




REVIEW ARTICLE

Immunomodulators: still having a role?

Jeffery M. Venner^{1,2} and Charles N. Bernstein ^{1,2,*}

¹University of Manitoba IBD Clinical and Research Centre, Winnipeg, Manitoba, Canada; ²Department of Internal Medicine, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

*Corresponding author. University of Manitoba, 804-715 McDermot Avenue, Winnipeg, Manitoba R3E3A4, Canada. Tel: 1-204-7893369; Fax: 1-204-7893972; Email: Charles.bernstein@umanitoba.ca

Abstract

Immunomodulators, particularly the thiopurines and to a lesser extent methotrexate, were standard of care for inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, for >40 years. While there has been a renaissance in available therapies with the advent of biologics and small molecules, an impetus remains for the ongoing use of thiopurines and methotrexate. This is particularly true for the maintenance of remission and when used in combination therapy with infliximab to suppress anti-biologic antibodies. This article summarizes the data behind immunomodulator use in Crohn's disease, focusing on the beneficial role these drugs still have while acknowledging their clinical limitations.

Key words: immunomodulators; 6-mercaptopurine (6-MP); azathioprine; Crohn's disease; methotrexate

Introduction

Inflammatory bowel diseases (IBDs), including Crohn's disease (CD) and ulcerative colitis (UC), have been subject to a rejuvenation of novel therapies over the past two decades. Prior to 1998, treatment for CD was mostly limited to corticosteroids, 5-aminosalicylic acid (5-ASA), thiopurines (6-mercaptopurine [6-MP] and azathioprine [AZA]), and methotrexate (MTX). Since the approval of the chimeric monoclonal antibody infliximab (antitumor necrosis factor [TNF]) by the Food and Drug Administration in 1998 for the treatment of CD, several other biologics have become available for patients with CD. As beneficial as many of these newer treatments have been for patients, they remain costly, carry their own risk profile, and do not guarantee disease remission. Thus, the so-called immunomodulators, particularly the thiopurines and MTX, remain a therapeutic option for many patients, especially those with mild to moderate disease activity in resource-limited settings. This review summarizes the

history of immunomodulators, their role in CD, and potential pitfalls with their use in clinical practice.

History in CD

Thiopurines

Thioguanine (TG) and, shortly after that, 6-MP and AZA were discovered and developed by Gertrude Elion and George Hitchings [1]. These drugs were initially used to treat acute lymphoblastic leukemia in children and remained common chemotherapy for >40 years. In 1960, Sir Roy Calne introduced 6-MP and AZA into the field of organ transplantation [2]. The first published trial using AZA in CD was reported in 1969 [3]. Seventy years after their initial discovery, 6-MP and AZA are still used for several chronic inflammatory conditions including IBD. In the past 10–15 years, it has become controversial whether thiopurines have a role in IBD treatment as monotherapy. Their

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use as monotherapy has diminished in North America but they have a prominent role in Europe and elsewhere [4].

Methotrexate

The role of Lederle Laboratories and Dr Yellapragada SubbaRow in the discovery of MTX is not as well recognized as the thiopurine story. This team was the first to isolate and synthesize folic acid [5], and subsequently developed an interest in folic acid antagonists for their potential role as chemotherapies. In the late 1940s under SubbaRow's guidance, MTX was synthesized [5]. MTX quickly became a highly regarded treatment option for not only leukemia, but also rheumatoid arthritis and psoriasis [5].

Mechanism of action

Thiopurines

Despite clinical use for >60 years, the mechanism of action of thiopurines has only more recently been better understood. The prodrug AZA is metabolized to 6-MP, which is then metabolized by three competitive enzymatic pathways (Figure 1) [6]. Two of these pathways result in the production of inactive metabolites, 6-methyl-mercaptopurine (6-MMP) and 6-thiouric acid (6-TU). The production of active metabolites results from the conversion of 6-MP into 6-thioguaninenucleotides (6-TGNs) via several enzymatic steps involving hypoxanthine-guanine phosphoribosyl transferase (HGPRT), inosine monophosphate dehydrogenase (IMPDH), and guanosine monophosphate synthase (GMPS) [6]. 6-TGNs technically refer to a group of metabolites that include tri-, di-, and monophosphate forms. 6-TG can also be converted to 6-TU (inactive) and 6-TGNs via HGPRT and intestinal microbiota, but the enzymatic pathway to get there is more direct than 6-MP [6, 7].

The mechanism of immunosuppressive action can be simplified into three processes, with intercalation being the most recognized. Further metabolism of 6-TGNs produces deoxy-6-thioguanosine 5' triphosphate (TdGTP) [8]. Incorporation of TdGTP into DNA triggers cell-cycle arrest and apoptosis through the mismatch repair pathway [8]. Second, an intermediary metabolite between 6-MP and 6-TGNs is 6-thioinosine monophosphate (6-TIMP). 6-TIMP is converted to methyl-thioinosine monophosphate (MeTIMP), an inhibitor of purine *de novo* synthesis [8]. Thus AZA and 6-MP are likely to have an additional antimetabolic effect not seen with 6-TG, attributed to its different metabolic pathway [6, 7]. The third mechanism involves inhibition of signal two of T-cell activation: conversion of CD28-mediated co-stimulation into an apoptotic signal through the binding of 6-thio-GTP (a 6-TGN) to Rac1 [9]. In summary, T-cell apoptosis is the overall main mechanism of action.

