Optimizing the use of thiopurines in inflammatory bowel disease

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***Abstract*:** Immunomodulator drugs, of which thiopurines can be considered the backbone, are widely used in the treatment of inflammatory bowel disease. They have been shown to be highly effective and safe; however, a significant proportion of patients are deemed to have a poor response or suffer adverse reactions. Knowing how to monitor and optimize thiopurine therapy in these scenarios is crucial to effective management. We discuss the metabolism of thiopurines, the use of enzyme/metabolite testing to guide treatment, as well as strategies to circumvent toxicity and side effects, such as allopurinol coprescription. The indications, use in pregnancy, safety profile and duration of thiopurine therapy are also discussed.

Keywords: gastroenterology, inflammatory bowel disease, ulcerative colitis, Crohn's disease, drugs, thiopurines, azathioprine

Introduction

Inflammatory bowel disease (IBD) comprises ulcerative colitis (UC) and Crohn's disease (CD). The disease courses are characterized by periods of relapse and remission. Both IBDs are clinically and histologically distinct but also share many common genetic and clinical factors.

UC was first described in 1859 by Samuel Wilks. Features of UC are confluent mural inflammation which begins in the rectum and extends proximally with sparing of the terminal ileum and small bowel. Sulfasalazine was the first drug introduced to treat the disease in the 1940s, followed by steroid treatment in the 1950s.

Patients with terminal ileitis were first reported in the early 1900s. Burrill Bernard Crohn described a series of cases in 1932 and subsequently this form of IBD became known as CD. Its hallmark is discontinuous chronic granulomatous inflammation, which commonly involves the terminal ileum but can affect any part of the gastrointestinal tract. In contrast with UC, CD may feature transmural inflammation which can result in penetrating or structuring disease.

The treatment for both diseases involves immunosuppressant medication. Mesalazine preparations are used as first-line treatment for UC, with corticosteroid therapy used in the early treatment of UC and CD. Thiopurine therapy is usually introduced as steroid-sparing therapy in UC when a patient's condition fails to respond to two courses of steroid therapy. In addition, certain clinical scenarios such as presentations of acute severe colitis treated with rescue therapy may require early thiopurine therapy to maintain remission. Thiopurines are generally used earlier in CD as they provide long-term disease course modification which steroid therapy does not provide. A recent prospective study concluded that early azathioprine (AZA) therapy (within 8 weeks of diagnosis) provided no benefit in sustaining steroid-free remission compared with placebo [Panés *et al.* 2013]. It did, however, show that AZA was more effective in preventing moderate–severe relapses. The main limitation of this study was the use of steroid-free remission as an endpoint. There is a wealth of evidence including a Cochrane Library analysis reporting the effectiveness of thiopurines in maintenance of remission in CD [Prefontaine *et al.* 2010]. This suggests that thiopurines provide long-term disease modification which steroids do not; however, steroid therapy has a role in early disease treatment as a bridge to maintenance thiopurine therapy. Surgery is usually indicated for patients in whom medical therapy has failed or is refractory.

AZA was first synthesized in 1957 by George Herbert Hitchings and Gertrude Elion. They hypothesized that the growth of rapidly dividing

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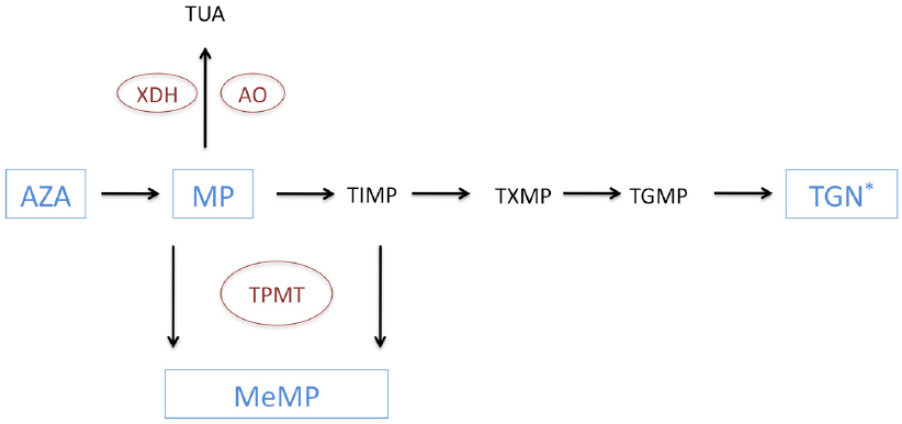

