Inhibition of glycinamide ribonucleotide formyltransferase results in selective inhibition of macrophage cytokine secretion *in vitro* and *in vivo* efficacy in rat adjuvant arthritis


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**Abstract**

**Objective**

To determine the effects of a glycinamide ribonucleotide formyltransferase (GARFT) inhibitor on macrophage inflammatory processes and *in vivo* in rat adjuvant arthritis.

**Methods**

GARFT inhibitors, LY309886 (6S-2’,5’-thienyl-5,10-dideazatetrahydrofolic acid) and LY329201 (R)-N-[[5-[2-(2-amino-1,4,5,6,7,8-hexahydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)ethyl]-2-thienyl]carbonyl]-L-glutamatic acid disodium salt, were investigated *in vitro* and *ex vivo* on primary murine peritoneal macrophages and in the RAW macrophage cell line for both purine depletion and inhibition of LPS induced monokine secretion. *In vivo* efficacy following GARFT inhibition was evaluated in modified rat adjuvant arthritis.

**Results**

LY309886 inhibited purine biosynthesis in the RAW cell line with an EC₅₀ of 90 nM, an effect readily reversible with exogenous hypoxanthine. LY309886 and LY329201 also inhibited LPS induced TNF alpha and MIP1 alpha in these cells and in primary macrophages. A similar effect could be demonstrated *ex vivo* with mice dosed for two days with 3 mg/kg of LY329201. LY329201 as well as methotrexate demonstrated a dose dependent reduction in both paw and spleen weight and improved joint histology following 2 weeks of dosing in a rat adjuvant arthritis study.

**Conclusion**

These results suggest that GARFT inhibitors should be tested in the treatment of rheumatoid arthritis by considering their mechanism of action, here successfully tested on activated macrophages.

**Key words**

inflammation, anti-folates, tumor necrosis factor, macrophage inflammatory protein 1.
GARFT inhibitors as anti-inflammatory agents / S. Chintalacharuvu et al.

Introduction
Mammalian glycinamidine ribonucleotide formyltransferase (GARFT), is a trifunctional enzyme involved in de novo purine biosynthesis. GARFT (EC 2.1.2.1) as a formyltransferase will result in the conversion of the glycinamidine ribonucleotide (GAR) in the presence of the cofactor 10-formyltetrahydrofolate to formylglycinamidine ribonucleotide (FGAR) and tetrahydrofolate (1-3). Mammalian GARFT will also catalyze the synthesis of GAR and aminomimidazole ribonucleotide as a synthetase. Whereas GARFT activity was first reported approximately 50 years ago (4), the demonstration that the antitumor agent 5, 10-dideazatetrahydrofolate (DDATHF) was a GARFT inhibitor (5) as well as the evaluation of GARFT inhibitors including lomotrexol (6R-5,10-dideaza-5,6,7,8-tetrahydrofolic acid) and LY309887 (6R-2',5'-thienyl-5,10-dideazatetrahydrofolic acid), a thiophene analogue of lomotrexol in oncology trials has led to the synthesis and evaluation of additional GARFT inhibitors (6-9). Although the dependence of multiple tumors for de novo purine biosynthesis suggested an obvious indication for this class of compounds, the utility for other indications was less apparent. Methotrexate represents a distinct type of anti-folate first used for oncology and then orally at lower doses with significant disease modifying activity in rheumatoid arthritis (10). The mechanism(s) by which methotrexate exhibits anti-inflammatory effects remains unclear although preclinical evidence would suggest a possible linkage to adenosine release (11-13). With the demonstration that methotrexate was also an anti-inflammatory agent in rodent arthritis models (14-16) it suggested that other anti-folates, distinct from methotrexate without dihydrofolate reductase (DHFR) inhibitory activity might also prove to be anti-inflammatory. To this extent, the GARFT inhibitor LY309887, $K_i = 6.5 \text{ nM}$, (7) was evaluated in a mouse collagen induced arthritis model (17). These authors reported that LY309887 inhibited disease progression as evidenced by lower arthritis index as well as reduced synovi-tis, cartilage erosion and pannus formation when compared to placebo treated animals with a similar arthritis index. The goal of the present study was to evaluate a similar GARFT inhibitor (LY329201, the disodium salt of LY309887) in the more severe rat adjuvant arthritis model as well as to explore the effects of the inhibitor in vitro on macrophage purine levels as well as its effects on LPS induced monokine secretion. The results demonstrate that LY329201 decreases both spleen and paw weight as well as reductions by histologic assessment in joint pathology, similar to that observed with methotrexate. Furthermore, macrophages treated either in vitro or ex vivo, demonstrated reduced ATP levels which were reversible in vitro by hypoxanthine as well as reduced secretion of both TNF alpha and MIP-1-alpha. These results provide additional support for the consideration of GARFT inhibitors for the treatment of rheumatoid arthritis and possibly other autoimmune and/or chronic inflammatory diseases.