Methotrexate

MTX likely has a spectrum of dose-dependent effects. In high doses (when given at 1–12 g/m² for the purpose of chemotherapy), MTX produces measurable and rapid cytotoxic effects, primarily through the inhibition of dihydrofolate reductase. Dihydrofolate reductase is a critical enzyme in folic acid metabolism, and interference in this pathway results in decreased purine and pyrimidine synthesis [10]. When used at lower doses (e.g. 7.5–20 mg weekly) in chronic inflammatory diseases such as CD, its immunomodulating effect is proposed to be more complex and not strictly associated with cytotoxicity, as supplementation with daily folate does not negate clinical benefit [10].

MTX is an inhibitor of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). Inhibition of AICAR results in the release of extracellular adenosine, and through binding adenosine receptors in paracrine fashion expression of several inflammatory mediators (e.g. TNF, nuclear factor-kappa beta, adhesion molecules [intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin]), and enzymes involved in extracellular matrix remodeling (e.g. matrix metalloproteins and tissue inhibitor of metalloproteinase) are decreased [10].

Induction of remission

Thiopurines

The efficacy of AZA or 6-MP monotherapy for induction in CD is modest at best and is not used for remission induction. A 2016 Cochrane meta-analysis of 1,211 patients from 13 randomized-controlled trials (RCTs) showed that 48% receiving AZA or 6-MP achieved remission compared with 37% in placebo arms (relative risk [RR] 1.23, 95% confidence interval [CI] 0.97–1.55) [11]. These studies were overrepresented with patients with mild to moderate disease, typically defined as Crohn's Disease Activity Index (CDAI) 150–450 with median CDAI closer to 200–250. The only trial to evaluate mucosal (endoscopic) healing was the Study of Biologic and Immunomodulator Naïve Patients in Crohn's Disease (SONIC) trial (described in more detail below) [12]. This study showed that 16.5% (18/109) of patients on AZA vs 30.1% (28/93) of patients on infliximab achieved mucosal healing ($P=0.02$) [12].

Methotrexate

Evidence for the induction of remission with MTX is less encouraging than with thiopurines, thus it is also not recommended for the induction of remission. A Cochrane review demonstrated that more patients in the placebo arm entered remission than those receiving low-dose MTX (12.5 or 15 mg per week) [13]. Nonetheless, in this meta-analysis, AZA or 6-MP was similar to MTX for induction of remission (RR 1.13, 95% CI 0.85–1.49) [11]. Patients who were either intolerant of or relapsed on thiopurines generally had good success at induction with MTX (30%–86% at 6 months), but only 20% were still in remission at 5 years [14]. However, this is all low-quality evidence, as a result of underpowering (i.e. <30 patients per treatment arm) and suboptimal study design (mostly retrospective) [11, 13, 14].

Maintenance of remission

Thiopurines

A 2016 meta-analysis of six studies (489 patients) showed that AZA was superior to placebo in maintaining remission over placebo (RR 1.19, 95% CI 1.05–1.34), with a number needed to treat of nine [15]. These data are relatively favorable for the use of thiopurines in remission maintenance. One of the larger studies in the meta-analysis included a double-blind RCT of newly diagnosed patients with CD, AZA ($n=68$) vs placebo ($n=63$) from 31 centers, and patients were followed up for 18 months [16]. While there was no difference in corticosteroid-free remission ($P=0.47$), more patients in the placebo group (19/63) experienced clinical relapse of moderate severity (CDAI of ≥ 220) than in the AZA group (8/68, $P=0.01$) [16]. At the time of patient enrollment, 25% of the study population had active disease (CDAI of >150), thus 75% were already in clinical remission. Furthermore, 70% were being treated with corticosteroids at the