Figure 1. A simplified schematic of the thiopurine metabolic pathway. AO, aldehyde oxidase; AZA, azathioprine; MeMP, methylmercaptopurine; MP, mercaptopurine; TGMP, thioguanine monophosphate; TGN, thioguanine nucleotide; TIMP, thioinosine monophosphate; TPMT, thiopurine-S-methyltransferase; TUA, thiouric acid; XDH, xanthine dehydrogenase.

cells could be arrested with antimetabolites of nucleic acid bases [Elion, 1989]. Their work led to the discovery of thioguanine (TG, 2,6-diaminopurine) and mercaptopurine (MP). AZA, a derivative of MP, was subsequently produced to increase the bioavailability of MP. Collectively, these drugs belong to a group known as thiopurine analogues.

Despite being unlicensed for IBD, thiopurines are indicated in the treatment of both UC and CD, primarily as corticosteroid-sparing therapy and disease-modifying drugs. For patients with UC taking AZA, steroid-free, clinical and endoscopic remission has been described in 53% of patients compared with 21% receiving mesalazine therapy [Aridizzone *et al.* 2006]. In patients with CD, two Cochrane reviews have shown that AZA/MP is more efficacious than placebo in inducing and maintaining remission [Mowat *et al.* 2010]. More recently, a meta-analysis has shown that thiopurine use is highly effective with an associated 40% reduction of surgical resection in CD [Chatu *et al.* 2014].

Biologic drugs are the latest addition to the therapeutic armamentarium in managing IBD. Strong evidence advocating combination therapy in treating moderate to severe CD comes from the SONIC trial, which found biologic with concomitant thiopurine therapy more efficacious than either drug as monotherapy [Colombel *et al.* 2010]. This finding indicates yet another use for thiopurine drugs in the evolving treatment of IBD.

Metabolism of thiopurines

AZA and MP are prodrugs that undergo extensive metabolism *via* a complex enzymatic pathway. Figure 1 shows a simplified version of the pathway illustrating the important enzymes and metabolites [Blaker *et al.* 2012]. Initially, the majority of absorbed AZA is metabolized to MP; however, a small amount is metabolized to purine bases implicated with hypersensitivity reactions [McGovern *et al.* 2002]. MP is then metabolized *via* three different pathways. It may be oxidized by xanthine dehydrogenase and aldehyde oxidase to form thiouric acid which undergoes urinary excretion [Remy, 1963]. Alternatively, MP can be methylated by thiopurine-S-methyltransferase (TPMT) to form methylmercaptopurine (MeMP). MP can also be metabolized by a group of enzymes known as the purine salvage pathway to produce the pharmacologically active metabolites, TG monophosphate, TG diphosphate and TG triphosphate. Collectively, these end metabolites are referred to as TG nucleotides (TGNs). MeMP may also be therapeutically efficacious, however it is widely accepted that TGNs are the primary mediators of therapeutic response.

TGNs are incorporated into DNA, inhibiting its synthesis and leading to DNA strand breakage thus hindering cell proliferation [Aarbakke *et al.* 1997]. However, the main mechanism of immunomodulation is by inducing T-cell apoptosis by modulating cell (Rac1) signalling [Tiede *et al.* 2003].

