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British Association of Dermatologists' guidelines for the safe and effective prescribing of azathioprine 2011

S.J. Meggitt, A.V. Anstey,* M.F. Mohd Mustapa,† N.J. Reynolds‡ and S. Wakelin§

Department of Dermatology, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP, U.K.
*Department of Dermatology, Royal Gwent Hospital, Cardiff Road, Newport, Gwent NP20 2UB, U.K.
†British Association of Dermatologists, Willan House, 4 Fitzroy Square, London W1T 5HQ, U.K.
‡Institute of Cellular Medicine, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, U.K.
§Department of Dermatology, St Mary's Hospital, Imperial College Healthcare Trust, Praed Street, London W2 1NY, U.K.

Correspondence
Simon Meggitt.
E-mail: s.j.meggitt@ncl.ac.uk

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1.0 Introduction

Azathioprine is a thiopurine immunosuppressant drug that occupies an important place in the management of many autoimmune and inflammatory skin diseases. Its parent drug 6-mercaptopurine (6-MP), and the closely related 6-thioguanine (6-TG), were originally developed for their anticancer properties, but thiopurines as a class are now more widely used for their anti-inflammatory and immunosuppressant effects. 6-MP and 6-TG have never found their way into routine dermatological practice and these guidelines relate to azathioprine and its extensive on- and off-label applications for inflammatory dermatoses.

Although azathioprine has been widely prescribed since the 1960s, there continue to be developments in understanding of drug action, pharmacogenetics and toxicology. These offer the potential for improved and individualized azathioprine prescribing, but have also created areas of controversy and resulted in contradictory information for clinicians. Nevertheless, a basic understanding of the issues relating to azathioprine metabolism and mode of action is important for the dermatologist, and should allow better explanation of treatment to patients with optimized prescribing and monitoring of therapy.

2.0 Purpose and scope

The overall objective of the guideline is to provide up-to-date, evidence-based recommendations for the safe and effective use of azathioprine. The document aims to update and expand on the previous guidelines by (i) offering a complete reappraisal of all relevant literature since 1966 and focusing on key developments over the past 5 years, in particular the applicability of thiopurine methyltransferase (TPMT) assessment to the clinical setting; (ii) addressing important, practical clinical questions relating to the primary guideline objective; (iii) providing guideline recommendations with an evaluation of their health economic impact; and (iv) discussing potential developments and future directions. The guideline is presented as a detailed review with highlighted recommendations for practical use in the clinic, in addition to updated patient information.

3.0 Stakeholder involvement and peer review

The guideline working group consisted of dermatologists and a patient representative. The draft document was circulated to the British Association of Dermatologists (BAD) membership, the British Dermatological Nursing Group (BDNG), an immunologist and a hepatologist for comments and peer reviewed by the Clinical Standards Unit of the BAD (made up of the Therapy & Guidelines and Audit & Clinical Standards Subcommittees) prior to publication.

4.0 Methodology

This set of guidelines has been developed using the BAD's recommended methodology[1] and with reference to the Appraisal of Guidelines Research and Evaluation (AGREE) instrument.[2] Recommendations were developed for implementation in the NHS using a process of considered judgment based on the evidence. PubMed, MEDLINE and EMBASE databases were searched up to January 2011 for randomized and nonrandomized controlled clinical trials, case series, case reports, open studies and research articles involving azathioprine and 6-MP. Due to the expected high number of results in the EMBASE search, which has a particular emphasis on drug literature, additional search protocols were used specifically to target key areas such as thiopurine-metabolizing enzymes and toxicity, as well as separating the results into predominantly dermatology- and gastroenterology-based publications. Search terms and strategies are detailed in Appendix S1 (see Supporting information). Searches were also carried out in the Cochrane, National Institute of Health and Clinical Excellence (NICE), Database of Uncertainties about the Effects of Treatments (DUET) and Royal College of Physicians (RCP) databases. Additional relevant references were also isolated from citations in reviewed literature, as well as independent targeted searches carried out by each co-author. All titles in the English language were screened, and those relevant for first-round inclusion were selected for further scrutiny; the abstracts were then reviewed by all members of the working group and the full papers of relevant material were obtained following selection by common agreement. Specific selection criteria were not deemed necessary as the number of selected abstracts was relatively small (< 150) and there was consensus that the full papers were needed in most cases. The structure of the guidelines was then discussed and different co-authors were allocated separate subsections. Each co-author then performed a detailed appraisal of the relevant literature, and all subsections were subsequently collated and edited to produce the final guideline.

5.0 Limitations of the guideline

This document has been prepared on behalf of the BAD and is based on the best data available when the document was prepared. It is recognized that under certain conditions it may be necessary to deviate from the guidelines, and that the results of future studies may require some of the recommendations herein to be changed. Failure to adhere to these guidelines should not necessarily be considered negligent, nor should adherence to these recommendations constitute a defence against a claim of negligence.

6.0 Plans for guideline revision

The proposed revision date for this set of recommendations is set for 2016; where necessary, important interim changes will be updated on the BAD website.

7.0 Azathioprine metabolism and pharmacogenetics

This section aims to give an overview of the basis for the biological effects of azathioprine and introduce some concepts related to dosing and toxicity which will be detailed later in the guideline. A widely experienced and important problem for the clinician using azathioprine is the large variability demonstrated by patients both in response to the drug and side-effects. In some patients this can be explained by increasingly well-characterized genetic differences in drug-metabolizing enzymes, but the role of other potential factors such as variability in drug absorption and bioavailability remains a matter for speculation.

