INTRODUCTION

Immunomodulators are central to the long-term management of patients with inflammatory bowel disease (IBD) (1). 6-mercaptopurine (6MP), azathioprine (AZA) and methotrexate are the most frequently used immunomodulators for inflammatory bowel disease are 6-mercaptopurine, azathioprine and methotrexate. The goals of using this class of medications are to control active inflammation, allow for the withdrawal of steroids, and ultimately to maintain long-term remission of Crohn’s disease and ulcerative colitis. To maximize benefit and minimize risk, it is important to use these medications properly and to monitor patients appropriately. This article outlines a practical approach for the use of these medications. In addition to dosing and monitoring recommendations, it reviews the available data on genetic testing, utility of measuring drug metabolites, and long-term risks and benefits.

6-MERCAPTOPURINE/AZATHIOPRINE

Evidence for Efficacy

Azathioprine has been studied in randomized clinical trials for Crohn’s disease dating back to 1971(2,3), and 6MP shortly thereafter in 1980 (4). A meta-analysis in 1995 evaluated nine randomized, placebo-controlled
trials studying AZA or 6MP for Crohn’s disease (5). In this study, compared with placebo, AZA or 6MP had an odds ratio of response of 3.09 (95% CI: 2.45–3.91) for the treatment of active Crohn’s disease and 2.27 (95% CI: 1.76–2.93) for maintaining remission. There are fewer data for AZA or 6MP for the treatment of ulcerative colitis, but two controlled trials confirmed efficacy for active disease(6) and maintaining remission (7) and more recently Ardizzone and colleagues showed that compared to 5-aminosalicylate therapy, AZA had an odds ratio of clinical and endoscopic remission of 4.78 (95% CI: 1.57–14.5) for steroid dependent ulcerative colitis(8). Drug-withdrawal studies in both Crohn’s disease and ulcerative colitis have shown an acceleration of relapse after stopping medication (7,9–13). Therefore, once started and effective, 6MP/AZA should be continued indefinitely until either it appears to no longer be working, or the patient experiences a significant adverse effect.

Adverse Effects

In general, serious adverse effects related to AZA and 6MP are rare. Adverse effects can be divided into direct or indirect toxicity. Direct toxicity refers to effects related to the medication itself, as opposed to indirect toxicity, where adverse effects are sequelae of some direct toxicity (e.g., infection due to bone marrow suppression). A report of 396 patients taking 6MP revealed evidence of direct toxicity including pancreatitis (3.3%), bone marrow suppression (2.0%), allergic reactions (2.0%) and drug hepatitis (0.3%) (14). Significant nausea occurs between 1.3%–6% (5,15), and may resolve with a switch from one agent to another (6MP $\leftrightarrow$ AZA). With mild adverse effects or another potential explanation for an event, a careful re-challenge or switch to the other agent is reasonable. Pancreatitis, severe allergic reactions or profound bone marrow suppression are contraindications to re-challenge.

Indirect toxic events include infections, such as viral or bacterial infections. Viral infections associated with use of 6MP/AZA includes herpes viruses, specifically Epstein-Barr virus (EBV), cytomegalovirus (CMV), varicella zoster virus (VZV) and herpes simplex virus (HSV). Most of these viral infections probably manifest as self-limited viral syndromes, but rare life-threatening complications have been reported (16–20). Serious bacterial infections are a concern, and rates are fairly consistent across different series (Table 1) (5,14,15,21–24). These infections are usually easily treated, but in a large series from Connell, et al of 739 patients, two patients died as a result of pancytopenia and sepsis.

Lymphoma has been a concern with use of 6MP/AZA as well. Although some have estimated either no or a very small increased risk of lymphoma associated with 6MP/AZA (25,26), others estimate a 3-fold relative risk compared with Crohn’s patients not taking these medications (27). The mechanism of lymphoma in this setting may be related to EBV-mediated lymphoma, which has been described in IBD patients on 6MP/AZA (28). In addition to the concern over lymphoma, cervical cancer (29) and non-melanoma skin cancer(30) appear to be slightly increased in patients taking 6MP/AZA. This has led some to recommend that patients taking 6MP/AZA have routine cervical cancer screening and added caution with sun exposure.

GETTING STARTED

Informed Consent

Based on the above efficacy and adverse effect data, a conversation with patients regarding benefits and risks is crucial. A decision analysis that looked specifically at the benefits of treatment against the risks of lymphoma found that in most patients, the benefits outweigh the risks (27). However, based on individual patient preferences and perceptions of risk, this decision may not be so straightforward. A formal informed consent document is not necessary, but documentation of a conversation with patients prior to beginning therapy should be incorporated into the patient record.

