12 Azathioprine/6-Mercaptopurine Metabolism in Ulcerative Colitis: A Guide to Metabolite Assessment—An Evidence-Based Approach

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Azathioprine • 6-Mercaptopurine metabolism • Ulcerative colitis • Metabolite assessment • Evidence-based

Introduction (Box 12.1)

Ulcerative colitis (UC) and Crohn's disease are chronic relapsing idiopathic inflammatory bowel disorders affecting over 1.7 million individuals in North America; about half have unremitting disease with symptoms of abdominal pain and diarrhea that impact patient's quality of life and work-related productivity [1, 2]. It stands to reason that the major goal of therapy for physicians caring for patients with inflammatory bowel disease (IBD) is to achieve and sustain a long-term disease remission with effective evidence-based corticosteroid-sparing therapeutic approaches that minimize the risk of drug-related toxicity.

Although 6-mercaptopurine (6-MP) and its prodrug azathioprine (AZA) have proven efficacy in the treatment of UC, the interpretation of clinical studies is often complicated by the heterogeneous nature of this bowel disorder [3] and the inter-investigator variability in the therapeutic end points used in monitoring clinical responsiveness to treatment. Over the last half century, a number of scoring systems have been developed to measure disease activity in patients with UC [4]. Most of these systems are based on a combination of clinical symptoms and endoscopic findings that are difficult to validate because of the myriad of symptoms overlapping with disease behavior [5]. Furthermore, the importance of tissue healing has become clinically relevant in light of recent reports correlating disease activity with a patient's overall risk of disease relapse and colorectal cancer [6]. Indeed, mucosal healing has now become an important predictor of clinical outcome, to the extent that all future-controlled clinical trials must now establish stringent primary end points of disease remission to include tissue healing in assessing treatment efficacy [4].

With the advent of pharmacogenomics and 6-MP metabolite monitoring in clinical practice, gastroenterologists have also found discordance between antimetabolite levels and their own assessment of disease activity [7–9]. The recent purported mucosal healing effect on AZA therapy in patients with UC [10] may require that clinicians redefine the therapeutic window of treatment efficacy based on the measurement of these antimetabolite levels. This review focuses on the role of antimetabolite therapy in sustaining long-term remission in patients with UC, as well as providing a guide on how to apply pharmacogenomics and metabolite monitoring in clinical practice based on a review of the literature.

Pharmacogenetics of 6-Mercaptopurine

Pharmacogenomics deals with the influence of genetic variation on drug response by correlating gene expression with a drug's efficacy or toxicity. Although the terms pharmacogenomics and pharmacogenetics tend to be used interchangeably, pharmacogenetics is generally regarded as the study or clinical testing of genetic variation that gives rise to differing responses to drugs, as it applies to either a single or at most a few gene polymorphisms.

Over the last 20 years, much has been learned about the pharmacogenetics of AZA and 6-MP metabolism in the clinical management of patients with leukemia and in

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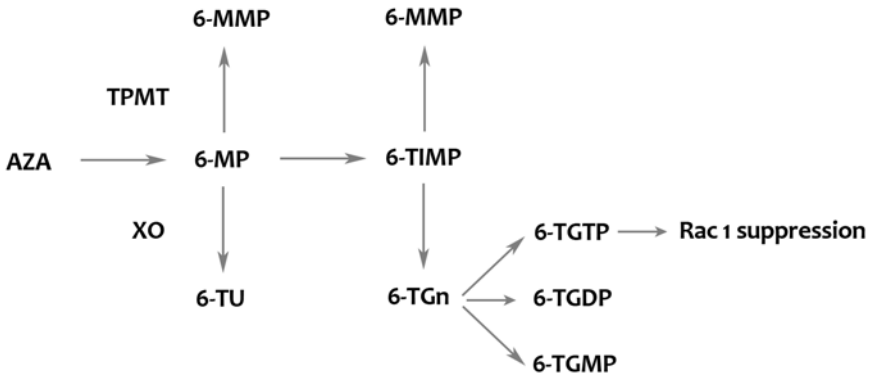

Fig. 12.1 Azathioprine (AZA) metabolism. *XO* xanthine oxidase, *6-TU* 6-thiouric acid, and *6-TIMP* 6-thioinosine monophosphate

IBD. Although most of our understanding has focused on the polymorphisms of thiopurine methyltransferase (TPMT) enzyme activity, recent studies have now also introduced potential polymorphisms in intracellular antimetabolite transport that influences clinical response despite presumed therapeutic drug dosing and metabolite levels [11].