Materials and methods
Macrophage cultures and cytokine assays
The RAW 264.7 macrophage cell line was maintained in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal calf sera (HyClone Laboratories, Logan, UT). Primary cultures of thioglycollate elicited macrophages were derived from BALB/c mice (Taconic, Germantown, NY) and maintained in culture in RPMI 1640 supplemented with 2% fetal calf sera. Both the RAW 264.7 macrophage cell line and the primary peritoneal macrophage cultures were stimulated with LPS (E. Coli O55:B5, Difco, Detroit, MI) for 5 or 24 hrs in the presence of varying concentrations of GARFT inhibitor or dexamethasone in select studies. Supernatants were assayed for TNF and MIP 1 alpha by ELISA (R & D Systems, Minneapolis, MN) as well as by multiplex analysis (LumineX, Lincoln Research, Inc., St. Charles, MO) to evaluate a broader spectrum of cytokines. LY309886 (6S-2',5'-thienyl-5,10-dideazatetrahydrofolic acid), and
LY329201 (R)-N-[5-[2-(2-amino-1,4,5,6,7,8-hexahydro-4-oxopyrido[2,3-d]pyrimidin-6-yl)ethyl]-2-thienyl]carbonyl]-L-glutamatic acid disodium salt, were synthesized at Lilly Research Labs (Indianapolis, IN). Other reagents unless specified were from Sigma (Sigma Chemical, St. Louis, MO).

ATP assay
Macrophages were stimulated with LPS at 100 ng/ml for 5-24 hrs with or without GARFT inhibitors. Cells were washed twice in phosphate buffered saline (PBS) and lysed in ATP releasing solution (Sigma). Supernatants were assayed for ATP using a luminometer (Perkin-Elmer Life Sciences, Gaithersburg, MD) following substrate addition (Sigma kit).

Ex vivo macrophage studies
BALB/c mice were thioglycollate elicited and at 3 days post thioglycollate injection, mice were dosed intra-peritoneal, i.p., with 3 mg/kg of LY329201 or vehicle (PBS) for two additional days. Peritoneal macrophages were harvested 24 hrs after the final dose of LY329201 and following minor adjustments to the leukocyte counts, similar densities of macrophages from LY329201 and vehicle injected mice were cultured in vitro. These cultures were then further treated or not with LY329201 at 20 uM in the presence of LPS and then supernatants were assayed for MIP1 alpha and cell lysates for ATP. In both instances reversal of the anti-folate effects were evaluated by coculture with 100 uM hypoxanthine.

Adjuvant arthritis
Male Lewis rats (180-200 grams, Harlan Laboratories, Indianapolis, IN) were used for these studies. A synthetic adjuvant, N, N-dioctyldecyl-N’-N-bis (2-hydroxyethyl) propanediamine (LA) was suspended in oil. On day 0, 0.1 ml of LA (7.5 mg/rat) was injected dermally at the base of the tail. On day 9, rats exhibit marked increases in paw swelling and were sacrificed on day 16 (18). On the day of sacrifice, total spleen and paw weights were measured. Histologic changes were examined by microscopy of formalin-fixed paws. Hind paws were trimmed, placed in decalifying solution for 24 hrs, embedded in paraffin, sectioned at 5 mm, stained with hematoxylin and eosin and studied under light microscopy. Pathologic changes were evaluated in the joint for cartilage erosion, joint space exudates, synovitis, and soft tissue inflammation. Histologic scoring for each parameter ranging from 0 (no signs) to 5 (severe) were conducted in a blinded fashion by a pathologist. This model is not characterized by significant pannus formation during the length of the study. Rats (n = 5/group) were treated from day 0 (day of disease induction) to day 15 (one day prior to sacrifice). Each day rats were injected i.p. with LY329201 (0.1 – 3 mg/kg), methotrexate (0.01 – 0.3 mg/kg), dexamethasone (0.1 mg/kg) or saline. Rats were housed in a controlled environment and provided with standard rodent chow and water. Animal care and studies were in compliance with IACUC rules and regulation and with institutional guidelines.

