. Efficacy of monotherapy with biologics and JAK inhibitors for the treatment of rheumatoid arthritis: a systematic review. *Adv Ther*. 2018;35:1535-1563.
16. Dennis G, Jr, Holweg CT, Kummerfeld SK, et al. Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. *Arthritis Res Ther*. 2014;16:R90.
17. Majumdar S, Aggarwal BB. Methotrexate suppresses NF- κ B activation through inhibition of I κ B α phosphorylation and degradation. *J Immunol*. 2001;167:2911-2920.
18. Seitz M, Zwicker M, Loetscher P. Effects of MTX on differentiation of monocytes and production of cytokine inhibitors by monocytes. *Arthr Rheum*. 1998;41:2032-2038.
19. Spurlock CF, 3rd, Gass HM, 4th, Bryant CJ, et al. Methotrexate-mediated inhibition of nuclear factor κ B activation by distinct pathways in T cells and fibroblast-like synoviocytes. *Rheumatology*. 2015;54:178-187.
20. Neurath MF, Hildner K, Becker C, et al. Methotrexate specifically modulates cytokine production by T cells and macrophages in murine collagen-induced arthritis (CIA): a mechanism for methotrexate-mediated immunosuppression. *Clin Exp Immunol*. 1999;115:42-55.
21. Hashizume M, Yoshida H, Tanaka K, et al. Interleukin-6 regulates anti-arthritis effect of methotrexate via reduction of SLC19A1 expression in a mouse arthritis model. *Arthritis Res Ther*. 2012;14:R96.
22. Hasko G, Pacher P. Regulation of macrophage function by adenosine. *Atheroscler Thromb Vasc Biol*. 2012;32:865-869. <https://doi.org/10.1161/ATVBAHA.111.226852>.
23. Li Y, Xu P, Xu K, et al. Methotrexate affects HMBG1 expression in rheumatoid arthritis, and the downregulation of HMGB1 prevents rheumatoid arthritis progression. *Mol Cell Biochem*. 2016;420:161-170.
24. Kuroiwa J, Takakusagi Y, Kusayanagi T, Kuramochi K, et al. Identification and characterization of the direct interaction between methotrexate (MTX) and high-mobility group box 1 (HMGB1) protein. *POLS One*. 2013;8:e63073.
25. Siemasko K, Chong AS, Jäck HM, et al. Inhibition of JAK3 and STAT6 tyrosine phosphorylation by the immunosuppressive drug leflunomide leads to a block in IgG1 production. *J Immunol*. 1998;160:1581-1588.
26. Kim NH, Lee MY, Park SJ, et al. Auranofin blocks interleukin-6 signalling by inhibiting phosphorylation of JAK1 and STAT3-. *Immunology*. 2007;122:607-614.
27. Thomas S, Fisher KH, Snowden JA, et al. Methotrexate is a JAK/STAT pathway inhibitor. *PLoS One*. 2015;10:e0130078.
28. Chinnaiya K, Lawson MA, Thomas S, et al. Low dose methotrexate in myeloproliferative neoplasm model. *Haematologica*. 2017 Sep;102(9):e336-e339.
29. Palandri F, Labate C, Sabattini E, et al. Low-dose methotrexate as treatment of myeloproliferative neoplasms: proof of principle of clinical activity. *Am J Hematol*. 2016;91:e329-30.
30. Goropevšek A, Holcar M, Pahor A, et al. STAT signaling as a marker of SLE disease severity and implications for clinical therapy. *Autoimmun Rev*. 2018;18:144-154.
31. Tarp S, Jurgensen TS, Furst TS, et al. Added value of combining methotrexate with a biological agent compared to biological monotherapy in rheumatoid arthritis patients: a systematic review and meta-analysis of randomised trials. *Semin Arthr. Rheum*. 2019;48:958-966.
32. O'Shea JJ, Gadina M. Selective Janus kinase inhibitors come of age. *Nat Rev Rheumatol*. 2019;15:74-75.
33. Kremer JM, Lawrence DA, Hamilton R, McInnes IB. Long-term study of the impact of methotrexate on serum cytokines and lymphocyte subsets in patients with active rheumatoid arthritis: correlation with pharmacokinetic measures. *RMD Open*. 2016;2:e000287.