Once absorbed into the plasma, AZA is rapidly converted to 6-MP by a nonenzymatic reaction. 6-MP is then taken up by a variety of actively replicating cells and tissues, including erythrocytes, T- and B-cell lymphocytes, as well as the bone marrow. The uptake of 6-MP is believed to be a rapid process. Once inside the cell, the metabolism of 6-MP occurs intracellularly along the competing routes catalyzed by hypoxanthine phosphoribosyltransferase and thiopurine S-methyltransferase (TPMT), giving rise to 6-thioguanine nucleotides (6-TGn) and 6-methyl-mercaptopurine (6-MMP), respectively (Fig. 12.1) [12]. 6-TGN is the active ribonucleotide of 6-MP that functions as a purine antagonist inducing lymphocytotoxicity and immunosuppression [13–15].

An apparent genetic polymorphism has been observed in TPMT activity in both the Caucasian and African-American population. Negligible activity is noted in 0.3 % of individuals and low levels (<5 U/mL of blood) in 11 % of individuals. TPMT enzyme deficiency is inherited as an autosomal recessive trait, and to date, 10 mutant alleles and several silent and intronic mutations have been described. In patients with the heterozygous TPMT genotype, 6-MP metabolism is shunted preferentially into the production of 6-TG nucleotides. Although 6-TG nucleotides are thought to be lymphocytotoxic and beneficial in the treatment of patients with leukemia and lymphoma, patients with low (<5) TPMT activity are at risk for bone marrow suppression by achieving potentially toxic erythrocyte 6-TGN levels on standard doses of 6-MP [16]. Despite low TPMT enzyme activity levels, presumed therapeutic erythrocyte 6-TGN metabolite levels can still be achieved without untoward cytotoxicity by lowering the dose of 6-MP 10- to 15-fold [17].

6-TGNs are active ribonucleotides that collectively function as purine antagonists, incorporating into DNA, thereby interfering with the ribonucleotide replication. Recent studies have also shown that one of these 6-TGN ribonucleotides, 6-TGTP, induces the apoptosis of both peripheral blood and intestinal lamina propria T-cell lymphocytes through the inhibition of Rac1, a GTPase that inhibits apoptosis. The specific blockade of CD28-dependent Rac1 activation by 6-TGTP is the proposed molecular target of 6-MP and its prodrug AZA (Fig. 12.1) [18].

The intracellular buildup of this specific 6-TGN metabolite may also be dependent on others, as yet undefined inherent genetic polymorphisms. Our recent studies have also proposed that there may also exist pharmacogenetic differences in the intracellular transport of 6-MP in peripheral blood lymphocytes that could potentially affect responsiveness to antimetabolite therapy. Our studies have shown an inherent variability in the transport of 6-MP in immortalized lymphocytes derived from patients with IBD. In these studies, seven inward and eight outward transporters were tested. One patient demonstrated the least amount of intracellular transport of 6-MP that correlated with the lowest susceptibility to 6-MP cytotoxicity. In this particular patient, multiple inward transporters, including the concentrative nucleoside transporters CNT-1, CNT-3, and the equilibrative nucleoside transporters ENT-3 and ENT-4 were notably low in expression. In comparison, a second patient exhibited robust 6-MP transport, an increased susceptibility to 6-MP cytotoxicity, and an increased expression of all influx transporters (except CNT-1), and equilibrative transporter ENT-4. Although no single transporter was either under- or overexpressed to explain these patterns of 6-MP transport, a correlation was shown between intracellular drug levels and the in vitro susceptibility to 6-MP-induced cytotoxicity. Interestingly, these differences were independent of 6-MP dose or erythrocyte 6-MP metabolite levels that were monitored clinically. Ongoing studies will also attempt to correlate these differences in drug transport with clinical responsiveness to antimetabolite therapy and drug metabolite levels. Identification of such transporters prior to initiating therapy may allow physicians to tailor therapy more effectively in patients with steroid-dependent IBD [11].