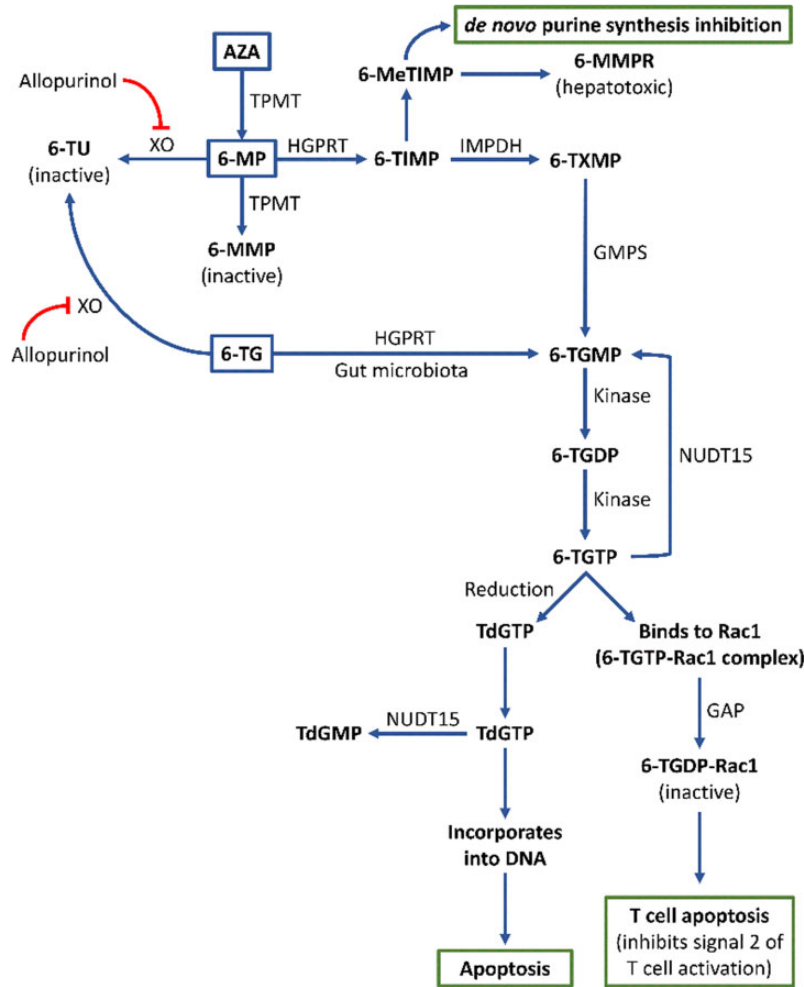


Figure 1. Thiopurine metabolism and proposed mechanisms of action. Green boxes indicate three immunosuppressing effects of thiopurines: TdGTP intercalation into DNA, inhibition of Rac1 and subsequent T-cell activation, and inhibition of *de novo* DNA synthesis. Red lines indicate inhibition of XO by allopurinol (can be used to shunt the pathway away from 6-TU in patients with high TPMT activity receiving low-dose thiopurines). AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; 6-TU, 6-thiouric acid; 6-TIMP, 6-thioinosine monophosphate; 6-TXMP, 6-thioxanthine monophosphate; 6-TGMP, 6-thioguanine monophosphate; 6-TGDP, 6-thioguanine diphosphate; 6-TGTP, 6-thioguanine triphosphate; TdGTP, deoxy-thioguanosine triphosphate; TdGMP, deoxy-thioguanosine monophosphate; 6-MMP, 6-methylmercaptopurine; Rac1, Ras-related C3 botulinum toxin substrate 1; 6-MMPR, 6-methylmercaptopurine ribonucleotides; TPMT, thiopurine methyltransferase; NUDT15, nucleoside diphosphate-linked moiety X-type motif 15; XO, xanthine oxidase; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; GMPS, guanosine monophosphate synthetase; IMPDH, inosine monophosphate dehydrogenase; GAP, GTPase-activating protein. This figure was adapted from [94], and subsequently revised and updated.

time of enrollment (while 30% were not on steroids). It is not known whether disease severity or corticosteroid use at the time of patient enrollment was associated with any of the outcomes, e.g. whether AZA was more (or less) successful at preventing clinical relapse or had steroid-sparing effects in those with active disease (CDAI of >150). Of note, more patients in the AZA group ($n = 14$) than in the placebo group ($n = 4$) experienced adverse events leading to drug discontinuation ($P = 0.02$), yet thiopurine methyltransferase activity (discussed below) was not assessed in study patients [16].

In another prospective open-label study, early treatment with AZA did not result in improved sustained clinical remission over 3 years vs conventional treatment (i.e. addition of AZA after development of corticosteroid dependency, poorly controlled disease, or severe perianal disease) [17]. However, this study assessed trimesters of being in remission. Persons randomized to early AZA had higher remission rates within the 9 months than placebo. When remission was compared after the first year, there was no difference between AZA and placebo users, but by this point, 40% of the placebo group was on AZA

[17]. The lack of difference between early users of AZA vs those whose therapy was at the discretion of the attending physician, who ultimately prescribed AZA to the majority of patients, likely reflected in the fact that both treatment groups were high users of AZA over time. This also likely explains why there was no difference in corticosteroid-free remission in patients treated with AZA or placebo in the Panés *et al.* study (described above) [16]. Nonetheless, these studies concluded that there is no benefit to using thiopurines as a pre-emptive therapy.

In a retrospective study of 6,960 patients with CD in the UK, 34% did not require therapy escalation (initiation of biologic or surgery), with a median time of thiopurine monotherapy to therapy escalation of 4 years [18]. Furthermore, thiopurine monotherapy was more successful in colonic than in ileocolonic disease (odds ratio [OR] 1.6, 95% CI 1.38–1.86) and less successful in those with perianal involvement (OR 0.7, 95% CI 0.61–0.80) [18].

Compared with budesonide, AZA is superior at maintaining remission at 1 year (RR 1.65, 95% CI 1.13–2.42), with both mucosal (83% vs 24%, $P = 0.0001$) and histologic ($P < 0.0001$) healing [19].

Methotrexate

There are few studies that have examined MTX as maintenance therapy in CD. There was no difference in MTX vs 6-MP in one study (RR 1.36, 95% CI 0.92–2.00, $n=50$ patients) [20]. Compared with placebo, weekly intramuscular MTX was more likely to maintain clinical remission (CDAI of ≤ 150 and corticosteroid discontinuation) at 16 weeks (39% in the MTX vs 19% in the placebo group, $P=0.025$) [21]. Overall disease activity was less in the MTX group (CDAI 162 ± 12) than in the placebo group (CDAI 204 ± 17 , $P=0.002$) [21]. In a follow-up study with a longer study period of 40 weeks, similar results were found [22]. Another study found that only 20% of patients will remain in remission on MTX at 5 years [14], but there is a paucity of robust long-term data. Furthermore, the two studies highlighted here that utilized intramuscular MTX [21, 22] underscore the observation that there may be a role for parental treatments, particularly in patients with severe small bowel involvement where absorption may be affected. Thus documenting the presence and extent of small bowel disease is likely to be important for future clinical trials and study design.