Azathioprine is a prodrug that is rapidly converted to 6-MP (Fig. 1), which is then metabolized by the purine salvage pathway. This is the usual cellular mechanism by which endogenous purines are interconverted and recycled. 6-MP is acted on by several competing pathways, and bioactivation occurs via a series of enzymes to form thioguanine nucleotides (TGNs). The major catabolic pathway is mediated by xanthine oxidase and produces thiouric acid. A third pathway of methylation by the enzyme TPMT produces several intermediates, most of which are therapeutically inactive. A common polymorphism in the *TPMT* gene, such that approximately 10% of individuals carry a low-activity variant allele, has been shown to be an important factor governing thiopurine toxicity. An increase in TPMT activity diverts metabolites from the activating pathway and fewer TGNs are formed. This effectively amounts to a reduction in azathioprine dose with a theoretical decrease in pharmacological (and toxic) effects. The converse situation occurs with decreased TPMT activity, such that in the extreme situation, those individuals (0·3%)[3,4] who inherit two low-activity variant *TPMT* alleles are highly likely to develop intense TGN-induced myelosuppression if given azathioprine at conventional doses. They are effectively receiving a massive thiopurine overdose and the profound and prolonged pancytopenia may be fatal.[5,6] A lesser degree of myelotoxicity, most commonly neutropenia, can also be seen in carriers of one variant *TPMT* allele who receive conventional thiopurine doses.[7,8] These findings and their relevance to both toxicity and efficacy have been elucidated in a series of seminal papers over the past 30 years,[3,9,10] and there are a number of excellent reviews of this topic.[11]

Other than TPMT, different enzymes and intermediates that may also be clinically relevant are gradually being described

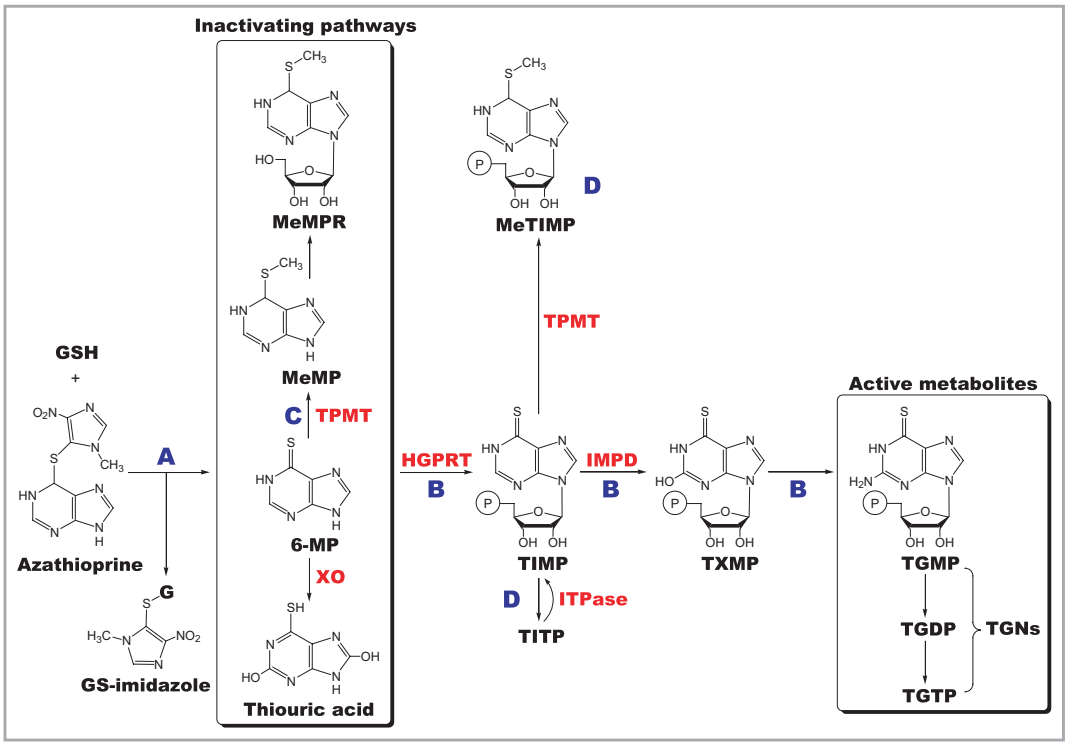

Fig 1. Thiopurine metabolism. The conversion of azathioprine to 6-mercaptopurine (6-MP) with subsequent metabolism by the endogenous purine salvage pathway is shown. The major active metabolites are thioguanine nucleotides (TGNs) and the major catabolic endpoints are thiouric acid and methylmercaptopurine (MeMP). This diagram is a significant simplification and only shows the key intermediates and enzymes that are of relevance to these guidelines. (A) Azathioprine reacts with glutathione and is cleaved to the glutathionyl derivative of 1-methyl-4-nitroimidazole and 6-MP. It is possible that the imidazole moiety may have a therapeutic effect,[12] but this has not been investigated *in vivo*. (B) The activation pathway is shown as a series of enzymatically mediated steps [hypoxanthine guanine phosphoribosyl transferase (HGPRT), inosine monophosphate dehydrogenase (IMPD)] from left to right in the diagram, and results in the formation of TGNs. These may exert their biological effects in several ways,[13,14] with incorporation of the false base into DNA being the most widely cited mechanism. (C) Thiopurine methyltransferase (TPMT) is a key enzyme in the pathway, as genetic variations in enzyme activity can explain differences in TGN profiles between individuals. Methylation by TPMT diverts metabolism away from TGN production, such that individuals homozygous for *TPMT* variant alleles will have absent TPMT activity and consequently develop very high TGN levels, which are myelotoxic. (D) Other enzymes [inosine triphosphate pyrophosphatase (ITPase)] and thiopurine intermediates [methylthioinosine monophosphate (MeTIMP)] may also affect toxicity and efficacy. For example, MeTIMP is an inhibitor of *de novo* purine synthesis *in vitro*.[15] However, the relevance of these factors in the clinical setting is much less certain than the TPMT/TGN paradigm (see section 13.0). XO, xanthine oxidase; MeMPR, methylmercaptopurine riboside; TIMP, thioinosine monophosphate; TITP, thioinosine triphosphate; TXMP, thioxanthine monophosphate; TGMP, thioguanine monophosphate; TGDP, thioguanine diphosphate; TGTP, thioguanine triphosphate; GSH, glutathione; GS-imidazole, glutathionyl derivative of 1-methyl-4-nitroimidazole.

and added to the multifaceted overall picture. Much of this literature is beyond the scope of the current guideline. However, those factors which either offer additional insight into efficacy and toxicity, or may affect future clinical practice will be reviewed, and are also shown in Figure 1.