Initiating and dosing thiopurine therapy

There is much inter-individual variation in the metabolism of thiopurines, both in their side effects and efficacy. This is due to differences in the amount of drug absorbed, idiosyncratic

reactions, drug–drug reactions and polymorphic variation in thiopurine enzyme metabolism. The appropriate ideal dose for AZA is 2–2.5 mg/kg/day and for MP is 0.75–1.5 mg/kg/day [Mowat *et al*. 2010]. The dose of TG is not dependent on the patient's weight and is usually prescribed at a dose of 20 mg/day.

The therapeutic onset after thiopurine initiation is delayed as it takes between 12 and 17 weeks for TGNs to be incorporated into DNA [Prefontaine *et al*. 2010]. This can have a great impact on clinical practice and acutely unwell patients often have to be bridged to thiopurine therapy with intermediary immunosuppressants such as corticosteroids.

MP is mainly metabolized by TPMT and has a pivotal role in determining the bioavailability of TGNs. TPMT activity has a trimodal phenotypic distribution according to allelic polymorphism. Within white populations, 0.3% of individuals have little or no activity, 12.4% have intermediate activity and 87.3% have normal activity.

Severe pancytopenia has been reported in patients with TPMT deficiency and it should therefore be measured prior to starting thiopurine therapy [Higgs *et al.* 2010]. It is worth noting that TPMT levels do not predict the majority of myelotoxicity cases and ongoing haematological monitoring is crucial [Gearry *et al*. 2003]. Reports indicate between 50% and 75% of thiopurine-related leucopenia occurs in patients with normal TPMT levels [Colombel *et al*. 2000; Ansari *et al*. 2002].

Prior to commencing thiopurine treatment, patients should be counselled on possible risks and side effects. As well as TPMT measurement, screening for opportunistic infections should be performed. Current European guidelines suggest serological screening for varicella zoster virus, hepatitis B virus, hepatitis C virus and human immunodeficiency virus [Rahier *et al*. 2009]. Screening for tuberculosis with a chest radiograph and interferon γ release assay should also be considered, particularly if risk factors are present. Young women should be encouraged to participate in national cervical cancer screening programs. Patients should also undergo vaccination prior to thiopurine initiation for the following diseases: varicella zoster virus, human papilloma virus, influenza, pneumococcus and hepatitis B [Rahier *et al*. 2009]. Naturally, live vaccines are contraindicated once immunomodulator therapy has begun.

Monitoring thiopurine therapy

When commencing thiopurine therapy, the full blood count (FBC) and liver function tests (LFTs) should be monitored every 2 weeks for the first 2 months followed by every 3 months for the duration of therapy. Pancreatitis and hepatotoxicity are uncommon, however the purpose of blood monitoring is primarily to detect thiopurine-induced leucopenia during treatment with approximately half of patients developing signs within 2 months and nearly two-thirds within 4 months of drug initiation [Colombel *et al*. 2000]. As a result, it is recommended that the FBC and LFTs be rechecked following any dose escalation. Patients developing flu-like illness, jaundice, abdominal pain and unexplained bleeding/bruising should report these signs to their physician immediately.

We recommend that measurement of red cell TGN levels from blood should be checked after 4 weeks of starting treatment with AZA/MP with the specific purpose of confirming adherence and guiding dose optimization. Subsequently, the levels should be checked again at 12–16 weeks once the TGN metabolites have reached a steady-state concentration. Although, it takes up to 4 months for the TGN tissue concentrations to plateau, early blood levels do appear to correlate with eventual tissue concentrations, enabling benefit in early blood level measurement. During maintenance treatment, TGN levels can be measured if patients experience a flare of their symptoms or if compliance or toxicity is suspected. TGN measurement is irrelevant for TG as the levels do not correlate with the dose. Figure 2 shows an algorithm illustrating when to measure TGN levels.

TGN levels have been shown to correlate well with clinical response. For IBD, TGN levels greater than 235 pmol/8×10^8 red blood cells (RBC) have been shown to maintain steroid-free remission in 65% of patients receiving thiopurines [Dubinsky *et al*. 2000]. Recently, a meta-analysis of nearly 2500 patients has supported this level as a therapeutic threshold [Moreau *et al*. 2013]. When the TGN level is less than 235 pmol/8×10^8 RBC, patients may still enter clinical remission but it is statistically unlikely [Dubinsky *et al*. 2000].