Thiopurine Methyltransferase Testing

Azathioprine is a prodrug that is converted to 6MP, which is then metabolized to an active metabolite, 6-thioguanine (6TG). 6TG appears to play an important role in drug efficacy, but in high levels contributes to bone marrow suppression. Thiopurine methyltransferase (TPMT) is an enzyme that converts 6MP to
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6-methyl-mercaptopurine (6MMP), diverting metabolism away from 6TG (Figure 1). Low TPMT enzymatic activity yields higher 6TG levels, and although this may increase the likelihood of a clinical response, it also increases the risk of significant bone marrow suppression (31,32).

It is now possible to measure TPMT activity to help predict an individual patient’s response to 6MP/AZA. TPMT activity can be measured either by looking at the genotype or the phenotype. Genotype testing will give a report that a patient is either normal wild type (2 normal TPMT alleles), heterozygous (1 normal, 1 variant allele) or homozygous recessive (2 abnormal alleles). This corresponds to high, intermediate or low (or no) TPMT enzymatic activity with phenotype testing. One or the other test is sufficient; both are not necessary. The frequencies of normal, heterozygous and homozygous recessive are 89%, 11% and 0.03% (1/300) respectively (33). Homozygous recessive (low or no enzymatic activity) patients should not be treated with 6MP/AZA as they are at significant risk for profound bone marrow suppression. Heterozygous (intermediate enzymatic activity) patients could be treated, but dosing should be with one-half of the standard dose. Patients with intermediate enzyme activity may respond successfully to therapy more frequently, while those with the highest TPMT activity levels have been shown to be resistant to standard doses of 6MP/AZA (34).

Many have adopted the strategy of TPMT testing prior to initiating therapy with 6MP/AZA. The largest benefit is to those patients who are homozygous recessive, as identifying them prior to initiating therapy can conceivably prevent severe, life-threatening leukopenia. Assuming that heterozygotes will benefit as well, the number needed to screen to prevent one adverse event is 100 (35). Furthermore, two decision analyses have now shown TPMT testing before starting 6MP/AZA therapy to be cost-effective (35,36).

Table 1
Patients Experiencing Toxicity from AZA/6-MP

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td># of patients</td>
<td>396</td>
<td>78</td>
<td>302</td>
<td>157</td>
<td>111</td>
<td>410</td>
<td>50</td>
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<tr>
<td>Stopped drug due to adverse effect (%)</td>
<td>n/a</td>
<td>10.3</td>
<td>8.9</td>
<td>5.7</td>
<td>18.0</td>
<td>n/a</td>
<td>22.0</td>
</tr>
<tr>
<td>Nausea (%)</td>
<td>n/a</td>
<td>1.3</td>
<td>1.3</td>
<td>3.2</td>
<td>3.6</td>
<td>n/a</td>
<td>6.0</td>
</tr>
<tr>
<td>Allergic reaction (%)</td>
<td>2.0</td>
<td>n/a</td>
<td>2.0</td>
<td>0</td>
<td>1.8</td>
<td>3.9</td>
<td>0</td>
</tr>
<tr>
<td>Pancreatitis (%)</td>
<td>3.3</td>
<td>1.3</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Hepatitis/abnormal LFT’s (%)</td>
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<td>1.3</td>
<td>n/a</td>
<td>1.3</td>
<td>0</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Leucopenia (%)</td>
<td>2.0</td>
<td>1.3</td>
<td>1.7</td>
<td>11.0</td>
<td>12.6</td>
<td>11.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Death related to leucopenia (%)</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection (significant) (%)</td>
<td>7.4</td>
<td>3.9</td>
<td>0.3</td>
<td>2.5</td>
<td>2.7</td>
<td>7.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Lymphoma (%)</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>0</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Death due to lymphoma (%)</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Blood dyscrasia (%)</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Death due to blood dyscrasia (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

*Meta-analysis, included some patients from Present, 1989. * This is an updated report from the Present, 1989 series.

n/a = not available

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(continued from page 34)

suppression, this only accounts for about 27% of myelotoxicity (37). It is important not to develop a false sense of security after normal TPMT testing, as nearly three-fourths of patients develop sporadic neutropenia later in treatment for unidentified reasons. Therefore, normal TPMT testing can help with initiation of therapy, but does not take the place of careful monitoring after starting therapy.