8.0 Effective use of azathioprine: review of the evidence

8.1 Indications

Licensed and unlicensed indications are listed in Table 1 (see Appendix 1 for Strength of recommendations and levels of evidence).

8.11 Licensed indications

Autoimmune bullous disorders

Although licensed for pemphigus vulgaris, the evidence for this indication is less than robust.[16] A recent systematic review of 11 studies of interventions for pemphigus vulgaris and pemphigus foliaceus concluded that although the quality of included studies was not high, there was evidence to support a steroid-sparing effect of azathioprine[17] which appeared greater than that of both cyclophosphamide or mycophenolate mofetil.[16] However, looking at induction of remission, there is insufficient evidence to indicate that azathioprine (or any other second-line agent including cyclophosphamide and mycophenolate) is more effective than glucocorticoids alone.[16] Two nonblinded, randomized controlled trials (RCTs) reported that mycophenolate appeared

Table 1 Licensed and unlicensed indications for azathioprine in the treatment of dermatological disorders

Licensed indications	Unlicensed indications
Systemic lupus erythematosus	Atopic dermatitis
Dermatomyositis	Psoriasis[a]
Pemphigus vulgaris[b]	Bullous pemphigoid
	Chronic actinic dermatitis
	Pyoderma gangrenosum
	Pityriasis rubra pilaris
	Wegener's granulomatosis
	Cutaneous vasculitis

[a]*Strength of recommendation D; level of evidence 3;* [b]*Strength of recommendation B; level of evidence 1* (see Appendix 1).

equally efficacious as azathioprine in inducing remission in pemphigus.[17,18] However, an intention-to-treat (ITT) analysis of one of these studies[18] suggested that mycophenolate may be more effective in achieving disease control than azathioprine.[16]

Lupus erythematosus

Azathioprine is licensed for use in systemic lupus erythematosus, and there is evidence to indicate superiority for maintenance compared with cyclophosphamide, following induction in patients with lupus nephritis.[19] In cutaneous lupus, there are no RCTs to indicate efficacy although several case series suggest that azathioprine may be a useful treatment.[20–22]

Dermatomyositis and polymyositis

Azathioprine appears effective as a second-line agent in patients with dermatomyositis (DM) or polymyositis (PM), with several case series showing improvement in 57–75% of patients.[23] Two RCTs have compared azathioprine with methotrexate (MTX), although the quality of this evidence was considered to be poor in a systematic review.[24] Efficacy appeared similar in a RCT ($n = 28$, published as an abstract only) although MTX showed a better side-effect profile.[25] In a 6-month crossover RCT ($n = 30$), a combination of weekly oral MTX plus daily azathioprine was compared with intravenous MTX alone (every 2 weeks) in resistant DM or PM.[26] Using ITT analysis, a greater number of patients improved with the MTX/azathioprine combination compared with intravenous MTX ($P = 0{\cdot}025$), although the study lacked the power to compare both treatments directly. In juvenile DM, azathioprine has been demonstrated to have a steroid-sparing effect[27] and may also be beneficial in patients who have failed other immunosuppressive therapies.[28]

8.12 Unlicensed indications

Autoimmune bullous disorders

Azathioprine is widely used as a steroid-sparing agent in autoimmune bullous disorders including bullous pemphigoid. For example, a recent survey in 42 German hospitals showed that azathioprine is used as a first-line therapy adjunctive to oral corticosteroids for pemphigoid in 69% of hospitals.[29] A systematic review of therapeutic modalities for pemphigoid concluded that the combined effectiveness of azathioprine adjunctive to corticosteroids had not been established although there was evidence to support a steroid-sparing effect (by up to 50%).[30] Since then, a multicentre, randomized, nonblinded trial showed mycophenolate mofetil and azathioprine to have similar efficacy in combination with corticosteroids in inducing remission of bullous pemphigoid.[31] Liver toxicity was seen at a higher frequency in the azathioprine group whereas infections appeared more common in patients treated with mycophenolate mofetil.[31,32] Thus, while the evidence base to support the use of azathioprine as an adjunctive treatment for bullous pemphigoid is lacking, this is also true for other second-line agents that may be considered. For the group of patients who have incomplete control with oral prednisolone and require alternative therapeutic regimes, adjunctive azathioprine will continue to be used in clinical practice until further evidence is forthcoming, particularly as dermatologists are usually familiar with azathioprine and its side-effect profile.