TGN levels greater than 450 pmol/8×10^8 RBC have been found to be associated with a greater occurrence of myelotoxicity and leucopenia [Dubinsky *et al*. 2000, 2002; Osterman *et al*. 2006].

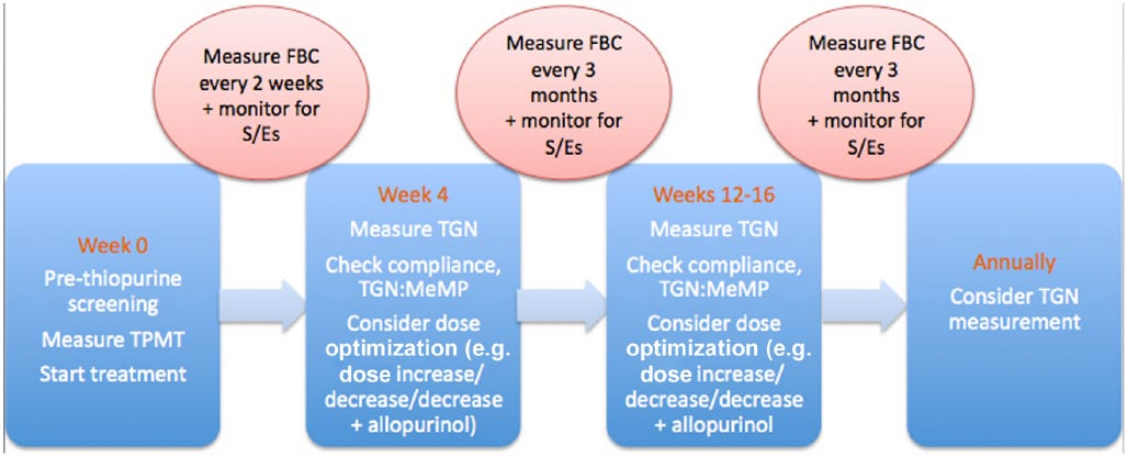

Figure 2. When to measure thioguanine nucleotides and blood test monitoring. FBC, full blood count; MeMP, methylmercaptopurine; S/Es, side effects; TGN, thioguanine nucleotide; TPMT, thiopurine-S-methyltransferase.

TGN level measurement is particularly useful for monitoring how much of the drug is converted to the potentially toxic metabolite, MeMP. Raised levels of MeMP indicates that the patient preferentially metabolizes the thiopurine to MeMP as opposed to therapeutically active TGN. This phenomenon of skewed drug metabolism is known as thiopurine hypermethylation and occurs in approximately 18% of patients with normal TPMT levels.

Optimizing thiopurine therapy

Clinically relevant dosing strategies to optimize treatment are further discussed. Table 1 summarizes the interpretation of TGN levels in thiopurine maintenance treatment [Irving, 2014].

When commencing a thiopurine, it should be noted that the therapeutic benefit is usually only achieved after approximately 3 months. During this time, patients should be supported with alternative therapies (e.g. corticosteroids) until TGN levels reach a therapeutic steady state.

TPMT measurement allows for those patients with intermediary or absent levels of enzyme to be identified. Patients with intermediate TPMT activity should receive 50% of their thiopurine dose, whereas patients with low TPMT activity should avoid thiopurines, though they may tolerate 5–10% of their standard dose [Kaskas *et al.* 2003; Relling *et al.* 2011].

The commonest cause of an inadequate response to thiopurine treatment is underdosing [Seidman, 2003] or poor compliance. Measurement of TGN levels can detect when this occurs and dose incrementation or patient education usually achieves therapeutic levels [Gearry and Barclay, 2005]. A prospective study of steroid-dependent patients with IBD showed that dose optimization of AZA to achieve levels greater than 250 pmol/8×10^8 RBC was significantly associated with a higher rate of disease remission [Roblin *et al.* 2005]. This provides strong evidence for TGN level monitoring and dose optimization and our experience indicates that this usually correlates with clinical response. Rarely, TGN levels may be low due to poor absorption of the drug. The mechanism for this is not fully understood and switching to another class of drug may be necessary [Irving, 2014].