Inosine triphosphate pyrophosphatase (ITPA) is another enzyme involved in the metabolism of 6MP/AZA (Figure 1). Although earlier reports found that ITPA deficiency may predict pancreatitis (38), the same authors later found this not to hold true (39). There are some data that ITPA deficiency may predict flu-like symptoms (38,39) and leukopenia (40), but more work is needed on this before determining if this is clinically relevant. Currently, ITPA testing is not commercially available.

Figure 1. Thiopurine metabolism. AZA = azathioprine; 6MP = 6-mercaptopurine; XO = xanthine oxidase; HPRT = hypoxanthine phosphoribosyltransferase; TPMT = thiopurine methyltransferase; IMP = inosine monophosphate; IDP = inosine diphosphate; ITP = inosine triphosphate; IMPDH = inosine monophosphate dehydrogenase; ITPA = inosine triphosphate pyrophosphatase.

Figure reprinted with permission from CA Siegel, BE Sands. Review article: practical management of inflammatory bowel disease patients taking immunomodulators. Alimentary Pharmacology & Therapeutics. 2005;22 (1):1-16.

Dosing

6MP/AZA have been used interchangeably in clinical practice, but once an agent is chosen it should be continued. The general recommendation for the goal dose of 6MP is 1.5 mg/kg and for azathioprine 2.5 mg/kg. If switching from 6MP to azathioprine, a conversion factor of 2.07 can be used (41). It is worth noting that strictly speaking, 1.5 mg/kg of 6MP multiplied by 2.07 is just over 3 mg/kg of azathioprine. This discrepancy points out the fact that there are still questions regarding optimal dosing. To date, the optimal weight-based dose for 6MP/AZA has not been established in prospective randomized trials, but such a dose-ranging
study is currently underway (B.E. Sands, personal communication). Branded AZA or 6MP may have slightly different bioavailability than generic versions (42). Although there are no data to suggest that this is clinically important, switching back and forth between products should as much as possible be avoided.

Different strategies have been suggested for initiating and titrating to the full weight-based dose. Although slow titration with monitoring of the white blood count (WBC) has been a common strategy, when evaluated prospectively, WBC did not correlate with efficacy or metabolite levels (32,42,43). Although reasonable, starting at a low dose with slow titration to the goal dose to monitor for adverse effects will both delay an already lengthy time for the medication to reach full effect and is unlikely to decrease the rate of toxicity. Idiopathic toxicities (pancreatitis, fever, rash, allergic reactions or nausea) are dose-independent, so a low starting dose will not necessarily protect against these. Dose-dependent toxicities (bone marrow suppression, hepatitis) are unlikely until a cumulative dose has been reached, so slow titration will delay, but not prevent adverse effects (41). As long as TPMT genotype is normal, starting at 50 mg daily for both 6MP and AZA for two weeks before pushing up to the full weight-based dose appears to be safe and decreases the amount of time required before therapeutic effects are seen (Figure 2).

**MONITORING**

**Laboratory Testing**

Routine laboratory monitoring is required for all patients taking 6MP/AZA. As previously noted, although TPMT testing can prevent severe bone marrow suppression in some, the majority of neutropenia occurs sporadically throughout the course of therapy (37). Therefore, TPMT testing should not take the place of routine monitoring of CBC. A general approach is to perform a CBC two weeks after starting therapy and then every two weeks for three months. If stable at 3 months, lab frequency can be decreased to monthly. If stable at 6 months, labs every three months for the duration of treatment is appropriate (see Figure 2). If TPMT testing is not performed, weekly CBC should be performed for the first month. Liver function tests (LFTs) can probably be performed less frequently, but to avoid confusion in laboratory ordering, they can be obtained at the same time intervals. If dose increases are made over the course of therapy, labs should be performed every two weeks for the next month. There is some evidence to suggest that following amylase and lipase along with CBC and LFTs for the first few months can pick up patients at risk for pancreatitis before clinical symptoms present (44). Some patients with IBD have an elevated baseline amylase and lipase for unclear reasons (45,46). Therefore, it can be very helpful to measure a baseline amylase and lipase before initiating therapy to avoid unnecessarily stopping 6MP/AZA for elevated values mistakenly attributed to the medication.