Inflammatory skin diseases

Eczema

Although azathioprine is not licensed for use in atopic eczema, there is now strong evidence (from two RCTs) for a statistically significant and clinically meaningful response to azathioprine. Both studies used the drug as oral monotherapy in moderate-to-severe, refractory disease.[33,34] One study showed that overall, at 12 weeks, azathioprine induced a 37% improvement in disease activity compared with a 20% improvement with placebo.[34] This was accompanied by parallel improvement in quality of life and patient symptoms. A double-blind, placebo-controlled trial has also shown azathioprine to be of benefit in chronic actinic dermatitis.[35]

Psoriasis

There is limited evidence to suggest that azathioprine may be effective as a monotherapy in the treatment of moderate-to-severe psoriasis[36] but it is now rarely used in clinical practice. However, a recent retrospective review suggests azathioprine may be combined with biologics such as infliximab as an alternative to MTX for long-term maintenance.[37]

Vasculitis

Azathioprine shows therapeutic efficacy in a variety of vasculitides and Behçet disease.[38] In Wegener's granulomatosis, a RCT has shown that azathioprine is as effective as cyclophosphamide in maintaining remission following induction by cyclophosphamide plus prednisolone.[39] Similarly, a prospective, open-label trial showed azathioprine to be as effective as

MTX for maintenance therapy.[40] There is also limited evidence for azathioprine use in rheumatoid vasculitis.[41]

In severe cutaneous leucocytoclastic vasculitis unresponsive to first-line therapy including dapsone, treatment with systemic corticosteroids combined with azathioprine may be considered, although evidence for this is limited to case series.[20] There is insufficient evidence to support the use of azathioprine in the management of Henoch–Schönlein purpura nephritis.[42] No studies have addressed whether azathioprine affects the development of kidney disease in Henoch–Schönlein purpura, but this is also the case for other immunosuppressive agents and corticosteroids.[43]

Other indications

There is a lack of formal studies but there is limited evidence that azathioprine may be effective in other inflammatory skin conditions such as pyoderma gangrenosum[44] and pityriasis rubra pilaris.[45]

Recommendations: unlicensed indications for azathioprine

There is evidence to support the use of azathioprine outside its product licence for the following indications:

- Atopic eczema (*Strength of recommendation A; level of evidence 1+*)
- Maintenance therapy for Wegener's granulomatosis (*Strength of recommendation B; level of evidence 1+*)
- Behçet disease (*Strength of recommendation B; level of evidence 1+*)
- Bullous pemphigoid (*Strength of recommendation B; level of evidence 1−*)

8.2 The role of thiopurine methyltransferase measurement in azathioprine prescribing

This section investigates the evidence for a link between TPMT and both azathioprine effectiveness and toxicity. Although these are theoretically affected by variations in TPMT activity, the results of studies are sometimes conflicting, and consequently the clinical importance of TPMT in certain situations remains uncertain.

8.21 Thiopurine methyltransferase and azathioprine toxicity

A growing number of studies clearly support the association between absent TPMT activity and acute severe neutropenia in patients receiving conventional doses of azathioprine or 6-MP.[5,6,9] A meta-analysis of 67 studies, the majority retrospective cohort in design, showed that 86% of patients with two variant *TPMT* alleles developed myelosuppression.[46] Recently, a controlled trial in 333 patients attempted to clarify the value of pretreatment TPMT genotyping in predicting haematological adverse events due to azathioprine.[4] The recruitment target ($n = 500$) was not met, but in agreement with other reports, the one patient who developed profound neutropenia (nongenotyped arm) was subsequently shown to have a homozygous *TPMT* null mutation. Prevention of potentially life-threatening myelosuppression by assessing pretreatment TPMT status offers the most compelling argument for the use of TPMT testing in the clinic. For a discussion of the evidence for the cost-effectiveness of this test, see section 12.4.

Importantly, many studies have also highlighted that patients with one variant *TPMT* allele (intermediate-range TPMT activity) who receive 'conventional' doses of thiopurines may be at greater risk of toxicity from therapy.[7,8,47–49] Unfortunately, there are only limited publications pertaining to patients with dermatological conditions, including one retrospective review of 139 patients with pemphigus vulgaris which failed to demonstrate an association.[50] Therefore, for the purposes of these guidelines, relevant literature for nondermatological indications is also reviewed.

Whether patients heterozygous at the *TPMT* locus have an increased risk of adverse events in general remains unclear from the literature. A few studies have suggested that nausea is associated with TPMT status,[51,52] but others have not,[53–55] and all of these studies were relatively small. One larger, prospective study of azathioprine for inflammatory bowel disease (IBD) indicated that 17% of 33 patients with intermediate-range TPMT activity reported nausea/vomiting, compared with 8% of 366 patients with normal TPMT status.[47] However, the authors of the study did not discuss this finding or report the statistical significance of the association.

The evidence for haematological adverse events in patients with *TPMT* mutations is much stronger. In a recent meta-analysis of 67 studies, the odds ratio (OR) for developing azathioprine-induced leucopenia for those with intermediate-range TPMT compared with normal activity has been calculated to be 4·2 [95% confidence interval (CI) 3·2–5·5].[46] This is similar to the result of the largest single study to assess TPMT activity and haematological toxicity, which examined 394 consecutive patients with IBD treated with azathioprine 2·0–2·5 mg kg^{-1}. The probability of myelotoxicity in the normal TPMT activity group was shown to be 3·5% compared with 14·3% in the intermediate TPMT activity group (95% CI 1·37–14·9, OR 4·5).[47] This is an important study, as the sample size was large, the design was prospective, and the result was statistically significant. The authors subsequently suggested the need for a 50% dose reduction in those with intermediate TPMT activity. However, a prospective study by the same authors (Gisbert *et al.*[56]) in 131 patients with IBD whose azathioprine dosage was determined by TPMT status reported that three of the four patients who suffered from myelotoxicity had normal baseline TPMT activity, with the fourth having intermediate levels. It can be concluded from this result that dose reduction for intermediate-range TPMT activity does not necessarily prevent the occurrence of neutropenia, as myelotoxicity can occur in the presence of normal TPMT activity. This is borne out by several other reports which suggest that the occurrence of bone marrow toxicity is often independent of TPMT status. One study

indicated that TPMT mutations were absent in 73% of patients with Crohn disease who had experienced severe myelosuppression with azathioprine,[57] indicating that pretreatment TPMT measurement should not be seen as a substitute for standard haematological monitoring.[58]