Statistical studies
Differences between the vehicle and compound treated cell cultures were evaluated for statistical significance by a paired, two tailed Students T test. Statistical significance between the vehicle and compound treated rats for changes in paw and spleen weight as well as in histologic scores were determined by a Dunnett’s T test.

Results
Cellular ATP depletion by the GARFT inhibitor LY309887 has been previously used to predict inhibition of human tumor cell line growth and hence sensitivity to this anti-folate (19). Accordingly, the GARFT inhibitor LY309886 was first evaluated for its ability to deplete intracellular ATP levels in the RAW 264.7 macrophage cell line as a predictor for primary macrophages. As demonstrated (Fig. 1), LY309886 treatment of RAW 264.7 cells for 5 hrs resulted in a dose dependent reduction in ATP with an IC50 of 90 nM. This effect was reversed by coculture of the cells in 100 uM hypoxanthine suggesting that the ATP depletion was not due to cell toxicity, but rather through the expected inhibition of de novo purine synthesis. In the presence of hypoxanthine the cells were able to maintain purine levels via the scavenger pathway (20). The effect on ATP depletion was also associated with a concentration dependent decrease in TNF alpha levels following a LPS challenge. As observed, (Fig. 2), 100 nM LY309886
resulted in a maximal inhibition of TNF alpha production with no further effect observed with higher concentrations of compound. Dexamethasone at 1 uM, however was able to further reduce TNF alpha production (from 8.7 ng/ml in the vehicle group to 2.5 ng/ml, a 70% reduction), consistent with the multiple levels by which glucocorticoids exert their anti-inflammatory effects.

Similar studies were performed in vitro with primary cultures of mouse peritoneal macrophages to determine whether the effects of LY329201 and LY309886 on purine biosynthesis and ATP depletion could also be demonstrated in non-transformed macrophages. As seen (Fig. 3A), both GARFT inhibitors depleted ATP levels in the primary macrophage cultures (IC50 values of 5.7 and 27.5 nM respectively) and furthermore, supernatants from these LPS stimulated cultures demonstrated a significant inhibition in both TNF (Fig. 3B) and MIP1 alpha synthesis (Fig. 3C) with maximal inhibition observed at 100 nM, similar to that seen with the macrophage cell line. This effect as with the ATP levels was reversible by coculture with hypoxanthine, even at 10 times the maximal effective concentration for cytokine inhibition for both GARFT inhibitors. Therefore, in both primary macrophages and macrophage cell lines, the effects of GARFT inhibitors on macrophages extend beyond inhibition of purine biosynthesis but also to mitigate the magnitude of the production of both TNF and MIP1 alpha cytokines in response to a LPS stimulus.

In an attempt to determine whether the anti-inflammatory effects of GARFT inhibitors are limited to the effects on

![Fig. 2.](image-url) Inhibition of LPS induced TNF alpha synthesis in RAW264.7 cells by LY309886. RAW264.7 cells were stimulated with 100 ng/ml of LPS for 5 hrs in the presence of varying concentrations of LY309886 and supernatants quantitated for TNF alpha by ELISA. Brackets indicate standard deviation of the mean. The difference in TNF alpha levels when compared to the LPS stimulated controls was significant P < 0.01 by a two tailed Student's T test.