**6-TG and 6-MMP Monitoring**

6-TG and 6-MMP are metabolites of 6MP that appear to correspond to clinical efficacy and potential hepatotoxicity respectively (32). Now that measurement of 6-TG and 6-MMP levels is commercially available, it is appealing to consider routine monitoring of these metabolites to predict response or toxicity and to guide dose titration. Although studies evaluating this tactic have yielded mixed results (32,47–52), a recent meta-analysis showed that higher 6-TG levels were associated with a significantly higher rate of clinical remission (53). Furthermore, a cost-effectiveness analysis showed that routine measurement of 6-TG and 6-MMP levels may improve outcomes and decrease the cost of care of steroid treated chronically active Crohn’s disease patients (36). These data are interesting, but still do not answer the question if measuring metabolites will be clinically more useful than routine practice. A prospective trial examining this critical question is currently underway (S.B. Hanauer, personal communication). At the current time, the data are insufficient to support routine monitoring of 6MP metabolites.

One approach that currently does appear clinically helpful is to consider metabolite testing when patients are not achieving therapeutic efficacy despite adequate weight-based dosing and enough time to expect effect (3–4 months). Very low or undetectable levels of 6-TG and 6-MMP in this setting signify non-compliance, or...
very rarely, poor absorption (as can be seen in short-gut syndrome). A 6-MMP:6-TG ratio >10 indicates preferential shunting to 6-MMP and has been suggested as a threshold representing unfavorable metabolism which is unlikely to result in therapeutic efficacy (54). If 6-TG levels are sub-therapeutic (6-TG <230–260 pmol/8 × 10^8 red blood cells without evidence of significant shunting to 6-MMP), an increase in 6MP/AZA dose may be indicated to achieve optimal dosing.

**Drug Interactions**

As patients may be taking 6MP/AZA for many years, it is likely that at some point they will be on concomitant medications that may interfere with drug metabolism. 5-aminosalicylates (5ASAs) drugs have gained the most attention. They are frequently used in IBD and in vitro, act as non-competitive inhibitors of TPMT (55) potentially leading to higher levels of 6TG. There have been conflicting data as far as the clinical significance of this effect (51,54,56,57), but it is worth keeping in mind if patients experience toxicity or loss of effect of 6MP/AZA after respectively starting or stopping 5ASAs. Oftentimes patients inquire if they can stop 5ASAs once becoming therapeutic on 6MP/AZA. Although this can be done successfully in most cases, caution needs to be taken as the lost TPMT inhibition from 5ASAs leading to therapeutic 6TG levels could drop patients below their fine line of therapeutic efficacy.

Allopurinol, frequently used for the treatment of gout, is a xanthine oxidase inhibitor that can have dramatic effects on 6MP/AZA metabolism (Figure 1). Specifically, a higher proportion of 6MP is metabolized to 6TG potentially leading to profound bone marrow suppression. In most cases, allopurinol should not be used in patients taking 6MP/AZA. Recently, Sparrow, et al have taken advantage of this interaction in attempts to increase 6TG levels (and drug efficacy) in those patients who preferentially shunt towards 6MMP. They have shown that in these “shunters,” co-administration of low-dose AZA with allopurinol leads to an increase in 6TG levels to the therapeutic range (58) and enhanced clinical effectiveness (M. Sparrow, personal communication). Although in the future this may provide a reasonable strategy, until the safety and true clinical significance is further studied, these medications should not be used together.

One study has shown that infliximab administration along with 6MP/AZA leads to higher 6TG levels and lower WBC (59). It is unclear if this is due to an effect on drug metabolism, improved absorption of 6MP/AZA or some other mechanism. These data need to be confirmed, but it is reasonable to consider more careful monitoring after infliximab is started. Other medications that should be used with caution in conjunction with 6MP/AZA include other immunosuppressants, warfarin (decreased International Normalized Ratio possibly due to increased synthesis of prothrombin), furosemide (risk of increased bone marrow suppression by TPMT inhibition), and angiotensin converting enzyme (ACE) inhibitors (increased bone marrow suppression by unknown mechanism).

**METHOTREXATE**

**Evidence of Efficacy**

Methotrexate is effective in Crohn’s disease for treating both active steroid dependent disease (60) and maintaining long-term remission (61). There have not been similarly convincing results for the treatment of ulcerative colitis, but suboptimal dosing, oral administration (as opposed to subcutaneous or intramuscular) and type II error may have played a role (62–66). Although not standard, if failing other medical alternatives, methotrexate could be considered in patients with steroid-dependent or refractory ulcerative colitis.