The issue of whether to reduce the dose in individuals with intermediate-range TPMT activity (in order to minimize the risk of bone marrow toxicity) remains a matter for debate. In addition to Gisbert *et al.*,[56] others have reported the use of this approach.[34,59] Based on a retrospective study of 28 patients with dermatological conditions, Snow and Gibson[60] proposed a TPMT-based, three-tier azathioprine dose schedule, with one dose for patients with TPMT in the heterozygote range and two incremental doses for TPMT activity in the homozygote range. Subsequently, this regime was adapted in a pilot[61] and then a RCT of azathioprine for atopic eczema using a two-tier regime.[34] In the RCT, no patients with intermediate TPMT activity receiving reduced azathioprine doses developed neutropenia, yet efficacy seemed to be maintained.[34] However, patient numbers with TPMT in the intermediate range were small, and toxicity was not a primary outcome measure of this study. Two studies in patients with autoimmune bullous disorders have addressed the issue of TPMT activity and dosing. A retrospective study ($n = 35$) showed complete remission with no leucopenia in two intermediate-range patients (azathioprine mean dose 1·7 mg kg^{-1} daily).[62] In a prospective study ($n = 27$), patients with normal TPMT activity received azathioprine up to 250 mg per day and intermediate-range patients received 25–75 mg per day, with no myelotoxicity occurring over a median of 13 months.[63] For suggestions on TPMT-based azathioprine dosing based on these and other studies see section 10.1 and Table 2.

Recommendations: TPMT and azathioprine toxicity

- There is strong evidence that baseline testing predicts severe neutropenia in patients with absent TPMT activity (*Strength of recommendation A; level of evidence 1+*)
- There is good evidence that intermediate TPMT activity is associated with myelotoxicity in patients receiving conventional azathioprine doses (*Strength of recommendation B; level of evidence 2++*)
- TPMT testing only identifies a proportion of individuals at increased risk of haematological toxicity, hence the continued need for regular monitoring of blood counts irrespective of TPMT status (*Strength of recommendation B; level of evidence 2++*)
- TPMT screening should not be declined by healthcare providers on the basis of cost-effectiveness (see section 12.4) (*Strength of recommendation B; level of evidence 2++*)

8.22 Thiopurine methyltransferase, thioguanine nucleotides and azathioprine efficacy

The link between low TPMT levels and increased risk of myelotoxicity was elucidated in patients with acute childhood lymphoblastic leukaemia receiving 6-MP.[9] With this came the parallel understanding that the concomitant high levels of TGNs (see Fig. 1) were also associated with better survival.[10]

Table 2 Suggested thiopurine methyltransferase (TPMT)-based maintenance doses for dermatological conditions

TPMT range	Azathioprine maintenance dose (mg kg^{-1} daily)
Absent	In general unsuitable for azathioprine
Intermediate	1·0–1·5
Normal	2·0–3·0

Since then the relationship between efficacy and these cellular factors has been investigated in inflammatory conditions treated with lower doses of thiopurines. Retrospective studies in IBD have confirmed that pretreatment measurement of TPMT might predict clinical response to azathioprine.[51,64] In a prospective study of 207 patients with IBD, intermediate-range TPMT activity was associated with a greater chance of clinical response compared with higher enzyme activity.[65]

Many studies in IBD[66–68] and after renal transplantation[69] have now correlated TGN levels with efficacy. For example, in a prospective study of 92 paediatric patients with IBD, TGN levels > 235 pmol per 8×10^8 red blood cells (RBCs) were highly correlated with a positive therapeutic response.[67] Although other studies[70] (a number retrospective) have failed to demonstrate a relationship, a meta-analysis of 12 studies on IBD provides evidence that higher TGN levels (> 230–260 pmol per 8×10^8 RBCs) are associated with increased efficacy (remission).[71] Consequently, a therapeutic range for TGNs in IBD of 235–450 pmol per 8×10^8 RBCs is now widely cited, although there is less evidence to support the recommended 'toxic' upper limit.[72]

Unfortunately, studies measuring TGNs in dermatological disease are limited; one report showed the average TGN level associated with clinical response in immunobullous disease to be 179 pmol per 8×10^8 RBCs.[63] Although conclusions should be drawn from a single study with caution, this suggests that the therapeutic threshold for TGNs, at least for immunobullous disease, might be a little lower than IBD.

9.0 Safe use of azathioprine: review of the evidence

Although azathioprine is effective in many inflammatory dermatological diseases, side-effects are common and can restrict use of the drug. An understanding of the potential toxic effects is important both for safe usage and to maximize efficacy. Side-effects can be split into dose-dependent, nonallergic and idiosyncratic dose-independent, presumed allergic. The majority of adverse events cannot be explained by variations in TPMT activity or thiopurine metabolite patterns. It is helpful to view side-effects broadly as those occurring in the short, medium and long term. When starting patients on azathioprine the emphasis during initial consultations should be on vigilance for potential early toxicity. Minor adverse effects are relatively common, and either resolve spontaneously or respond to simple measures such as dose adjustment. Patients should be prepared for this eventuality in order to maximize compliance.

9.1 Short-term toxicity

9.11 Nausea

The most frequently observed adverse effect of azathioprine is isolated, dose-dependent nausea. Patients with true azathioprine hypersensitivity also exhibit nausea as part of a wider symptom complex, but management of these patients is different and is described separately. Nausea early in the course of azathioprine treatment is common and usually resolves after a few weeks without any alteration of dose. This tendency is reflected in the empirical approach of gradual dose escalation which has been practised by prescribers for years. There are insufficient data to allow exact guidelines to be formulated for this process. However, two recent RCTs of azathioprine for atopic eczema differed in their use of fixed dosing[33] ($2{\cdot}5$ mg kg^{-1}) vs. dose escalation (e.g. $2{\cdot}0$ mg kg^{-1} for 4 weeks increasing to $2{\cdot}5$ mg kg^{-1} in patients with normal-range TPMT activity).[34] There was a 20% difference in dropout rate between the studies due to toxicity and nonadherence; greater efficacy overall was demonstrated in the group receiving a lower initial dose. However, even with dose escalation, one-quarter of patients had nausea which limited maximum achievable dose or resulted in treatment withdrawal.[34] Several commonly used approaches to reduce nausea in this situation include taking azathioprine with or after food, splitting the daily dose, and co-prescription of antiemetics. If these strategies fail, then there is also evidence that switching to 6-MP can reduce gastrointestinal side-effects.[73]