Clinical Application of Metabolite Testing

In patients with UC, the aim is to optimize antimetabolite therapy early in the course of the disease in order to minimize the overall risk for disease progression. The factor with the most significant direct correlation with disease progression is severity of colitis early in the course of the illness [5]. In a large population-based cohort study, patients with severe active UC were 14.8 times more likely to have disease progression compared to patients without severe colitis. Patients with left-sided colitis at diagnosis are 2.5 times more likely to progress to extensive colitis than patients with isolated proctitis progressing to either extensive colitis or left-sided disease [19]. Although disease progression can occur in patients of all age groups, most children will present clinically with extensive colitis at diagnosis, while those children presenting with either proctosigmoiditis or left-sided disease will rapidly progress to pancolitis within 6 years of the diagnosis [20]. In general, pediatricians regard ulcerative colitis as a rapidly progressive disease in children, with an associated increased likelihood of requiring proctocolectomy. The rapid induction and maintenance of disease remission remain the primary goal therapy in patients with UC. Using a Markov model, there is an 80–90 % probability that a patient with clinically inactive disease would remain in remission for a year, with a 20 % chance of relapse in the following year. By contrast, data from patients with clinically active disease demonstrate a 70 % probability of having a relapse during the year following diagnosis [21]. The same results were shown within the post hoc analysis of the combined ACT I and ACT II data among the infliximab-treated patients. Interestingly, mucosal healing was the primary end point of long-term remission in those studies [22]. The importance of tissue healing was also underscored by Froslie and coworkers. In that study, patients with UC that achieved tissue healing at 1 year were less likely to require colectomy in the subsequent 5-year follow-up period [23].

Although 6-MP and AZA have clinical efficacy in maintaining disease remission in patients with UC, the wide therapeutic dosing range used in clinical practice today would suggest that pharmacokinetic differences in drug metabolism may also influence responsiveness to therapy. Moreover, a true separation between immunosuppression and cytotoxicity has yet to be defined since the dosing of 6-MP and azathioprine has been based largely on clinical outcome. Indeed, the wide range in azathioprine dose used in clinical practice would suggest that a safe and established therapeutic dose has yet to be determined. The situation is further complicated with recent evidence that would suggest that mucosal healing of the affected bowel decreases the risk of disease relapse and progression.

Table 12.1 Clinical responsiveness to 6-MP and AZA therapy based on threshold (235–250[a]) erythrocyte 6-TGN metabolite levels

Study	Patients (response)	6-TGN response threshold		Odds ratio
		Above	Below	
Dubinsky [26]	92 (30)	0.78	0.40	5.0
Gupta [27]	101 (47)	0.56	0.43	1.7
Belaiche [28]	28 (19)	0.75	0.65	1.6
Cuffari [7]	82 (47)	0.86	0.35	11.6
Achkar [8]	60 (24)	0.51	0.22	3.8
Lowry [9]	170 (114)	0.64	0.68	0.9
Goldenberg [29]	74 (14)	0.24	0.18	1.5

[a]pmoles/8×10^8 RBCs

Conventional dosing strategies must now be redefined based on these new end points of clinical remission that includes mucosal healing. Nevertheless, immunosuppression is not without its risk. The clinician must always remain aware of potential adverse effects, including allergic reactions, hepatitis, pancreatitis, bone marrow suppression, and lymphoma while attempting to achieve an optimal therapeutic response irrespective on how the physician chooses to define it clinically [24, 25].

The measurement of erythrocyte 6-TG and 6-MMP metabolite levels by means of high-pressure liquid chromatography (HPLC) has now become a useful clinical tool for documenting patients' compliance to therapy. In our preliminary study, erythrocyte 6-TG metabolite levels showed a strong inverse correlation with disease activity, where the lack of clinical response was clearly associated with low (<50) erythrocyte 6-TGN metabolite levels. To date, a number of studies in both the pediatric and adult literature have supported the notion of therapeutic drug monitoring in patients with IBD. However, a uniform consensus has not yet been reached on account of the absence of well-controlled clinical trials (Table 12.1) [7–9, 26–29]. Although a meta-analysis by Osterman and colleagues has shown that higher metabolite levels correlated with a more favorable clinical response, no clearly defined therapeutic window of efficacy and toxicity has been established based on 6-MP metabolite levels [30]. Since mucosal healing has now become the salient end point for clinical remission, metabolite testing should now be considered just as a guide to therapy. The notion of using the existing threshold 6-TGN metabolite levels would seem antiquated. At present, the existing technology should only be used in identifying pharmacogenomic differences in drug metabolism, monitoring patient compliance with antimetabolite therapy, and avoiding excessive immunosuppression in patient with recalcitrant disease, high (>400) 6-TGN levels, and normal white blood cell counts.