**Adverse Effects**

Liver toxicity has been a prime concern for the use of methotrexate. Although active hepatitis and cirrhosis are a problem in a significant number of patients with psoriasis (67) taking methotrexate, this appears to be much less common in rheumatoid arthritis (68) and in IBD (69). The rate of methotrexate induced hepatotoxicity appears to increase with known risk factors for liver disease including obesity, alcohol overuse and diabetes (all more common in psoriasis than IBD). Although methotrexate induced liver toxicity is rare in
IBD, caution should still be used in those patients with presence of these risk factors. There are no specific practice guidelines for Crohn’s disease or ulcerative colitis, but pre-treatment liver biopsy is not generally recommended unless there is a suspicion of underlying liver disease. Hypersensitivity pneumonitis has also been a concern with the use of methotrexate. It appears to be more common in patients with rheumatoid arthritis and although reported in IBD (63,70), it is very rare.

Nausea is common with the use of methotrexate, but is usually manageable. Strategies to manage nausea include changing to nighttime dosing, higher doses of folic acid (2 mg daily) and if necessary, the addition of antiemetics around the time of the weekly dose. Mouth sores are not infrequent, and are also helped with higher dose folic acid.

Finally, and most important of the adverse effects is the teratogenic and abortifacient nature of methotrexate. Women are required to use at least one form of highly effective birth control. As methotrexate can also be toxic to sperm, men should stop methotrexate at least 3 month before trying to conceive (71,72).

Getting Started
As noted for 6MP/AZA, before commencing therapy with methotrexate a conversation with patients regarding risks and benefits is essential. The starting dose for induction of remission is 25 mg weekly (Table 2). Although the Feagan studies (60,61) used intramuscular (IM) dosing, subcutaneous (SQ) dosing is more easily tolerated by patients and has been shown to be bioequivalent (73). Oral methotrexate has variable absorption that becomes more unreliable at higher doses (74). Reflecting the Feagan data, if patients are in clinical remission by 16 weeks, the dose can be decreased to 15 mg weekly for maintenance.

Injectable methotrexate is provided as 25 mg/mL with or without preservatives. If the formulation with preservatives is available, it can be supplied in 10 mL vials. If not, 1 mL or 2 mL vials without preservatives can be prescribed (discarding the unused portion each week). A 3 cc syringe with a 25 gauge, 5/8th inch needle seems most manageable for patients to give SQ injections, either 1 mL (25 mg) for induction or 0.6 mL (15 mg) for maintenance dosing.

Co-administration of folic acid 1 mg taken by mouth daily prevents clinically significant folate deficiency, and as previously noted may diminish nausea and mouth sores. Methotrexate probably takes about the same amount of time as 6MP/AZA to show full efficacy (3–4 months), but there has been a suggestion that methotrexate may act more quickly (75).

Monitoring
Routine laboratory testing is required while on methotrexate, but less often than with 6MP/AZA primarily because significant leukopenia is uncommon. CBC and LFTs with albumin should be performed monthly for the first 2 months and then every 1–2 months for the duration of therapy (Table 2). Reflecting the recommendation of the American College of Rheumatology (68), liver biopsy should be performed only if there are persistent elevations of AST above the upper limit of normal in five of nine (or six of twelve if performed monthly) lab tests in a given year, or a decrease in albumin below the normal level in the setting of improving inflammatory disease. Methotrexate should be stopped if liver biopsy reveals moderate-to-severe fibrosis or cirrhosis, or with persistent elevation of AST in a patient who refuses liver biopsy (68).

At the doses used in IBD, significant drug interactions with methotrexate are uncommon. Medications with immunosuppressant effects should be used with caution (e.g., sulfonamides). Since adenosine may play a role in the mechanism of action of methotrexate, it should be noted that adenosine receptor antagonists (caffeine, theophylline) could decrease therapeutic benefit (76).

CONCLUSION
6-mercaptopurine and azathioprine are cornerstones in the long-term management of Crohn’s disease and ulcerative colitis. Methotrexate is an effective 2nd line agent in Crohn’s disease. Trends in early aggressive management and “top-down” therapy are bringing these medications to patients at an earlier point in their disease. Safe and effective use of immunomodulators is imperative to optimize the risk/benefit profile. These
recommendations reflect current knowledge in 2006. As more data emerge in regards to pharmacogenetics, metabolite monitoring and optimal dosing, we will hopefully be able to fine-tune this approach even further in the future.
Safe and Effective Use of Immunomodulators for IBD

INFLAMMATORY BOWEL DISEASE: A PRACTICAL APPROACH, SERIES #2