Thiopurine hypermethylation appears to occur in up to 20% of the population [Smith *et al.* 2012], where TGN levels are found to be low with conversely raised MeMP levels. An MeMP:TGN ratio of greater than 11 has been shown to greatly increase the risk of toxicity [Dubinsky *et al.* 2002; Smith *et al.* 2012]. Specifically, an MeMP level greater than 5700 pmol/8×10^8 RBC is associated with hepatotoxicity [Dubinsky *et al.* 2000]. Unfortunately, the initial thiopurine dose or TMPT level does not predict the risk of hypermethylation [Cuffari *et al.* 2001; Blaker *et al.* 2012]. Increasing the dose in patients who exhibit thiopurine shunting can increase the levels of MeMP with no rise in TGN levels [Cuffari *et al.* 2001]. Indeed, this practice can increase the risk of toxicity and highlights the importance of TGN/MeMP measurement. A clinically important strategy to circumvent this problem is to reduce the thiopurine dose to

Table 1. Interpretation of TGN levels in patients on maintenance AZA/MP therapy.

TGN level	MeMP level	Interpretation	Action
Zero	Zero	Poor/variable compliance	Patient education (consider poor absorption: rare)
Low ($<235 \times 10^8$ pmol/RBC)	Low	Subtherapeutic dosing	Increase dose and recheck levels
Low ($<235 \times 10^8$ pmol/RBC)	High (MeMP:TGN $>$ 11)	Thiopurine hypermethylator	Reduce drug dose to 25–33% + commence allopurinol and recheck levels
Therapeutic ($>235 \times 10^8$ pmol/RBC)	Any (<5700 pmol/8×10^8)	Therapeutic or thiopurine/class resistance	Continue drug or change thiopurine/class if no response
High ($>450 \times 10^8$ pmol/RBC)	High (>5700 pmol/8×10^8)	Supratherapeutic dosing	Reduce dose and recheck levels

AZA, azathioprine; MeMP, methylmercaptopurine; MP, mercaptopurine; RBC, red blood cell; TGN, thioguanine nucleotide.

25–33% and coprescribe the xanthine oxidase inhibitor, allopurinol (100 mg daily) [Sparrow *et al.* 2007; Leung *et al.* 2009; Smith *et al.* 2012]. The precise mechanism of this observation remains unknown but may be related to the finding that allopurinol increases thioxanthine levels which in turn inhibits TPMT activity [Blaker *et al.* 2013]. Recognition of thiopurine hypermethylation is clinically relevant and steroid-free remission at 1 year has been described in up to 73% of patients in a recent large series [Smith *et al.* 2012]. A crucial footnote is that failure to reduce the thiopurine dose in conjunction with allopurinol coprescription carries a great risk of myelotoxicity. Another benefit of low-dose AZA/MP and allopurinol can be to bypass certain thiopurine-related adverse effects, including gastrointestinal upset, flu-like symptoms, myalgia and hepatoxicity [Ansari *et al.* 2010].

Thiopurines are generally well tolerated, however approximately 9% of patients are thiopurine resistant and a further 15–28% develop an adverse drug reaction [Schwab *et al.* 2002]. The drug reaction may be related to drug metabolism, in which case TPMT and TGN measurement can guide treatment, but between 1% and 6.5% of patients experience idiosyncratic reactions [de Boer *et al.* 2007]. Most reactions (such as flu-like illness, arthralgia, rash, headache) seem to occur in the first 2–3 weeks of treatment and improve after a few weeks if the drug is continued. If symptoms persist, the patient can be informed that they stop rapidly upon drug withdrawal. Commencing therapy at a lower dose such as 50% of the ideal weight-based therapeutic dose may reduce the severity of an adverse reaction and allows for early identification and intervention. Once tolerated, the dose can then be increased with FBC, LFT and TGN monitoring guiding treatment. In addition, there is now robust evidence that subdividing the total ideal thiopurine dose into two smaller daily doses can be of benefit in reducing some side effects such as nausea in individuals who are preferential methylators [Shih *et al.* 2012; Pavlidis *et al.* 2014]. This simple intervention has also been shown to improve long-term remission rates [Shih *et al.* 2013].