TPMT Testing

Low and Intermediate (<5 U/mL Blood) TPMT

Eleven percent of the population is considered heterozygous carriers of the TPMT-deficient allele and potentially at risk for drug-induced leukopenia. In the patient who is homozygous recessive with absent TPMT enzyme activity, there is the added risk of severe, irreversible bone marrow suppression. Since then, there have been a number of similar cases of irreversible bone marrow suppression both in patients with IBD on maintenance azathioprine therapy and in patients with leukemia on standard doses of 6-MP. It remains the author's opinion that these patients should not be considered candidates for antimetabolite therapy.

A number of secondary malignancies, including acute myelogenous leukemia and brain tumors, have been insinuated to be related to the use of maintenance 6-MP therapy in patients with leukemia and the heterozygous TPMT genotype. Although 6-TG and 6-MMP metabolites were not measured in these patients, it may be assumed that these patients were potentially exposed to high-maintenance 6-TG metabolite levels despite presumed therapeutic 6-MP dosing and were thus overly immunosuppressed.

In IBD, Black and coworkers showed that patients with Crohn's disease and a "mutant" TPMT allele also incurred significant drug-induced leukopenia on standard doses of azathioprine therapy and were compelled to discontinue treatment. In contrast, patients with the wild-type allele achieved a good clinical response while on azathioprine therapy without untoward cytotoxicity [31]. This study and others would suggest that all patients with the heterozygous allele are at an increased risk for drug toxicity and should not be prescribed azathioprine or 6-MP therapy. However, this would exclude 11 % of the population who could potentially benefit from 6-MP therapy. It has been shown in prospective open-label clinical trials that by identifying these patients prior to initiating AZA therapy and adopting a moderate dosing strategy (6-MP, 0.5–1 mg/kg/day; AZA, 1–1.5 mg/kg/day), most patients may achieve a favorable clinical response while avoiding potential bone marrow suppression. It remains the author's opinion that these patients be monitored carefully with serial CBCs.

High (>16 U/mL Blood) TPMT

The genetic polymorphism in TPMT activity observed in the general population may also have far-reaching implications regarding patient responsiveness to therapy and clinical response time. Twenty percent of the population is considered to be rapid (>16) metabolizers of 6-MP and AZA and in theory would require larger than the standard doses of drug in order to achieve any therapeutic drug benefit [21]. In these patients, 6-MP metabolism is shunted away from 6-TGN production and into the formation of 6-MMP (Fig. 12.1). In patients with leukemia, high TPMT activity is associated with an increased risk for disease recurrence [17].

In a prospective open-label study in adults, just 20 % of patients with either UC or Crohn's disease and erythrocyte TPMT levels >16 U/mL of blood responded to AZA therapy despite therapeutic drug dosing (2 mg/kg/day). In comparison, 30 % of patients with TPMT levels between 12 and 16 U/mL blood responded to therapy. These were also more likely to require higher dosages (2 mg/kg/day) of AZA from the outset in order to optimize their erythrocyte 6-TGN metabolite levels [21].

In comparison, patients with TPMT activity levels ≤12 U/mL blood achieved high (>250) mean erythrocyte 6-TG levels after 16 weeks of induction AZA. This occurred even though both groups received a similar dosage of AZA. In this patient population, 69 % of patients achieved a favorable clinical response with presumed therapeutic erythrocyte 6-TGN metabolite levels after 4 months of continuous AZA therapy [21].

High hepatic TPMT activity may draw most of the 6-MP from the plasma, thereby limiting the amount of substrate available for the bone marrow and peripheral leukocytes. This concept of rapid AZA metabolism interfering with therapeutic response could explain the low response rate in a controlled clinical trial in Crohn's disease that compared high-dose oral (2 mg/kg/day) azathioprine therapy with and without initiating a short course of high-dose intravenous (1.6 g/36 h infusion) AZA therapy. That study was confined to individuals with upper normal or high levels of TPMT enzyme activity so that the intravenous azathioprine treatment group could be studied safely. Even at 2 mg/kg/day of oral azathioprine therapy, only 20 % of these rapid metabolizers in both groups achieved clinical remission, a clinical response that is lower than that reported in most consecutive patient publications [32].