Combination therapy

The SONIC trial showed that combination therapy of AZA and infliximab at Week 26 achieved greater rates of clinical and endoscopic remission (57%) compared with monotherapy with either drug (AZA, 30%; infliximab, 44%) [12]. Post hoc analysis of the SONIC trial suggested that combination (AZA and infliximab) therapy was likely to be more successful at achieving clinical and endoscopic remission when implemented early in the disease course (time post-diagnosis of <18 months without fistulization) rather than later or when associated with complications (time post-diagnosis of >18 months or presence of fistulae) [23].

A retrospective Canadian study of 8,129 patients with CD starting infliximab, combined with or without an immunomodulator (thiopurine or MTX), found similar results: combination infliximab and immunomodulator was associated with a decrease in composite outcomes (hospitalization, surgery, corticosteroid use, or switch of biologic; hazard ratio [HR] 0.77, 95% CI 0.66–0.90) [24]. There was a signal towards greater hazard of treatment failure in the infliximab and MTX group compared with the infliximab and thiopurine group, but this did not reach statistical significance [24]. This may be attributable to underpowering of the infliximab and MTX combination group, as they made up 16% of the combination cohort (and infliximab and thiopurine comprised 84% of the combination cohort). A retrospective Australian study also found similar results: combination infliximab and thiopurine therapy yielded a high rate of induction (74%) vs infliximab monotherapy (47%, $P=0.04$), as well as three-times longer time to require treatment escalation or failure vs infliximab monotherapy (29 vs 9 months, $P=0.01$) [25].

Adalimumab and AZA combination therapy was not significantly better than adalimumab monotherapy in an open-label randomized control trial (the DIAMOND study) [26]. The combination group also experienced more frequent side effects, which subsequently resulted in a greater dropout rate from the trial [27].

Combination of thiopurines or MTX with vedolizumab or ustekinumab in CD is currently not supported [28]. Available studies are retrospective, observational, and/or contain inappropriate case mix (i.e. combining UC and CD), often with small sample sizes [29].

Contrary to the findings of the SONIC trial, combination MTX and infliximab therapy is not currently supported by the literature [13]. The largest placebo-controlled RCT of 126 patients showed no difference in infliximab monotherapy vs combination therapy for induction (HR 1.35, 95% CI 0.68–2.67) [30]. The study population had relatively mild to moderate disease (mean CDAI 208), with 29% ($n=37$) having a CDAI of <150 at the time of study enrollment [30]. Nonetheless, MTX is often used in combination with infliximab to suppress antidrug antibodies. This combination gained popularity because of concerns for the rare but lethal development of hepatosplenic T-cell lymphoma in young males using thiopurines.

Mechanism of action in combination therapy

In the SONIC trial, patients in the combination therapy arm (infliximab and AZA) had a lower incidence of anti-infliximab antibody detection (1 of 116 patients or 0.9%) than those receiving infliximab alone (15 of 103 patients or 14.6%) [12]. This corresponded to both higher median trough levels for patients receiving combination therapy vs infliximab monotherapy (3.5 vs 1.6 $\mu\text{g/mL}$, $P<0.001$) and greater rates of corticosteroid-free remission [12]. This was probably an expected finding when considering the immunogenicity of the chimeric infliximab antibody compared with humanized adalimumab, and explains why there is a lack of additional benefit in adalimumab and thiopurine combination therapy over adalimumab monotherapy alone. Interestingly however, in the DIAMOND study (combination therapy with AZA and adalimumab), the combination therapy resulted in higher trough levels of adalimumab than adalimumab monotherapy, despite the absence of clinical benefit [26]. Thiopurines have a complex metabolism and mechanism of action as indicated above. Through downstream effects, they result not only in decreased antibody synthesis, but also in reduced antibody catabolism [31, 32], thus having a complex role in the half-life of biologics and anti-biologic antibodies, in addition to their other known immunomodulating effects.

Fistulizing disease

There are few studies that specifically assess the effects of thiopurines on fistula healing, thus most data are derived from secondary outcomes. One older meta-analysis demonstrated that AZA or 6-MP was more successful in fistula healing (54%, 22 of 41 patients) than placebo (21%, 6 of 29 patients; OR 4.44, 95% CI 1.50–4.44) [33]. This was also associated with a steroid-sparing effect [33]. It is regarded that simple fistulae (intersphincteric or low transphincteric involving $<30\%$ of the external sphincter) respond well to AZA or 6-MP [34]. There is a paucity of data assessing thiopurine monotherapy for complex perianal fistulas (extrasphincteric or suprasphincteric), anterior location in females, multiple external openings, or when associated with an abscess. For those with complex fistulae or concurrent luminal disease, biologic or combination therapy (biologic with thiopurine) is recommended [35]. Thiopurines as maintenance therapy do not appear to prevent the development of fistulas compared with placebo [16], but event rates are low and this has never been studied as a primary outcome.