Recommendations: managing nausea

(Strength of recommendation D; level of evidence 4)

- Early, mild nausea is a common and often self-limiting side-effect of azathioprine
- Gradual dose escalation may be useful in minimizing initial nausea
- Moderate nausea can be managed by
  - Using divided daily doses
  - Taking azathioprine after food
  - Temporary dose reduction
  - Antiemetics
- Nausea associated with other symptoms such as fever, myalgia or arthralgia suggests hypersensitivity and should be managed differently (see section 9.12)

9.12 Hypersensitivity

Azathioprine hypersensitivity is an idiosyncratic, immunologically mediated reaction that presents with a distinct symptom complex within weeks of starting the drug. It is probably underdiagnosed, as symptoms are easily confused with infection or underlying disease.[74] Hypersensitivity is a potentially serious adverse event, although fatality appears to be rare.[75] Reports are confined to retrospective case series and consequently the incidence is unknown. However, in a prospective series of 79 patients with atopic eczema treated with azathioprine, five developed symptoms suggestive of hypersensitivity.[76] It was speculated that the abnormal immunity in these patients may increase the likelihood of drug hypersensitivity over nonatopic individuals.[34]

Hypersensitivity can manifest with generalized or organ-specific symptoms. Fever, myalgia, arthralgia and nausea are common features; more rarely hepatitis, interstitial nephritis[77] or renal failure[78] are seen. In severe cases hypotension and shock can occur.[79] Rash, usually maculopapular, has been described, but it is possible that some reported eruptions, such as erythema nodosum,[80] relate to the underlying condition rather than the hypersensitivity reaction. Pneumonitis has been reported infrequently, mainly in renal transplant patients and patients with IBD.[81] Azathioprine-induced pancreatitis is also rare[82] and appears to be restricted to patients with Crohn disease[83] (see section 10.83).

Confirming hypersensitivity with rechallenge can produce more severe symptoms, especially with conventional azathioprine doses; extreme caution is therefore recommended when considering this approach,[84] with the use of the smallest possible azathioprine dose. If symptoms of hypersensitivity were severe, then rechallenge in a hospital setting with access to resuscitation facilities is advised. In up to 60% of azathioprine-hypersensitive patients 6-MP may be a safe alternative,[85–88] suggesting that in these individuals immunological sensitivity is directed against the imidazole rather than the thiopurine moiety of the azathioprine molecule.

9.2 Medium-term toxicity

9.21 Myelotoxicity

Bone marrow suppression, usually manifested as neutropenia, is a potentially serious and not uncommon dose-dependent side-effect of azathioprine. Detailed analysis of early trials on azathioprine (which included rates of haematological adverse events) have previously been collated and summarized.[89] The range of azathioprine-induced neutropenia in these 10 studies was 5–30% with a mean of 19%. For a review of the relationship between myelotoxicity and TPMT pharmacogenetics, see section 8.21.

9.22 Susceptibility to infection

It is possible that azathioprine may increase susceptibility to infection even in the absence of neutropenia, although evidence for this is limited. Mild lymphopenia is quite commonly seen in patients receiving thiopurines[90] and this may be a relevant factor. Organ transplant recipients receiving azathioprine in conjunction with other immunosuppressants do have an increased risk of infections, presumably due to the degree of immunosuppression achieved. Varicella zoster virus (VZV) infections have been shown to occur more commonly in patients with IBD receiving azathioprine.[91] VZV infection (chicken pox/shingles) is usually a benign and self-limiting

disease, but patients taking immunosuppressant medication are susceptible to more severe disease and its complications[92] (see Recommendations for management of VZV in this group). However, an increase in infections in general has not been demonstrated in cohorts of patients with IBD[93] or atopic eczema[34] receiving azathioprine monotherapy. Nevertheless, this remains a theoretical risk and careful selection of patients is required prior to starting azathioprine; reactivation of latent infections such as tuberculosis has been reported.[94]

Recommendations: managing VZV in patients receiving azathioprine[92]

(*Strength of recommendation D; level of evidence 4*)

- Consider temporary withdrawal of azathioprine
- Prompt use of oral antivirals (aciclovir, valaciclovir or famciclovir) in all patients
- Intravenous antiviral therapy desirable for disseminated or ophthalmic VZV

9.23 Hepatotoxicity

Mild derangement of liver blood tests due to azathioprine is not uncommon and usually has no serious clinical implications. In contrast, severe hepatotoxicity is rare. Liver injury occurs in two patterns: (i) acute idiosyncratic drug-induced liver injury (DILI) and (ii) nodular regenerative hyperplasia. The former may either be cholestatic (bilirubin and alkaline phosphatase disproportionately raised compared with transaminases) or hepatocellular (transaminases raised disproportionately). Previous classifications of DILI as either hypersensitivity or dose-dependent are now not considered helpful [G. Aithal (University Hospitals NHS trust, Nottingham, U.K.), personal communication].

Nodular regenerative hyperplasia seems to be exclusive to patients with IBD and organ transplant recipients, and can occur after several years of azathioprine therapy. In contrast, other forms of thiopurine-induced liver injury occur most commonly during the first few months of therapy and usually resolve completely on azathioprine withdrawal.[95] In comparison with hepatocellular DILI, cholestatic injury takes longer to resolve after stopping azathioprine and in some cases has progressed despite drug withdrawal.[95] For both forms of DILI, there is no incidence data specific to dermatological conditions in azathioprine-treated patients, although useful inferences can be drawn from a recent systematic review of hepatotoxicity in patients with IBD. The study included 2992 patients and demonstrated a mean annual DILI rate (abnormal liver blood tests per patient-year) of 1·4%.[95] However, patients with inflammatory skin disease and patients with IBD may have different susceptibilities to azathioprine-induced liver damage.