Furthermore, high (>15) erythrocyte TPMT levels may also explain the rather low clinical response noted in the AZA treatment arm of the SONIC trials. In that study, despite optimized induction dosages (2.5 mg/kg/day) of AZA, just 30 % of patients responded to therapy [33], a clinical response that is lower than what has been generally concluded from the Cochrane meta-analyses of AZA therapy in treating patients with IBD [34].

Clinical Application of TPMT Testing

Most physicians will monitor CBC and serum aminotransferases monthly during the first 3 months of initiating

therapy. Although TPMT measurement has been shown to predict leukopenia in up to 20 % of patients, TPMT monitoring may be used clinically to increase the level of physician comfort in prescribing antimetabolite therapy, in general, and in minimizing the perceived need for monitoring CBC, and for dose titration, all of which may increase clinical response time.

For example, knowing the TPMT status in a patient may aid the physician in utilizing a variable AZA dosing strategy in patients with IBD. Patients with absent TPMT should not receive AZA therapy. Those with very low (<5) TPMT activity can be effectively treated with 1.0–1.5 mg/kg/day of AZA while monitoring CBC and erythrocyte 6-TG levels. Patients with TPMT activity between 5 and 12 U/mL blood have an increased likelihood of responding to a more moderate dosing strategy, such as 1.5–2.0 mg/kg/day. In patients with above average (>12) TPMT activity, AZA therapy may have to be started at 2.0 mg/kg/day in order to achieve a favorable clinical response. However, higher dosages, such as 2.5 mg/kg/day, may be needed for those with very high (>16) TPMT enzyme activity. Physicians must be cognizant of the potential refractoriness to antimetabolite therapy among those patients with high TPMT enzyme activity despite presumed therapeutic drug dosing. It remains the author's opinion that although empiric drug dosing remains an acceptable standard of care based on TPMT genetic polymorphisms, the clinician must be sensitive to potential phenotypic differences in TPMT activity that may influence responsiveness and or toxicity to antimetabolite therapy. Among those patients with either recalcitrant disease or drug-induced toxicity, the measurement of erythrocyte 6-MP metabolites may facilitate a more cogent clinician response to therapy (Textbox).

Box 12.1: Key Summary

1. Measure TPMT genotype/phenotype prior to initiating anti-metabolite therapy;
2. TPMT:
   (a) homozygous recessive—consider an alternate therapy;
   (b) heterozygous—consider 1.0–1.5 mg/kg/day of AZA;
   (c) homozygous dominant—consider 2.0–2.5 mg/kg/day of AZA;
3. Follow CBC q2weeks ×2, then q4weeks ×2, then with each follow-up;
4. If after 2 months patient remains either steroid dependent or has a disease exacerbation, check 6-TGn/6MMP metabolites (please see Table 12.2);
5. Toxicity:
   (a) Pancreatitis—discontinue anti-metabolite therapy (idiosyncratic reaction to anti-metabolites);
   (b) Hepatitis (ALT>3×N)—if 6-MMP/6-TGn ratio > 1/50 lower dose of AZA by 25 mg/day and repeat ALT in 2 weeks;
   (c) Leukopenia: high 6-TGn (>250)—consider lowering dose of AZA by 25 mg/day and repeat WBC in 2 weeks.

Disclaimer: This is a suggestion by the author and has not been assessed in prospective randomized placebo-controlled trials.

(continued)

Table 12.2 Metabolite profiles, clinical impression, and therapeutic decision

Group A	Absent/very low (<50) 6-TGN absent 6-MMP	Nonadherence	Patient education
Group B	Low (<250) 6-TGN Low (<2,500) 6-MMP	Sub-therapeutic dose	Dose titration
Group C	Low (<250) 6-TGN High (>5,700) 6-MMP	Rapid metabolizer	Switch therapy vs allopurinol
Group D	High (>400) 6-TGN High (>5,700) 6-MMP	Thiopurine resistant	Switch therapy

