

# Folate receptor $\beta$ : a novel target for therapeutic intervention in rheumatoid arthritis?

*"FR $\beta$  harbors full potential to serve as an attractive target for therapeutic interventions of activated synovial macrophages in RA patients by small-molecule folate antagonist drugs."*

For more than five decades, the folate antagonist methotrexate (MTX) has served as an effective, convenient and cheap drug, eliciting anti-rheumatic activity as either a single agent or in combination regimens with other DMARDs and, more recently, with anti-TNF $\alpha$  biological agents [1-4]. Intriguingly, despite the long-term clinical application of MTX, the ultimate molecular mechanism(s) via which MTX exerts its anti-arthritic effect(s) has not been fully resolved [2,5-7]. In fact, multiple mechanisms of action have been reported [2,7], of which the most dominant mechanisms are considered to be: inhibition by MTX-polyglutamates of the folate-dependent purine biosynthesis *de novo* enzyme, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (AICARTFase), provoking an extracellular release of adenosine and anti-inflammatory signaling via adenosine receptors; and induction of apoptosis of activated T cells [6,8]. The extended knowledge concerning the cellular pharmacology of MTX, its inhibitory effects on folate and purine metabolism as well as downstream signaling pathways, and insight into mechanisms of resistance to MTX, has been explored to find associations for either clinical responses or toxic effects during treatment of rheumatoid arthritis (RA) patients with MTX. In fact, analysis of at least two types of parameters appeared to harbor potential predictive value: a pharmacogenetic index combining common polymorphisms of genes coding for key enzymes in folate transport and folate and purine metabolism [9-12], and cumulative levels of polyglutamate forms of MTX in red blood cells of RA patients during treatment [10,13]. Predictive models based on these parameters deserve further exploration and validation in prospective clinical studies, but they may also face some intrinsic shortcomings [14]. For example, genetic polymorphisms are generally not informative regarding the enzymes/proteins they are

encoding with respect to their functional activity, regulation and modulation of expression, differential levels of expression in immune cells to be targeted, and the influence of environmental factors influencing functional activity. Moreover, MTX-polyglutamate accumulation in red blood cells of RA patients is considered to mimic MTX accumulation in targeted immune cells (e.g., T cells, B cells, monocytes, macrophages and dendritic cells), but this does not take into account interactions between these cells and the fact that various immune cells may have differential levels of expression of multiple folate/MTX transporters, enzymes involved in polyglutamate synthesis and hydrolysis, and extracellular and intracellular folate homeostasis, all of which can influence cellular MTX-polyglutamate accumulation [7,15]. Furthermore, it is conceivable that whenever predictive models may have the power to anticipate efficacy at the start of treatment, this may not be the case when predicting long-term efficacy upon chronic treatment. Many RA patients still have to discontinue treatment after serial dose escalations due to loss of efficacy, drug-related toxic effects or both [16-19]. As such, there is still room to improve the efficacy of MTX and/or delay the onset of the resistance phenomena [7,20-22], either using second-generation folate antagonists, or MTX-conjugates with a better therapeutic window or long-term efficacy over MTX [23,24]. Alternatively, folate antagonists that exert less of a systemic effect due to more selective targeting of specific proinflammatory cytokine-producing immune cells could provide a rationale for a potentially more effective therapy with fewer toxic side effects.

Given the hydrophilic and anionic nature of MTX and most other folate antagonists, specific transport systems are required to facilitate their cell entry. In a recent study, we explored the potential role of a receptor-mediated transport route for the uptake of MTX and



**Gerrit Jansen<sup>†</sup>**

<sup>†</sup>Author for correspondence:  
Department of Rheumatology,  
Room 3A64, VU University  
Medical Center,  
De Boelelaan 1117, 1081 HV  
Amsterdam, The Netherlands  
Tel.: +31 204 446 685;  
Fax: +31 204 442 138;  
g.jansen@vumc.nl



**Joost W van der Heijden**

Department of Rheumatology,  
VU University Medical Center,  
Amsterdam, The Netherlands



**Ben AC Dijkmans**

Department of Rheumatology,  
VU University Medical Center,  
Amsterdam, The Netherlands

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second-generation folate antagonists in synovial macrophages [25]. Folate receptors (FRs) represent one of the three transport routes that can mediate the cellular uptake of folate and folate antagonist. The two other routes include carrier-mediated systems: the reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT). PCFT is primarily involved in the intestinal uptake of folic acid and 5-methyltetrahydrofolate (the main circulating plasma folate) but in addition, folate antagonists such as MTX serve as a low micromolar substrate for PCFT, which functions optimally at an acidic pH of pH 5.5 [15,26]. The RFC is constitutively expressed in most mammalian cells and tissues and is characterized by having a high (low  $\mu\text{M}$ ) affinity transport for 5-methyltetrahydrofolate and MTX, but a poor affinity for folic acid [15,27]. FR expression, on the contrary, is restricted to selected cells/tissues [28].

At least three isoforms of FR have been documented; two isoforms,  $\alpha$  and  $\beta$ , are linked to the cell surface via a glycosylphosphatidyl inositol anchor, whereas the  $\gamma$ -isoform lacks the glycosylphosphatidyl inositol anchoring signal and is constitutively secreted in circulation [29]. At the cell surface, FRs can reside either in clathrin-coated vesicles [30] or in special microdomains – designated lipid rafts that also harbor proteins involved in signaling processes [31].