In the relatively common situation of mild derangement of liver blood tests, values often return to normal without alteration of dose or drug withdrawal, a phenomenon termed adaption. The following approaches should be used if abnormalities persist or worsen:[95] when initial abnormalities are not transient or are marked (a precise cut-off point has not been determined, but a guide for transaminases would be greater than twice the upper limit of normal), an initial dose reduction of 50% is recommended;[95] if values normalize, the initial dose may cautiously be prescribed again with more frequent monitoring of liver blood tests thereafter.[95] This approach was used in the only prospective study of thiopurine hepatotoxicity to date; almost half the patients were subsequently able to continue on the full dose.[96]

In contrast to the increased likelihood of myelotoxicity with low TPMT activity, several studies suggest that TPMT activity in the high normal range may confer an increased risk of liver damage from thiopurine drugs, probably due to elevated levels of methylated thiopurine metabolites [predominantly methylmercaptopurine riboside (MeMPR), see Fig. 1].[97,98] In a study of 173 patients with IBD treated with azathioprine or 6-MP, 4·6% developed hepatotoxicity; mean MeMPR levels were significantly higher in these patients compared to those with no adverse effects.[99] However, 90% of patients with high MeMPR levels above the third quartile had no hepatotoxicity, while 40% of patients with hepatotoxicity had normal MeMPR levels below this cut-off. Therefore, with such poor sensitivity and specificity the measurement of MeMPR is neither superior to, nor should it replace, the routine monitoring of liver blood tests to screen for azathioprine-induced hepatotoxicity.

Recommendations: managing hepatotoxicity

(*Strength of recommendation B; level of evidence 2++*)

- Mild derangement of liver blood tests is not uncommon and may not require alteration of therapy
- Various patterns of serious liver injury can more rarely be seen at any stage of azathioprine therapy
- Detection of any abnormal liver blood tests should prompt both careful evaluation and increased frequency of repeat testing; dose reduction or drug withdrawal may be needed

9.3 Long-term toxicity

9.31 Carcinogenesis

Background

One paradox of the action of thiopurine drugs is their efficacy against some malignancies such as acute lymphoblastic leukaemia, but carcinogenicity in other situations. However, with the possible exception of skin cancer, it is unlikely that azathioprine, when used within certain constraints for dermatological diseases, results in any measurable or clinically important increase in risk of developing malignancy. This area is both controversial and complex and requires careful discussion with patients (see section 10.82).

Before prescribing any immunosuppressant drug, it is worth considering that the past few years have seen a revolution in the treatment of some inflammatory skin diseases. There has

been a vast increase in the use of novel immunosuppressants with unknown long-term safety profiles. These drugs are now superseding more traditional therapies such as thiopurines. In contrast, epidemiological data on the long-term toxicity of thiopurines are available from several medical disciplines. This safety record should be borne in mind and be part of the general discussion with a patient when choosing an immunosuppressant therapy.

Ultraviolet radiation and skin cancer

It is widely recognized that the risk of developing nonmelanoma skin cancer (NMSC) is increased by the long-term administration of azathioprine to solid-organ transplant recipients. The co-prescription of several immunosuppressants in this situation is likely to be a major contributor to this risk,[100,101] which may be elevated more than 200-fold.[102] There is also evidence of skin cancer risk in thiopurine-treated patients with IBD.[103] Recently, a nested case–control study[104] of 742 cases of NMSC and 2968 matched controls (both groups of IBD patients) showed there to be a significant association with new NMSC and thiopurine use for longer than 1 year (adjusted OR 4·3; 95% CI 3·1–6·0). The use of antitumour necrosis factor (TNF) agents, but not MTX, mycophenolate or ciclosporin, was also significantly associated with NMSC development. These results implicate thiopurines above other immunosuppressants in the development of NMSC in the IBD population. Although there are no studies addressing this issue for inflammatory skin disease, the results from the IBD study clearly have important implications for dermatology patients receiving azathioprine for more than 1 year.

Considerable progress has recently been made in determining the mechanism of photocarcinogenesis by the combination of thiopurine drugs and ultraviolet (UV) radiation. UVA wavelengths (320–400 nm), which account for 95% of solar UV radiation, are poorly absorbed by purines in DNA and are normally considered to be less harmful than UVB. However, 6-TG has a maximum absorbance at 340 nm. Absorption of UVA photons by 6-TG-substituted DNA generates reactive oxygen species which cause lethal and mutagenic DNA damage and may then permit the development of NMSCs.[105,106] Consistent with this, azathioprine administration has been shown to confer increased UVA sensitivity in normal skin, demonstrated by reduced minimal erythema doses to UVA.[107]

Taken together, both epidemiological and laboratory data suggest that UV exposure is an important carcinogenic hazard for thiopurine-treated patients. This has major implications for dermatology patients receiving long-term azathioprine therapy and the need for education about rigorous photoprotection is highly important.