To our knowledge, there are no specific studies that assess the efficacy of MTX in fistulizing disease, compared with either placebo or other standard-of-care agents. Thus MTX cannot be recommended for fistulizing CD.

Post-operative relapse

The American Gastroenterological Association and American College of Gastroenterology currently recommend early initiation of thiopurines and/or an anti-TNF agent for those at higher risk of disease recurrence [36, 37]. Higher risk carries an 80% risk of endoscopic recurrence and is impacted by disease duration of >10 years, patient age of <30 years, being a smoker, or at least two surgeries for penetrating disease [36, 37]. While AZA and 6-MP are superior to placebo (RR 0.81, 95% CI 0.69–0.96) at reducing the likelihood of relapse and equivalent to 5-ASA (RR 0.95, 95% CI 0.81–1.11), they are inferior to adalimumab and infliximab (RR 2.89, 95% CI 1.50–5.57) [36, 38]. Unfortunately, the data comparing thiopurines vs anti-TNF therapy in the post-operative setting are deemed of low-quality evidence (mostly small observational studies). Hence, the current recommendation is to initiate either thiopurines and/or an anti-TNF agent.

There are currently insufficient data to suggest combining thiopurines (or MTX) and an anti-TNF agent in the post-operative setting. A recently published open-label RCT comparing adalimumab monotherapy to combination therapy (thiopurine plus adalimumab) for patients with *de novo* or post-operative anastomotic stricture found no statistical difference in clinical disease activity or requirement for stricture surgery treatment [39]. There are no studies examining MTX monotherapy in the post-operative setting.

Other immunomodulators

Mycophenolate mofetil

There is little research exploring mycophenolate mofetil (MMF) in CD. MMF is the prodrug for mycophenolic acid, which inhibits inosine monophosphate dehydrogenase, a key enzyme in *de novo* purine synthesis (which is critical for T- and B-cell function). MMF has replaced AZA in organ transplantation (typically used in conjunction with another agent, such as a calcineurin inhibitor), as MMF was found to be superior to AZA in preventing rejection of kidney transplants and generally has an equivalent to more favorable side-effect profile [40]. Notably, long-term MMF use is associated with a decreased incidence of malignancy, particularly non-melanoma skin cancers compared with AZA [40]. However, MMF is associated with higher rates of gastrointestinal upset (particularly diarrhea) and there are case reports of MMF-induced enterocolitis [40, 41]. Despite this, there is observational evidence for its use in the induction and maintenance of remission in CD [42, 43]. There is a potential use for MMF in mild to moderate CD, or possibly in combination with other therapies for severe or refractory disease, although at present the use of MMF in CD would have to be considered experimental.

Calcineurin inhibitors

The calcineurin inhibitors include cyclosporin and tacrolimus. Calcineurin is a phosphatase and inhibition of its enzymatic activity prevents activation of nuclear factor of activated T cells (NFAT), thus these drugs primarily inhibit signal one of T-cell activation. While cyclosporin is indicated for rescue therapy in severe UC and small retrospective studies indicate cyclosporine or tacrolimus may have a role in inducing remission in severe CD (especially when used in combination therapy with vedolizumab) [44], several RCTs have shown that calcineurin inhibitors do not have a role in the induction of remission in CD [45]. In small observational studies, topical tacrolimus has been

shown to induce at least a partial response in those with perianal CD [46]. There are no studies demonstrating a role for calcineurin inhibitors in maintenance therapy.

PRE-THERAPY ASSESSMENT

Genetic and phenotype testing for thiopurine metabolizing enzymes

Thiopurine methyltransferase (TPMT) and nucleoside diphosphate-linked moiety X-type motif 15 (NUDT15) are important enzymes in thiopurine metabolism (Figure 1). However, mutations in these enzymes can result in severe thiopurine-induced myelosuppression.

TPMT catalyses the conversion of 6-MP to 6-MMP, one of the inactive metabolites. However, TPMT activity is variable across the population, as it is controlled by codominant polymorphisms and is responsible for some of the interpatient differences observed in the toxicity and therapeutic efficacy [8]. Patients treated with conventional doses of AZA or 6-MP who have TPMT deficiency or low enzyme activity are at increased risk of developing life-threatening leukopenia as drug metabolism is shunted significantly towards the 6-TGN pathway [8]. Conversely, high activity of TPMT can result in subtherapeutic drug levels and hepatotoxicity [8]. More than 40 TPMT variant alleles have been reported, and most are associated with lower TPMT activity (compared with the wild type). TPMT*2, -*3A, and -*3C are the most common mutant alleles that result in reduced TPMT activity. Both genotype (expressed allele) and phenotype (enzyme level) testing can be done in the clinical setting. The enzyme level measurement is preferred since there are racial differences that impact genotype. An undetectable TPMT level is an absolute contraindication to thiopurines. Conversely, normal TPMT levels indicate a low risk for myelopoeitic adverse effects, especially leukopenia. With a normal level, there is low risk for dramatic cytopenias and hence full dosing (i.e. 2–2.5 mg/kg AZA or 1–1.5 mg/kg 6-MP) can be initiated. However, even with a normal TPMT enzyme level, cytopenias may occur and hence the complete blood count (CBC) still requires monitoring. An intermediate TPMT level would indicate the dosage initiation should be halved. Although a normal TPMT level is not without risk per se, TPMT mutations are relatively rare, especially homozygote TPMT mutant alleles (0.01% in Asians and 0.6% in Caucasians) [47, 48]. If measuring enzyme levels, it is essential to inquire about recent blood transfusions (within the past 3 months), as these can result in falsely normal values of TPMT, thus potentially impacting thiopurine dosing [49].