AZA is usually the first line thiopurine to be prescribed. Intolerance due to side effects may lead to early discontinuation of the drug. In this instance, benefit may be derived from using a metabolic descendant such as MP or TG [Nagy *et al.* 2008; Amin *et al.* 2014]. This strategy has been shown to benefit certain patients who are intolerant of the initially trialled thiopurine. In patients who experience AZA/MP-induced pancreatitis, both further AZA/MP use is contra-indicated, however, patients have been shown to subsequently respond to and tolerate TG [Amin *et al.* 2015].

Risks and benefits in continuing or withdrawing thiopurine therapy

Generally, thiopurine therapy should be continued indefinitely once remission has been achieved. A randomized controlled trial of withdrawal of AZA therapy in patients with IBD found that 21% of patients who ceased AZA therapy experienced a relapse of their disease compared with 8% on ongoing therapy [Lémann *et al.* 2005]. The median duration of treatment and clinical remission was 5.7 and 5.3 years respectively. Logically, raised inflammatory markers (C-reactive protein) and anaemia were found to predict relapse. Further follow up of the cohort which ceased AZA found high rates of just over 50% relapse at 3 years with almost two-thirds of patients relapsing after 5 years [Treton *et al.* 2009].

Despite having a US Food and Drug Administration (FDA) category D rating (risk of adverse events to foetus), thiopurines are thought to be generally safe in pregnancy [Mowat *et al.* 2010]. The FDA rating is based on anecdotal evidence of high abortion rates; however, studies of AZA/MP in IBD exist, showing no increased risk of adverse events in pregnancy [Van Assche *et al.* 2010]. Patients who are in clinical remission at the time of conception are less likely to suffer flare of their disease during the course of their pregnancy. It is for this reason that it is generally advised that thiopurine therapy be continued in pregnancy. At present, there are few data to support that thiopurines are relatively contraindicated in breastfeeding. A recent small study has shown that there are negligible concentrations of thiopurine metabolites expressed in breast milk [Sau *et al.* 2007].

All immunomodulators carry a slightly increased risk of infection with a specifically increased susceptibility to viral infections seen with thiopurines [Toruner *et al.* 2008]. Fungal and mycobacterial infections are more associated with corticosteroid and biologic use respectively. In the case of an acute infection, the thiopurine can be withdrawn, the infection treated, and the thiopurine safely reintroduced once the infection has been cleared. It is important to note that there is a much greater risk of infection when immunomodulators are used in combination therapy [Toruner *et al.* 2008].

Postoperative patients do not appear to have an increased complication rate with thiopurine use and the drugs can be used safely in high-risk patients. Interestingly, preliminary data from the POCER trial suggest a reduction in postoperative endoscopic recurrence in patients treated with thiopurines or adalulimab [Kamm *et al.* 2014].

