

# Folate supplementations for methotrexate therapies in cancer and arthritis: rationales revisited

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For many decades, the folate antagonist methotrexate (MTX) has served as anchor drug in the treatment of selected cancer types, e.g., (pediatric) leukemia, and chronic inflammatory and joint destructive diseases like rheumatoid arthritis (RA). MTX treatment of leukemia commonly includes high dose MTX therapy (1–10 g/m<sup>2</sup>) [1], whereas RA treatment is based on low-dose MTX therapy (7.5–30 mg/wk) [2]. In both treatment modalities, therapy-induced toxicities, e.g., mucositis with high dose MTX and liver toxicities with long-term low dose MTX, are antagonized by post-supplementation of folates; leucovorin (LV) after HD-MTX and folic acid with low-dose MTX. Despite longstanding experience with various MTX/folate supplementation schedules in clinical practice, it is still an unresolved issue what is the most optimal schedule of MTX and folate supplementation is in terms of dosing and timing of folate supplementation. Furthermore, given the large variation in folic acid supplementation dosages prescribed with MTX treatment of RA patients worldwide, awareness for folate over-supplementation and concomitant long term adverse effects is called for. This commentary will address this issue in light of recent insights from laboratory, nutritional and clinical studies.

The rationale for HD-MTX and leucovorin rescue treatment is based on tumor cell uptake of MTX via the reduced folate carrier and subsequent intracellular conversion to MTX-polyglutamates (MTX-PGs) by the enzyme folylpolyglutamate synthetase (FPGS) [3]. These MTX-PGs are potent inhibitors of key enzymes in folate metabolism, including dihydrofolate reductase (DHFR), thymidylate synthase (TS), 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICARFT) and glycinamide ribonucleotide formyltransferase (GARTF), thereby impairing de novo biosynthesis of purines and thymidylate (for DNA synthesis), amino acids and methylation reactions. Sustained MTX inhibition of DHFR (>99% for 24 h) is considered necessary to initiate antitumor effects. It is assumed that normal cells harbor lower FPGS activities than tumor cells and thus accumulate lower MTX-PG levels will thus impose a less sustained DHFR block. Consequently, upon administration of the reduced folate cofactor leucovorin the blockade of DHFR will be re-

versed more easily in normal cells than in tumor cells thereby reducing toxic effects. From this perspective, it is evident that optimal dosing of MTX and timing of leucovorin administration after MTX dosing is crucial to obtain highest efficacy and minimize toxicity. In most standard protocols, LV administration is commonly initiated at the time (between 24–48 h) when plasma levels of MTX had dropped below 1–2 μM. However, to allow more personalized approach during the course of treatment, it would be of added value to monitor additional MTX cellular pharmacology parameters (cellular transport capacity, MTX-PG accumulation and FPGS activity) as well as intracellular folate status. Notably, MTX-PG analyses in leukemic blast cells revealed a large inter-patient variability of MTX-PG accumulation during HD-MTX therapy [1]. In addition, a recent study analyzed MTX-PG and folate levels in erythrocytes (RBC) during the course of HD-MTX/LV treatment of leukemia patients, again demonstrating a large interpatient variability with the standard dose of MTX [4]. Whether leukemic blast cells also harbor large inter-patient variability in folate levels needs further exploration and thus advocate the inclusion of a therapeutic drug monitoring arm in future HD-MTX/LV treatments to identify whether patients are either under- or over-dosed with MTX and/or LV accounting for differential therapy efficacies.

Dedicated studies related to MTX toxicity in mucosal tissues may also benefit from the recent availability of mucosal organoid models [5], which may help to optimize timing and dosing of LV after MTX administration to minimize toxicity.

In RA treatment, LV (at a dose of 2.5–5 mg/once a week) also proved to be effective in controlling MTX toxicity without compromising MTX efficacy at MTX dosages <15 mg and >15 mg, respectively [6]. In the same study, 1–2 mg of folic acid (daily after MTX) demonstrated almost equal results as for LV. For cost-effective reasons, folic acid is nowadays the preferred choice to manage MTX toxicity. Although initially 1 mg daily dosages of FA were commonly used to manage MTX toxicity in RA treatment, during the past 2 decades variable and increasingly higher dosages of FA (up to 30 mg weekly [7]) have employed in clinical practice. In some cases, these changes were indicated because of social health

care reimbursements reason, whereby (e.g., in the Netherlands) 1 mg FA tablets for daily use were not reimbursed but 5 mg FA for once weekly use were. Remarkably, this change in policy was not backed up with a proper clinical trial comparing the 2 dosages for MTX efficacy, MTX toxicity and possible long term (adverse) effects.

A systematic review by Liu *et al.* [8] reported that folic acid supplementation dosages of <10 mg/week and dosages of >25 mg/week were equally effective in reducing MTX toxicity and sustaining MTX efficacy in RA patients. Notwithstanding this fact, the wide variation of folic acid supplementation dosages and scheduling calls for reflection as one hallmark folate nutritional study has provide compelling evidence that humans can only metabolize 1 mg of folic acid/day because of the very low DHFR activity in the liver [9]. Thus, administering dosages of >1 mg folic acid/day will result in an increase of unmetabolized folic acid (UMFA) in the circulation. This was clearly shown in elderly persons who were administered 5 mg folic acid daily for 3 weeks after which UMFA reached plasma levels of 15–209 nmol/L [10]. Similarly, in a clinical RA setting, patients on low dose MTX therapy with either weekly 10 or 30 mg folic acid supplementation, serum UMFA levels were also markedly increased (up to 300 nmol/L) after 24 weeks of therapy [7]. It is well recognized that long-term circulating UMFA may impose a diversity of (adverse) effects, e.g., impairing uptake of circulating 5-methyltetrahydrofolate in vascular endothelial cells, impairing NK cell function, impairing neurodevelopment, increasing the risk of gestational hypertension, providing a growth advantage for premalignant cells, and epigenetic effects [11–13]. A recent prospective cohort study of >10,000 subjects also revealed an age-dependent relationship between UMFA serum levels and an increased risk of mortality [14].

Altogether, these observations call for awareness of potential adverse effects associated with prolonged folic acid over-supplementation at daily dosages exceeding 1 mg/day in MTX treatment protocols for RA, even though MTX efficacy and toxicity may not be compromised. This notion also relates to complementary folic acid intake via folate food fortification programs and or multi-vitamin preparations. Like for cancer patients, there is accumulating evidence of a large interpatient variability of blood cell accumulation of MTX-PGs of RA patients who all received a standard dose of 15–25 mg MTX [15]. This would argue not only for personalizing MTX therapy, but also folic acid supplementation. To this end, emerging therapeutic drug monitoring of folates/UMFA levels in plasma and MTX-PG and folate levels in blood cells of RA patients during the course of treatment could further guide optimal MTX therapies, reduction of MTX toxicities and avoidance of adverse effects initiated by folic acid over-supplementation. Notwithstanding decades of successful clinical use of MTX in cancer and RA treatment, deciphering the complexity of regulation of folate metabolism and MTX cellular pharmacology in cancer cells and immune cells may further assist in

fine-tuning current MTX treatment protocols and designing novel MTX/antifolate-based protocols. Targeting mitochondrial folate metabolism [16] deserves future exploration as therapeutic option.

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## Conflict of interest

The author declares no conflict of interest.

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