The enzyme product of NUDT15 catalyses (shunts) 6-TGTP and TdGTP away from the DNA intercalation pathway (Figure 1). Thus decreased NUDT15 activity results in increased cytotoxicity (analogous to decreased TPMT activity). The variant that has probably been studied the most, NUDT15 R139C, seems to play an important role in the development of early leukopenia as well as alopecia [50], but is most prevalent in Asian populations over other ethnicities.

Safety and efficacy of thiopurines depends on several patient factors. Generally, increasing age is associated with adverse effects (e.g. infection and malignancy). However, Epstein-Barr virus (EBV)-related T-cell lymphoma and hepatosplenic T-cell lymphoma can be devastating conditions and are more common in young adults. There is an increased risk of hepatotoxicity and pancreatitis, the latter of which is amplified with concurrent smoking [51].

Pre-therapy blood work consisting of a CBC, liver function and enzyme tests, kidney function, and blood glucose (and also consideration of a pregnancy test) are recommended for assessment of baseline parameters and comorbid illness. A chest radiograph or pulmonary function tests are also recommended prior to initiation of MTX for anyone at risk of pulmonary disease (e.g. positive smoking history), as lung abnormalities are predictive of the development of MTX-induced pneumonitis.

It is important to screen for hepatitis viruses (HBV, HCV), human immunodeficiency virus (HIV), varicella zoster virus (VZV), EBV, and tuberculosis (depending on risk) prior to initiating therapy with an immunomodulator [37, 52]. Patients with active HBV (HBsAg- and DNA-positive) should receive prophylactic antiviral therapy with nucleos(t)ide analogs, starting 1–2 weeks before starting a thiopurine and continue for 12 months after cessation of the thiopurine [53]. HCV infection is not an absolute contraindication to initiating immunosuppressive therapy, but patients who fulfill criteria for treatment should be treated with directly acting antivirals. Thiopurines and MTX can be safely used in those with HIV on active antiretroviral therapy, but careful counseling and monitoring are required as these patients have an additional risk of infection and leukopenia [54]. Active VZV infection is a contraindication to administering immunosuppressive agents. If there is no history of chickenpox, shingles, or vaccination against VZV, patients should be tested for VZV IgG antibodies. Seronegative patients should receive a complete VZV vaccination series ≥ 3 weeks prior to initiation of thiopurines or MTX. Thiopurines and MTX should also be avoided in EBV-seronegative (EBV-determined nuclear antigen or viral-capsid antigen IgG) patients. EBV reactivation is associated with development of lymphoma, and *de novo* infection is associated with an aggressive and often fatal form of post-mononucleosis lymphoma [55, 56]. Vaccination for HBV, VZV, human papillomavirus, pneumococcal pneumonia, influenza, and COVID-19 are all recommended prior to initiation of immunosuppressive therapy. While the typical hepatotoxicity response to thiopurines is a rise in transaminases, in the setting of infectious mononucleosis, there may be a predominant cholestatic enzyme rise [57]. Rarely, hepatic veno-occlusive disease or Budd–Chiari syndrome can occur.

Drug interactions and a role for allopurinol

Thiopurines have several drug interactions, particularly some that can result in myelosuppression. The xanthine oxidase (XO) inhibitors allopurinol and febuxostat, 5-aminosalicylates, angiotensin-converting enzyme inhibitors, loop diuretics, and ribavirin can worsen or result in myelosuppression when combined with a thiopurine. However, low-dose thiopurine with allopurinol has been shown to induce a better clinical response and tolerance to the drug with lower hepatotoxicity as it shifts the metabolism of 6-MP and 6-TG away from 6-TU production and the production of 6-TGNs (Figure 1) [58, 59]. Furthermore, a subset of patients with high TPMT activity (>35 pmol/h/mg of hemoglobin) metabolize thiopurines rapidly leading to a sub-therapeutic response and a predisposition to hepatotoxicity [60]. Administration of low-dose thiopurine along with allopurinol can correct the rapid metabolization and reduce the hepatotoxicity risk. Allopurinol does carry other risks however (e.g. toxic epidermal necrolysis, fever, gastrointestinal intolerance, and hematuria) that dictate appropriate counseling and monitoring. Warfarin typically requires a dose increase during concurrent thiopurine use, thus international normalized ratio monitoring is also required.

MTX has relatively few drug interactions. Most notably, concurrent use of MTX and high dose non-steroidal anti-inflammatory drugs (e.g. ASA of >1 g/day) or with trimethoprim-sulfamethoxazole is associated with cytopenias [61].

Dosing regimens for thiopurines

There is no objective evidence for picking one thiopurine over the other (i.e. 6-TG, 6-MP, or AZA). The choice of agent will largely be based on local practice, and physician and patient preference, and possibly cost. As indicated above, however, most of the larger trials (i.e. SONIC) used AZA, and there is theoretical mechanistic reasoning to use 6-MP or AZA over 6-TG. Metabolism of the former two agents results in the intermediate product, MeTIMP, an inhibitor of purine *de novo* synthesis, which may provide additional immunosuppression benefit.