![Fig. 3.](image-url) LY309886 and LY329201 reduce ATP levels and the synthesis of TNF and MIP1 alpha in primary cultures of murine peritoneal macrophages. Thioglycollate elicited macrophages were incubated with varying concentrations of either LY329201 or LY309886 for 24 hrs in the presence of 100 ng/ml of LPS. Following incubation, ATP levels were quantitated on macrophage lysates (A) and supernatants were assayed for TNF alpha (B) and MIP1 alpha (C). (A) Reductions in ATP levels were significant (P< 0.01) for the LY309886 treated cells at concentrations greater than 50 nM, and for the LY329201 treated cells at concentrations greater than 1 nM. Reductions in both TNF and MIP1 alpha were apparent in the cultures treated (trt) with compounds but were reversed in compound treated cells co-cultured with 100 uM hypoxanthine (HPX). Brackets indicate the standard deviation of the mean. ‘*’ and ‘**’ indicates statistical significance P< 0.01 and 0.001 respectively compared to the vehicle treated cells by a two tailed Student's T test.
TNF and MIP 1 alpha, primary macrophages were stimulated with LPS for 24 hrs in the presence of 1 uM LY309886. Supernatants were then evaluated by multiplex to determine whether all LPS induced cytokines were similarly inhibited. As seen, (Table I), LY309886 reduced IL-6, MCP-1 and KC levels, whereas IL-1 beta and RANTES were not effected. In contrast, dexamethasone, at 0.1 uM, was clearly a more potent anti-inflammatory agent in terms of both the number of cytokines inhibited and the magnitude of this inhibition. These results suggest that the anti-inflammatory effects of GARFT inhibitors may be more specific and less broad spectrum than those effects mediated by glucocorticoids.

Table I. Selective inhibition of cytokine secretion by LY309886 in LPS activated macrophages.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>IL-1α</th>
<th>IL-6</th>
<th>TNF</th>
<th>MIP-1α</th>
<th>MCP-1</th>
<th>KC</th>
<th>RANTES</th>
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<tr>
<td>Vehicle</td>
<td>0.76</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.2</td>
<td>1.4</td>
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<td>13.4</td>
<td>1.1</td>
<td>62.4</td>
<td>10.0</td>
<td>18.1</td>
<td>1.1</td>
</tr>
<tr>
<td>LPS + LY309886</td>
<td>63</td>
<td>5.9</td>
<td>0.47</td>
<td>27.6</td>
<td>4.8</td>
<td>9.7</td>
<td>1.2</td>
</tr>
<tr>
<td>LPS + dexamethasone*</td>
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<td>0.5</td>
<td>0.2</td>
<td>10.0</td>
<td>2.4</td>
<td>4.8</td>
<td>0.46</td>
</tr>
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</table>

* Murine peritoneal macrophages were incubated in the absence or presence of LPS at 100 ng/ml for 24 hrs and supernatants harvested for cytokine quantitation by multiplex analysis.

Fig. 4. Macrophages from LY329201 treated mice demonstrate reduced ATP levels ex vivo. Mice were injected with thioglycollate broth and 72 hrs later dosed i.p with LY329201 at 3 mg/kg or vehicle for 2 successive days. Macrophages were harvested 24 hrs after the second dose of LY329201 and cultured in vitro, in the presence or absence of 20 uM LY329201. Cells were lysed after 24 hrs and ATP levels quantitated. Brackets indicate the standard deviation of the mean. * Indicates significance, relative to the in vitro untreated groups, by a Student's two tailed T test P < 0.001.

Fig. 5. Hypoxanthine reverses the ex vivo inhibitory effects of LY329201 on ATP depletion and cytokine secretion. Macrophages from vehicle or LY329201 treated mice (see figure 4 legend) were cultured in the presence or absence of 100 uM hypoxanthine (HPX) and stimulated with LPS at 100 ng/ml. (A) ATP levels were determined on the macrophage lysates from the LPS stimulated cultures and supernatants were collected for MIP 1 alpha (B) and TNF alpha (C) quantitation. Unless otherwise indicated all cultures were stimulated with LPS in vitro. Brackets indicate the standard deviation of the mean. * Indicates significance P< 0.001 from the LPS stimulated vehicle treated mice by a two tailed Student's Ttest.
tained from mice injected i.p. with 3 mg/kg of LY329201 for two days prior to harvest when placed in culture showed reduced ATP levels in comparison to the vehicle treated animals. The vehicle treated macrophages did demonstrate reduced ATP levels when treated in vitro with 20 uM LY329201 whereas the macrophages from the LY329201 treated animals did not demonstrate any further reduction in ATP when treated in vitro with compound. These results suggest that the in vitro effects of GARFT inhibitors on ATP depletion could also be demonstrated ex vivo. The ex vivo effects of LY329201 were reversible when cells were cocultured with 100 uM hypoxanthine and these cells were indistinguishable from the vehicle group (Fig. 5). Furthermore, in agreement with the in vitro studies, macrophages from the LY329201 treated mice demonstrated reduced levels of MIP 1 alpha (Fig. 5B) and TNF alpha (Fig. 5C) when compared to vehicle treated mice and here too, these ex vivo inhibitory effects were reversible with hypoxanthine.