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Characteristically, all FRs bind folic acid with a nanomolar affinity, but FR $\alpha$  and FR $\beta$  differ in their relative binding affinities for reduced folates and folate antagonists [32–34]. In addition, FRs bind MTX with an affinity that is over 100-fold lower than their affinity for folic acid. Each of the FRs has a restricted expression profile; FR $\alpha$  is expressed in certain normal epithelial cells and is overexpressed in some tumor tissues (e.g., ovarian carcinomas) [28]. FR $\beta$  is dominantly expressed in blood cells of the myelomonocytic lineage [35]. However, the functional ability of FR $\beta$  to bind ligand is confined to activated (synovial) macrophages or malignant acute myeloid leukemia cells, rather than to normal hematopoietic cells [29,36–38], which would thus minimize potential toxic side effects of FR-targeted therapies. In RA pathogenesis, activated macrophages

in the synovial membrane play a central role in the chronic course of the disease, by facilitating a persistent proinflammatory state of other inflammatory cells and synovial fibroblasts, and the bone-destructive activity of osteoclasts [39].

Based on their intrinsic properties and selective expression/ligand-binding profile, FRs have been exploited for imaging purposes with  $^{99\text{m}}\text{Tc}$ -folate, as well as for therapeutic targeting of FR-positive cancer cells by immunotherapy, folate-conjugated toxic drugs, liposomes and nanoparticles [40–44]. In an RA setting, this technology has been partly adopted for imaging and therapeutic targeting of FR $\beta$  on activated synovial macrophages; however, most of these studies were performed at an *in vitro* level or in animal models of arthritis [45–47], and need to be extended to a clinical level.

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 “The nonpolyglutamatable TS inhibitor BGC945, displayed selective targeting against FR $\beta$  transfected cells as it harbored, in contrast to other evaluated folate antagonists, a concomitant poor affinity for the RFC.”  
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In a study by Van der Heijden *et al.*, the specific aim was twofold: to identify whether folate antagonist drugs, to which FR $\beta$  binds with a higher affinity than the poor binding affinity it has for MTX, would represent more potent drugs; and to assess whether these selected drugs would bind specifically to FR $\beta$  and not to constitutively expressed RFC, thereby constituting more selective targeting and reducing potential toxic side effects [25]. Screening of a series of folate antagonists revealed that FR $\beta$  had a poor binding affinity for inhibitors of dihydrofolate reductase (50–100-fold lower than for folic acid). However, four compounds were identified for which FR $\beta$  had a high binding affinity (20–77-fold higher than for MTX and close to the binding affinity for folic acid). Two of these folate antagonists were inhibitors of glycinamide ribonucleotide formyltransferase (GARTFase) and were involved in purine biosynthesis *de novo*, and two others were inhibitors of thymidylate synthase (TS). Only one of these, the nonpolyglutamatable TS inhibitor BGC945 [48], displayed selective targeting against FR $\beta$ -transfected cells as it harbored, in contrast to other evaluated folate antagonists, a concomitant poor affinity for the RFC. BGC945 has recently been renamed as ONX0801 after Onyx Pharmaceuticals (CA, USA) obtained a worldwide license for this drug. Obviously,

BGC945/ONX0801 deserves further preclinical exploration for selective activated macrophage-directed therapy in arthritic animal models. In this respect, three novel folate-based GARTFase inhibitors with moderate FR $\beta$  binding affinities recently demonstrated anti-arthritic potency in arthritic animal models [49,50], but since these folate antagonists are also efficiently transported via RFC, these drugs lack full FR $\beta$  targeting selectivity. One other issue that needs to be addressed is whether second-generation folate antagonists (both polyglutamatable and nonpolyglutamatable) that primarily inhibit dihydrofolate reductase, TS or GARTFase are equally or more efficient than MTX in evoking an anti-arthritic response, and whether this involves a mechanism that is similar or distinct to that of MTX. In fact, evidence has been presented that the folate-based TS inhibitor raltitrexed, and GARTFase inhibitors, could elicit an anti-arthritic response in an arthritic animal model [49–51]. Since it is not anticipated that these folate antagonists efficiently inhibit AICARTFase and provoke an adenosine release as observed for MTX-polyglutamates [6], this suggests that other mechanisms are operative. In this context, studies by Van der Heijden *et al.* demonstrated that folate-based TS and GARTFase inhibitors were capable of inducing apoptosis in activated T cells from RA patients and suppressing proinflammatory cytokine production [52]. One final aspect that may be of importance for successful FR-targeted therapies is extracellular/intracellular folate status, which may influence receptor occupancy and competition [34]. To this end, folic acid or leucovorin supplementation [53], as well as dietary intake

of folates by folate food fortification and/or vitamin supplements by RA patients should be carefully monitored [54,55].

Collectively, FR $\beta$  harbors full potential to serve as an attractive target for therapeutic interventions of activated synovial macrophages in RA patients by small-molecule folate antagonist drugs. Such a targeted approach could have a better therapeutic window when a prototypical folate antagonist harbors a good affinity for FR $\beta$  in conjunction with a poor affinity for RFC. Candidate drugs meeting this criterion, for example BGC945/ONX0801, have now been identified [25]. This, together with strategies that were recently described to upregulate FR $\beta$  expression by low-dose all-*trans* retinoic acid [56], histone deacetylase inhibitors [57] and glucocorticoids [29,58], may even further improve such a therapeutic strategy. Ideally, noninvasive positron emission tomography imaging technology [59] with folate-based tracers could be utilized to select RA patients for FR $\beta$  expression on macrophages at inflamed sites, which may then be eligible for treatment with FR $\beta$ -targeted folate antagonist drugs.

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