Standard weight-based dosing

The American Gastroenterological Association recommends a daily dose of 2–3 mg/kg AZA and 1–1.5 mg/kg 6-MP, whereas the European Crohn's and Colitis Organisation recommends a daily dose of 1.5–2.5 mg/kg AZA and 0.75–1.5 mg/kg 6-MP [62]. Recommended dosing for 6-TG is 0.2–0.3 mg/kg [63].

Low dose or split dosing

Low-dose AZA (< 2 mg/kg, or 25%–50% of standard dose is generally regarded as low dosing) is generally preferred when used in conjunction with 5-ASA, allopurinol, or in combination therapy with infliximab. Conversely, split dosing can maintain more steady serum concentrations and avoids the peak that is associated with greater affinity for TPMT. Thus split dosing is an option for those with hepatotoxicity.

Incremental dosing

Starting with 25–50 mg/day of AZA or 6-MP with incremental 25 mg increase every 2–4 weeks can help reduce gastrointestinal intolerance.

Therapeutic drug monitoring

Thiopurines are typically considered therapeutic with a 6-TGN concentration of 235–450 pmol/ 8×10^8 red blood cells (RBCs). Lower levels of 6-TGN (>125 pmol/ 8×10^8 RBCs) are considered therapeutic in infliximab combination therapy, but only if therapeutic infliximab levels are also present [64]. Patients who are within a therapeutic range but have active disease are considered treatment-refractory. Myelosuppression is associated with a 6-TGN titer of >450 pmol/ 8×10^8 RBCs [65], while hepatotoxicity is associated with 6-methylmercaptapurine ribonucleotides titer of $>5,700$ pmol/ 8×10^8 RBCs [66]. Typically metabolites should be measured every 4 weeks after a dose modification and subsequently every 12 weeks until a therapeutic level is achieved [67]. There is no evidence to suggest that routine measuring of serum metabolite levels is beneficial, and that reactive monitoring (i.e. in those with suspected treatment failure or adverse effects) is reasonable. A regular CBC is recommended to monitor for myelosuppression. Thiopurine metabolites build up in RBCs, thus a megaloblastic erythropoiesis is expected to develop after initiation of therapy. It has been suggested that a mean corpuscular volume (MCV) increase of ≥ 7 fl or MCV ≥ 101 fl reflects that the thiopurine is having a biologic effect. Absence of macrocytosis may reflect an absence of a biological effect or alternatively non-adherence. Very low 6-TG and 6-MMP levels reflect non-adherence [68].

Malignancy risk

Thiopurines are associated with increased risk of malignancies, primarily non-Hodgkin and Hodgkin lymphomas and non-melanoma skin cancers (basal and squamous cell carcinomas). A recent meta-analysis that included four studies of >200,000 patients with CD or UC found the incidence rate ratio (IRR) per 1,000 patient-years for the development of lymphoma to be 2.32 (95% CI 1.79–2.79, $P < 0.001$) for those who received thiopurines compared with no thiopurines or anti-TNF agents [69]. Importantly, the lymphoma risk appears to be compounded in those receiving thiopurine and anti-TNF agent combination therapy compared with either anti-TNF monotherapy (IRR 2.49, 95% CI 1.39–4.47, $P = 0.002$) or thiopurine monotherapy (IRR 1.70, 95% CI 1.03–2.81, $P = 0.039$) alone [69]. The highest absolute risk for thiopurine-associated lymphoma is in those >50 years of age and reduces to that of the general population after the thiopurine is discontinued [70]. However, there appear to be ethnic differences as Japanese and Northern Indian patients with IBD on thiopurines do not seem to have an increased risk of lymphoma, regardless of dose or duration [71, 72].

EBV is highly implicated in the development of thiopurine-associated lymphoma. Approximately 50% of IBD patients who develop lymphoma are EBV-seropositive. These lymphomas resemble post-transplant lymphoproliferative disorder and are of B cell in origin. Patients who are EBV-seronegative can develop mononucleosis and fatal forms of post-monomucleosis T-cell lymphoma (that is associated with hemophagocytic lymphohistiocytosis) [55, 56]. Thus thiopurine use should be strictly limited in EBV-seronegative patients.

Hepatosplenic T-cell lymphoma is a rare gamma-delta T-cell lymphoma that is non-EBV-related and is typically fatal. The overall risk of developing hepatosplenic T-cell lymphoma is rare (<1:20,000 person-years) and is highest in young males receiving thiopurine and anti-TNF agent combination therapy [73].

There is limited evidence to suggest that thiopurine use in IBD also carries an increased risk of myelodysplastic syndrome and acute myeloid leukemia [74]. However, this the absolute risk of myeloid disorders was only 1:10,000, thus the risk needs to be balanced against that of alternative treatments.

The increased risk of non-melanoma skin cancers in patients on thiopurines with IBD has been well established [75, 76]. However, ultraviolet exposure, skin type, and age are stronger risk factors than thiopurine use for the development of non-melanoma skin cancers [77]. Thus it is critical to emphasize the importance of patient education towards safe sun exposure for those on thiopurines. Cervical and urinary tract malignancies have also been associated with thiopurine use [78, 79].