The anti-inflammatory effects both in vitro and ex vivo demonstrated for GARFT inhibitors in the present study as well as a previous study reporting that LY309887 mitigated disease progression in the mouse collagen induced arthritis model (17) raised the question as to whether GARFT inhibitors would have disease modifying activity in other preclinical arthritis models. To this extent, LY329201 from 0.1 to 3 mg/kg, and methotrexate at 0.01 to 0.3 mg/kg were administered for 15 days i.p. in the modified rat adjuvant arthritis model. As demonstrated (Fig. 6), both LY329201 and methotrexate significantly inhibited the increase in spleen weight (Fig. 6A) with ED50 values of 0.2 mg/kg and <0.1 mg/kg respectively. Similar effects were also observed when paw weight changes were measured (Fig. 6B). These effects were comparable at the highest doses to the magnitude of inhibition observed with 0.1 mg/kg dexamethasone.

A similar effect was observed following a histologic assessment of joint pathology (Fig. 7). In the vehicle control ankle joints, there was marked soft tissue swelling, edema, fibroblast proliferation, and new capillary vessel formation along with influx of numerous lymphocytes and macrophages. This inflammation extended up into the perosteum of the long bones and into the synovial lining. Often there was exudation of this inflammation into the synovial cavity and into the articular cartilage of the bones contributing to articular cartilage erosion and secondary inflammation. Histologic scores (cartilage erosion, joint space exudates, synovitis and soft tissue inflammation) were significantly reduced in the affected joints with LY329201 at 3 and 1 mg/kg. A reduction in cartilage erosion and synovitis was also observed at 0.3 mg/kg although the remaining indices were not different from the vehicle control. Similarly, methotrexate at 0.3 and 0.1 mg/kg also significantly reduced histologic scores while the lower doses were without effect. Therefore, the efficacy demonstrated in adjuvant arthritis would suggest, as with glucocorticoids and methotrexate that GARFT inhibitors should have disease modifying activity in rheumatoid arthritis patients.

Discussion

The development of folic acid antagonists that target folate binding enzymes including dihydrofolate reductase, thymidylate synthetase, and as in the pre-
sent study GARFT has been a major focus in drug discovery for over 50 years with the primary application towards oncology. With the dependence of many tumors on increased de novo purine and pyrimidine biosynthesis, it is believed that the primary mechanism(s) for the cytostatic and cytotoxic effects of anti-folates is through inhibition of DNA and RNA synthesis (9, 21, 22). These cytotoxic effects of anti-folates have been demonstrated in vitro on a variety of tumor cell lines. For example, the potent GARFT inhibitor LY 309887 has been demonstrated to decrease ATP levels and to be cytotoxic against leukemic cell lines including CCRF-CEM with an IC50 in the low nM range (19, 23).

In the present study LY309886, as well as LY329201 have also been demonstrated to decrease cellular ATP levels and to be cytotoxic against leukemic cell lines including CCRF-CEM with an IC50 in the low nM range (19, 23).

GARFT inhibition on macrophage ATP levels were reversed by exogenous hypoxanthine, suggesting the ability to bypass the block in de novo purine biosynthesis via the scavenger pathway. Furthermore, these effects were also associated with a reversible decrease in the secretion of monokines including TNF and MIP 1 alpha following LPS challenge, an effect not previously reported. These effects were not global in contrast to the effects on a broader group of cytokines as seen when macrophages were treated with dexamethasone.