Thiopurine therapy has been shown to carry a slightly increased risk of malignancy, specifically lymphoproliferative disease. Studies indicate a four- to fivefold increased risk compared with the background population, although the absolute risk remains very low [Kandiel *et al.* 2005; Beaugerie *et al.* 2009]. A recent decision analysis study found that alternative therapy would be favoured if there were a tenfold risk in lymphoma [Vos *et al.* 2011]. The overall consensus is that the benefits of thiopurine therapy outweigh the risk of malignant lymphoma. Exposure to ultraviolet radiation, such as heavy sun exposure, has been shown to carry an increased risk of nonmelanomatous skin cancer. A retrospective study found that thiopurine exposure over 47 years was associated with an odds ratio of 5.0 for nonmelanomatous skin cancer. Caucasian descent carried an increased risk with an odds ratio of 12.4 [Setshedi *et al.* 2012]. More recently, a large retrospective analysis of thiopurine therapy both with and without anti-tumour necrosis factor (TNF) treatment has been published. Thiopurine monotherapy was found to be associated with an increased risk of malignancy compared with combination therapy with anti-TNF drugs. The rate of malignancy for patients aged more than 50 years receiving thiopurine therapy was 18.2%, significantly greater than the rate of 3.8% for patients aged less than 50 years. Thiopurine treatment duration of greater than 4 years was also shown to carry a greater risk for skin cancer and lymphoma [Beigel *et al.* 2014]. A large prospective observational study has recently shown that patients previously exposed to thiopurines have a sevenfold increased risk of developing a myeloproliferative disorder. Interestingly, patients receiving ongoing thiopurine therapy were not at greater risk [Lopez *et al.* 2014]; however, the results of this study should be considered when initiating and withdrawing therapy. Advancing age certainly seems to confer a greater risk of malignancy, particularly for patients over the age of 50 years. The risks and benefits should be carefully weighed up, with patients appropriately counselled and also considered for alternative therapy if appropriate. We currently practice annual outpatient screening of skin lesions for all patients receiving thiopurine therapy, with suspicious lesions referred for specialist dermatology assessment.

Historically, there has been concern regarding the risk of nodular regenerative hyperplasia (NRH) and the need for interval magnetic resonance imaging (MRI) screening. The cumulative incidence of NRH in patients with IBD treated with AZA is approximately 1% at 10 years. High doses of TG have been associated with frequencies of NRH of up to 62%; however, low-dose TG (20 mg once daily) appears safe with no cases of NRH seen [Musumba, 2013]. Until a prospective trial occurs reliably evaluating the risk of NRH with TG, our current practice is to survey for NRH with annual MRI scanning. The development of thrombocytopenia or raised liver enzymes detected by blood testing every 2 months may also be appropriate triggers to consider MRI screening.

There is no ideal duration of therapy, however 5 years would appear to be a pragmatic time to review the need for ongoing treatment based on

previous study data. Consideration should be given to the long-term risks with thiopurine therapy, which may become increasingly important with advancing age [Beigel *et al*. 2014]. As always, the risks and benefits need to be carefully discussed with each individual patient. Thiopurine withdrawal has been shown to be associated with a greater risk of relapse [Wenzl *et al*. 2014]. Most recently, a retrospective study reported that thiopurine withdrawal in patients in sustained remission was associated with a 1-year moderate to severe relapse rate of 23% in CD and 12% in UC [Kennedy *et al*. 2014]. A raised C-reactive protein level prior to thiopurine withdrawal was found to be highly predictive of relapse, with rates also cumulatively increasing with time [Kennedy *et al*. 2014]. However, in those who decide to stop therapy and suffer a relapse, therapeutic response can be recaptured in almost all patients with recommencement of the thiopurine [Treton *et al*. 2009; Kennedy *et al*. 2014]. This differs somewhat from biologic therapy when a 'drug-holiday' can be associated with antidrug antibodies, an increased incidence of drug reactions and possibly a reduced chance of recapturing therapeutic response.

Summary

Thiopurine therapy should be personalized and tailored to the individual being treated. Thiopurine metabolism remains complex but is becoming increasingly understood. It is important to understand and identify reasons as to why a thiopurine is ineffective or not tolerated. Dose changes, switching to another thiopurine/drug, allopurinol coprescription and management of side effects are crucial to ensure thiopurines are used in the best way. In addition, the use of TPMT and TGN measurement is clinically relevant and can help optimize existing thiopurine treatment. Further studies specifically to document the benefit of TGN monitoring, allopurinol coprescription and TG efficacy and safety will aid in guiding treatment and ensure thiopurines are used to maximize response.

Conflict of interest statement

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