There are very little data available on MTX-associated malignancies in IBD. Extrapolating from rheumatoid arthritis, there may be a small increase in risk of non-melanoma skin cancers [80, 81], but long-term follow-up studies are needed to assess the true risk.

Patients with a history of malignancy can be considered for thiopurines after 2 years of completion of cancer treatment and disease clearance [82]. However, this interval should be increased to 5 years in those who had a malignancy with high risk of recurrence [82]. Any history of an EBV-associated malignancy is a contraindication to thiopurine use. If already on a thiopurine at the time of developing a neoplastic disease, treatment can continue uninterrupted if the tumor(s) can be surgically resected with clean margins. If the malignancy is not amenable

to complete surgical resection or chemo- or radiotherapy is required, then the thiopurine should be withdrawn.

Immunomodulators in pregnancy and lactation

Thiopurines were previously labeled as Class D by the Food and Drug Administration due to teratogenic effects in animals. Indeed, AZA does cross the placenta and its metabolites can be isolated from fetal RBCs. However, several studies have found no increase in the risk of congenital abnormalities, low birth-weight, early childhood infections, autism spectrum disorder, or attention deficit hyperactivity disorder when used during pregnancy [83–86]. There is possibly an increased risk of pre-term birth, but this may be due to IBD activity rather than thiopurine use itself [87]. Thus, thiopurine use for the maintenance of remission during pregnancy is considered effective and safe. However, combination therapy with a thiopurine and anti-TNF agent does carry an increased risk of infection for infants [84, 87]. Hence, it is recommended that one of these drugs, usually the thiopurine, be discontinued during pregnancy.

In breast milk, thiopurine levels peak 4 hours after drug ingestion and rapidly decline to 10% of this level 2 hours later [88]. Moreover, maximum exposure of drug levels in breast milk is <1% of the maternal dose [88]. No adverse effects on mental health, physical development, or infection rates have been associated in infants breastfeeding from women with IBD on a thiopurine [88]. Nonetheless, it is recommended that lactating mothers breastfeed ≥ 4 hours after drug ingestion. The practice of “pumping and dumping” after 4 hours is not likely effective and therefore is not a recommended harm-reduction strategy.

Due to the teratogenic effects of MTX, it should be avoided during pregnancy. Thus patients wanting to become pregnant should discuss with their physician drug discontinuation or rotation to another drug (e.g. thiopurine) during preconception planning. MTX is also not recommended during breastfeeding as it can accumulate in neonatal tissue, although even observational data are lacking [89].

Treatment withdrawal

There is no consensus decision on when to withdraw thiopurines. Discontinuation of thiopurines is associated with an average 1-year relapse rate of 38% in CD [90]. Conversely, the proportion of CD patients who relapse on thiopurine monotherapy increases with time: 36% at 1 year, 55% at 5 years, and 68% at 10 years after thiopurine initiation (median duration until relapse of 3.2 years, 95% CI 2.1–4.3) [91]. Thus early discontinuation of treatment is likely to be harmful to the patient, yet most patients on thiopurine monotherapy will eventually experience treatment failure. Ultimately, the decision to stop thiopurines is individualized. Patients with sustained mucosal healing and normal biomarkers may be considered for cessation of thiopurines after 4–5 years. Elderly patients (>60 years) should be considered for thiopurine withdrawal due to the increased risk of developing neoplastic disease. After thiopurine withdrawal in elderly patients, it is recommended they undergo close follow-up as they are still at increased risk of developing malignancies [92].

Recommendations

We are in need of well-powered RCTs with a properly defined case mix and long-term follow-up to better understand the utility of monotherapy with thiopurines or MTX in CD. There is a lack of data analysing treatment efficacy in perianal CD,

isolated colonic CD, isolated small bowel disease, and pouchitis. Studies generally focus on hard end points, such as the induction of remission or relapse requiring corticosteroids. However, preliminary data suggest that most treatment “non-responders” indeed improve [93]; they just are not reaching the primary end point of clinical remission. If monotherapy results in meaningful improvement in disease activity, this opens the door for more trials in combination and surgical-sparing therapies, and notably personalized medicine. Furthermore, most trials of immunomodulator monotherapy used clinical remission as the primary outcome. It is increasingly recognized that endoscopic (and even histologic or molecular) healing leads to more sustained remission with fewer adverse events or complications.

Authors' Contributions

J.M.V. wrote the manuscript and designed the figure. C.N.B. provided edits to the final manuscript prior to submission. Both authors have read and approved the final version of the manuscript.

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Conflict of Interest

Dr Bernstein has consulted with or served on advisory boards for Abbvie Canada, Amgen Canada, Bristol Myers Squibb Canada, JAMP Pharmaceuticals, Janssen Canada, Pfizer Canada, Sandoz Canada, and Takeda Canada, and has received unrestricted educational grants from Abbvie Canada, Janssen Canada, Pfizer Canada, Bristol Myers Squibb Canada, and Takeda Canada. He has been on the speaker's bureau of Abbvie Canada, Janssen Canada, Pfizer Canada, and Takeda Canada. He has received research grants from Abbvie Canada, Amgen Canada, Pfizer Canada, and Sandoz Canada, and contract grants from Janssen. Dr Venner has nothing to declare.

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