With the demonstration that GARFT inhibitors could significantly reduce the production of both TNF and MIP 1 alpha following an in vitro LPS challenge, and the association of TNF and MIP 1 alpha with the progression of many of the arthritides (24-27) the effect of LY329201 was investigated in the rat LA model. The disease, in this model, is morphologically indistinguishable from the classic adjuvant arthritis induced by Freund’s complete adjuvant (18). However, in our experience, the LA model produces a more consistent disease in rats. LY329201 was investigated in the rat adjuvant arthritis model. Efficacy, as demonstrated by reductions in paw and spleen weight as well as in histologic examination of the affected joints, was apparent and as with methotrexate, was dose dependent. While LY329201 at 3 mg/kg had similar efficacy as methotrexate at 0.3 mg/kg, it is not possible to extrapolate to relative potencies without a detailed understanding of the pharmacokinetics of both molecules in this model.

The efficacy observed with LY329201 is in agreement with the effect of the related GARFT inhibitor LY309887 in the mouse collagen induced arthritis model (17). In that study, LY309887 demonstrated a dose dependent effect on the production of IgG1 and IgG2a collagen antibodies and on a global arthritis score when mice were dosed every other day for 5 doses at 50 ug/kg i.p. In contrast, efficacy in the present study required higher doses, 1-3 mg/kg daily. The difference in potency between these two studies likely reflects differences in the severity of the arthritis models. Furthermore, the mouse
collagen induced arthritis study was performed with mice maintained on a low folate diet which will increase the potency of the compound whereas the present study was done using animals maintained on a normal diet without folate restriction. The rat adjuvant arthritis model is a T cell dependent chronic erosive arthritis for which efficacy of low dose methotrexate and glucocorticoids has been reported (14-16, 28). Low dose methotrexate, between 0.1 – 1 mg/kg weekly on an intermittent dosing paradigm, has been demonstrated to reduce limb swelling, and articular destruction based on radiographic and histopathologic scores (15, 28). While the anti-inflammatory effects of low dose methotrexate have been demonstrated both clinically and preclinically, the mechanism(s) by which methotrexate mediates these effects remains unclear. The demonstration that polyglutamated methotrexate, likely the form that accumulates intracellularly, is a potent inhibitor of 5-aminoimidazole-4-carboxamide-ribonucleotide transformylase, (AICARFT) and that the resulting accumulation of AICAR can contribute to adenosine accumulation by inhibiting adenosine deaminase and adenosine monophosphate deaminase (16, 29, 30) provides a possible mechanism of action. Previous studies have demonstrated that A<sub>2a</sub> and A<sub>3</sub> adenosine receptor agonists have anti-inflammatory effects in vitro and in the case of an A<sub>2a</sub> agonist, can delay the progression of arthritis and lessen the disease severity in the mouse collagen-induced arthritis model (31). Furthermore, the anti-inflammatory effects of methotrexate and an analog MX-68 in a mouse air pouch carrageenan model were less apparent in A<sub>2a</sub> or A<sub>3</sub> receptor deleted mice (30). Whether the anti-inflammatory effects of the GARFT inhibitors LY309886 and LY329201 are mediated through an adenosine related mechanism remains to be determined.

Intervention in the purine biosynthesis pathways both clinically with methotrexate and preclinically with methotrexate and GARFT inhibitors has suggested the potential for other inhibitors of de novo nucleoside biosynthesis to be effective in the management of rheumatoid arthritis. To this extent, Leflunomide was approved for the treatment of rheumatoid arthritis (32). Leflunomide, an inhibitor of dihydroorotate dehydrogenase, a key enzyme in de novo synthesis of pyrimidines results in uridine depletion and is believed to act primarily on T and B lymphocytes. However, Leflunomide’s anti-inflammatory effects extend beyond lymphocytes (33, 34). Treatment of synovial macrophages cultured from rheumatoid arthritis patients, for example, with A77 1726, the active metabolite of Leflunomide, resulted in reduced synthesis of TNF α, IL-1β, cyclo-oxygenase 2 and intercellular adhesion molecule-1 (33). Efficacy with Leflunomide in preclinical autoimmune models including rat adjuvant arthritis has also been reported (35, 36).

Despite significant advances in the management of rheumatoid arthritis, including the use of anti-TNF interventions plus methotrexate, there is still considerable room for improvement for this patient population. Whereas additional antibody approaches including anti B cell antibodies, IL-17 and IL-23 as well as other nonspecific cytokine interventions are being explored (37, 38), novel approaches targeting purine and pyrimidine biosynthetic pathways including GARFT inhibition will, if successful, provide patients with additional therapeutic options for the treatment of rheumatoid arthritis.

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