34. Bijlsma JWJ, Welsing PMJ, Woodworth TG, et al. Early rheumatoid arthritis treated with tocilizumab, methotrexate, or their combination (U-Act-Early): a multicentre, randomised, double-blind, double-dummy, strategy trial. *Lancet*. 2016;388:343-355.
35. Spindler SR, Li R, Dhahbi JM. Novel protein kinase signaling systems regulating lifespan identified by small molecule library screening using *Drosophila*. *PLoS One*. 2012;7(2):e29782.
36. Dowty ME, Jesson MI, Ghosh S, et al. Preclinical to clinical translation of tofacitinib, a janus kinase inhibitor, in rheumatoid arthritis. *J Pharmacol Exp Ther*. 2014;348:165-173.
37. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev*. 2009;228:273-287.
38. Clark JD, Flanagan ME, Telliez JB. Discovery and development of Janus Kinase (JAK) inhibitors for inflammatory diseases. *J Med Chem*. 2014;57:5023-5038.
39. Gadina M, Le MT, Schwartz DM, et al. Janus kinases to Jakinibs: from basic insights to clinical practice. *Rheumatology*. 2019;58:i4-i16.
40. Schwartz DM, Kanno Y, Villarino A, et al. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discovery*. 2017;16:843-862.
41. Fleischmann R, Mysler E, Hall S, et al. Efficacy and safety of tofacitinib monotherapy, tofacitinib with methotrexate, and adalimumab with methotrexate in patients with rheumatoid arthritis (ORAL Strategy): a phase 3b/4, double-blind, head-to-head, randomised controlled trial. *Lancet*. 2017;390:457-468.

42. Fleischmann R, Schiff M, van der Heijde D, et al. Baricitinib, methotrexate, or combination in patients with rheumatoid arthritis and no or limited prior disease-modifying antirheumatic drug treatment. *Arthritis Rheumatol.* 2017;69:506-517.
43. Hazlewood GS, Barnabe C, Tomlinson G, et al. Methotrexate monotherapy and methotrexate combination therapy with traditional and biologic disease-modifying antirheumatic drugs for rheumatoid arthritis: a network meta-analysis (Review). *Cochrane Database Syst Rev.* 2016; ;353:i1777.
44. Burmester GR, Kremer JM, Van den Bosch F, et al. Safety and efficacy of upadacitinib in patients with rheumatoid arthritis and inadequate response to conventional synthetic disease-modifying anti-rheumatic drugs (SELECT-NEXT): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet.* 2018;391:2503-2512.
45. Westhovens R, Taylor PC, Alten R, et al. Filgotinib (GLPG0634/GS-6034), an oral JAK1 selective inhibitor, is effective in combination with methotrexate (MTX) in patients with active rheumatoid arthritis and insufficient response to MTX: results from a randomised, dose-finding study (DARWIN 1). *Ann Rheum Dis.* 2017;76:998-100.
46. Winthrop KL. The emerging safety profile of JAK inhibitors in rheumatic disease. *Nat Rev Rheumatol.* 2017;13:234-243.
47. Pappas DA, Hooper MM, Kremer JM, et al. Herpes zoster reactivation in patients with rheumatoid arthritis: analysis of disease characteristics and disease-modifying anti-rheumatic drugs. *Arthr Care Res.* 2015;67:1671-1678.
48. Nakajima A, Urano W, Inoue E, et al. Incidence of herpes zoster in Japanese patients with rheumatoid arthritis from 2005 to 2010. *Modern Rheumatol.* 2015;25:558-561.

How to cite this article: Gremese E, Alivernini S, Tolusso B, Zeidler MP, Ferraccioli G. JAK inhibition by methotrexate (and csDMARDs) may explain clinical efficacy as monotherapy and combination therapy. *J Leukoc Biol.* 2019;106:1063–1068. <https://doi.org/10.1002/JLB.5RU0519-145R>