While TPMT testing may guide the physician's initial dosing practices, metabolite testing will allow them to clinically respond to patient's refractoriness to therapy despite presumed therapeutic dosing (Table 12.2). Patients that are clearly noncompliant (Group A) with low metabolite (6TGN, 6-MMP) levels should be educated and have the need for improved adherence to the therapy reinforced. Patients that are nonresponding and clearly sub-therapeutic (Group B) should have their dose of AZA titrated to improve overall clinical response. Previous studies have shown this approach to be highly effective in improving overall clinical response while avoiding unnecessary toxicity. In a study of 25 adult patients refractory to AZA and low (<250) erythrocyte 6-TGN metabolite levels, 18 were pushed into clinical remission by having their dose of AZA increased by 25 mg/day [21]. Among patients that are deemed rapid metabolizer (Group C), the possibility of changing the pharmacokinetics through the addition of allopurinol may be considered. However, the physician will need to be aware of the potential risk of toxicity [35]. It remains the author's opinion that this

therapeutic approach be restricted to tertiary care centers experienced with this approach and accessible to metabolite monitoring. Lastly, those patients clearly refractory to AZA despite therapeutic drug dosing should be considered for alternative therapies (Group D).

Combination Therapy

It has been the practice in many institutions, including our own, to initiate maintenance anti-TNF-α therapy in patients that have shown clear refractoriness to either long-term 6-MP or AZA therapy. All of the studies, including ACCENT, CHARM, and PRECISE, did not show a therapeutic benefit with combination therapy (anti-TNF-α with antimetabolite) to just anti-TNF-α therapy alone in maintaining disease remission in patients with moderate to severe Crohn's disease. In comparison, the SONIC study focused its attention on patients who were naïve to anti-TNF-α therapies and either naïve or had stopped (>3 months) AZA therapy prior to recruitment. In that study, combination therapy was shown to be superior to either infliximab or AZA monotherapy [33].

A similarly designed study was recently presented in abstract form in patients with moderate to severe UC. In that 16-week study, 40 % of patients on combination therapy achieved a steroid-free remission, significantly higher than those patients on monotherapy alone (22 % infliximab; 24 % AZA). Both the combination and the infliximab-only treatment arms were superior to AZA monotherapy in overall clinical response and mucosal healing [36].

The purported benefit of combination therapy in SONIC and the above-referenced study in patients with moderate to severe UC is balanced with the increasing concern of hepatic T-cell lymphoma among young (<18 years) patients on combination therapy. This concern has led many physicians to consider discontinuing either 6-MP or AZA with the introduction of biological therapy despite the potential for reducing antibody to infliximab formation. Although all anti-TNF-α have antigenic properties, thereby rendering patients susceptible to antibody formation, those patients on infliximab are most vulnerable. The concurrent use of immunosuppressive therapy has in the past been shown by Rutgeerts and coworkers to maintain a favorable clinical response to maintenance infliximab therapy, presumably due to the prevention of antibody formation. In that study, 75 % (12/16) of patients on concurrent 6-mercaptopurine maintained a favorable clinical response compared to 50 % (9/18) on no concurrent immunosuppressive therapy [37]. In the ACCENT 1 study, only 18 % of the patients on neither concurrent prednisone nor immunosuppressive drug therapy developed antibody to infliximab compared to just 10 % of patients on concurrent azathioprine or methotrexate therapy [38].

In a previously presented study of adult patients with IBD on combination therapy, high 6-TGN levels associated with an improved clinical responsiveness to maintenance anti-TNF therapy. In that study, patients in remission had higher (>300) median erythrocyte 6-TGN metabolite levels compared to patients (<100) with either a partial clinical response or ongoing corticosteroid dependency. Interestingly, patients with anti-TNF-associated side effects (SE) also had low (<100) median 6-TGN levels [39]. Although the concurrent use of either AZA or 6-MP may allow for a more protracted clinical response, the precise mechanism of action is unclear. Whether this purported benefit would justify the increased risk of hepatic T-cell lymphoma is debatable, especially since adalimumab and certolizumab pegol have proven efficacy of salvaging patients refractory to infliximab. Unfortunately, TPMT and 6-MP metabolite levels have shown no correlation with the 36 reported cases to date of hepatic T-cell lymphoma [40].

Conclusions

6-MP and AZA have proven efficacy in the maintenance of disease remission in patients with IBD. The application of pharmacogenetics and metabolite testing in clinical practice may improve the overall clinical response to antimetabolite therapy and reduce the risk of antimetabolite-induced side effects. The careful monitoring of complete blood counts and erythrocyte 6-TG metabolite levels is indicated in patients with either low (<5) or above average (>16) TPMT levels, and it remains the authors' opinion that relying on either total leukocyte counts or mean corpuscular volume as the sole measure of dosing adequacy should be used with caution.

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