One group of thiopurine-treated patients that deserves special consideration is organ transplant recipients who may have already developed multiple dysplastic keratoses and NMSCs. This group poses a particular therapeutic challenge; they should ideally be examined regularly in dedicated dermatology clinics by clinicians with an interest in skin cancer and all patients should be educated to report any skin lesions that develop in intervening periods. For new transplant patients, management should be proactive with education about sun protection beginning at the time of (or even before) transplantation.[108] For those transplant patients who continue to develop NMSCs, there is some evidence to suggest that switching from azathioprine to drugs with a lower theoretical risk of photocarcinogenesis, such as mycophenolate mofetil or sirolimus, may result in a reduced frequency of cancer and precancerous keratoses.[109]

Lymphoma risk

Prolonged use of azathioprine in combination with other immunosuppressants in solid-organ transplant recipients increases the risk of developing several malignancies, with non-Hodgkin's lymphoma occurring second only in frequency to NMSC.[110] Much of the risk may be attributable to the intensity of immunosuppression rather than azathioprine *per se*, and oncogenic viruses such as Epstein–Barr virus are thought to be a major factor.[110,111] Most malignancies occur early, usually in the first year after transplant. This chronology contrasts with UV-related NMSC development, which mainly develops after approximately 10 years of immunosuppression,[102] suggesting that there are different mechanisms for internal and cutaneous carcinogenesis.

Whether there is also a risk of internal malignancy, in particular lymphoma, in nontransplant patients treated with azathioprine monotherapy is controversial. There are no useful data for dermatology patients, but there is important literature from the IBD population. Two meta-analyses have addressed the issue and the conclusions are conflicting. Kandiel *et al.*[112] concluded that there was an approximately fourfold greater risk of developing lymphoma with long-term thiopurine therapy (3891 patients). This would translate to one additional lymphoma for every 300–4500 years of treatment, depending on the age of the patient. However, there have been criticisms of the method used in this meta-analysis,[113] and whether the calculated risk was due to treatment or the underlying disease was not convincingly demonstrated.[111] Masunaga *et al.*,[114] in a subsequent meta-analysis failed to find any increased risk of malignancy (4039 patients). Unlike the study by Kandiel *et al.*, which used data obtained from the general population as a control, Masanuga *et al.* used a control population of patients with IBD who had not received immunosuppression. Since then, a large prospective cohort study by Beaugerie *et al.*[115] in 19 486 patients with IBD has shown a significant association between thiopurine use and the incidence of lymphoma (hazard ratio 5·3; 95% CI 2·0–14). However, the authors acknowledge that the excess risk may also relate to the underlying IBD activity. The gut was affected in six of the 23 patients who developed a lymphoproliferative disorder, often in intestinal segments affected by IBD; this might also suggest that disease activity was an important factor in carcinogenesis. Most recently, in a retrospective cohort of 17 834 patients with IBD no overall increased risk of lymphoma was found.[116] Although

this study, unlike that by Beaugerie *et al.*, was not designed to investigate risk with azathioprine/6-MP, it is interesting to note that 11 out of 12 patients who developed Epstein–Barr-positive lymphoma had used thiopurines, compared with four of 21 patients with Epstein–Barr-negative lymphoma.[116]

Given that the results of these studies are contradictory, and that IBD itself may confer a risk of malignancy, it is difficult to know what advice to give regarding lymphoma risk to patients with dermatoses that may require prolonged azathioprine therapy. However, if there is an increased long-term risk, this would appear to be low in absolute terms. Furthermore, studies of azathioprine use in the short-to-medium term do not appear to show an excess of internal cancers.[117,118] Taken together these data suggest the best approach would be to restrict courses of azathioprine to the short-to-medium term. Those patients requiring long-term treatment who have no other therapeutic alternatives should be counselled about the possible malignancy risk but advised that this, if increased, is likely to be small.

10.0 How and when should azathioprine be prescribed?

10.1 Dosing

The Summary of Product Characteristics (SPC) for azathioprine recommends a starting dose of 1–3 mg kg^{-1} daily (with larger doses recommended in transplantation).[119] The dose should be adjusted within these limits depending on response and haematological tolerance, with subsequent reduction for maintenance therapy following clinical response. The therapeutic effects of azathioprine often take several months to become apparent after initiation of therapy, and similarly the effects of dose reduction or cessation of therapy may also be delayed, possibly due to persistence of active drug metabolites. Doses at the lower end of the range are recommended in patients with renal and/or hepatic impairment or in the elderly (see section 10.51, 10.52 and 10.65).

The doses required for skin diseases largely conform to the general recommendations of the SPC. Apart from studies relating dose to TPMT status (see below), there have been no recommendations on dosimetry related to either the indication for use or whether monotherapy or combination with oral corticosteroids is needed. A strategy for dose reduction for patients in remission has not been addressed in studies of dermatological diseases, and remains a matter for empirical titration by the clinician.

Although it is clear that patients with absent TPMT activity (TPMT null) should in general not receive azathioprine (or 6-MP), in rare circumstances a greatly reduced dose may be used (approximately 5–10% of standard), with very careful monitoring of the full blood count (FBC) and metabolites,[11,120] but even then patients may develop leucopenia.[121]

Table 2 shows suggested TPMT-based maintenance dose ranges for the treatment of dermatological conditions (see section 8.21). The use of lower initial doses (e.g. for the first 4 weeks of therapy) is also recommended in order to minimize early side-effects such as nausea (see section 9.11). Table 2 is adapted from Snow and Gibson,[60] Meggitt and Reynolds,[61] Meggitt *et al.*,[34] Gardiner *et al.*[122] and Bezier *et al.*,[62] and reflects the range of doses successfully used in studies comparing patients with heterozygous vs. homozygous wild-type TPMT phenotype. It should be emphasized that collectively the number of heterozygous range patients in these reports was small. Consequently, these recommendations should serve as a guide for current use, in anticipation of information from future studies with larger patient numbers.

Recommendations: azathioprine dosing

- Patients with normal TPMT activity are at low risk of profound neutropenia and can be prescribed azathioprine at conventional doses (see Table 2) (*Strength of recommendation A; level of evidence* 1+)
- Patients with intermediate (heterozygous) range TPMT activity treated with conventional thiopurine doses have an increased risk of neutropenia and should receive a lower azathioprine maintenance dose (see Table 2 for suggested dose regimen) (*Strength of recommendation C; level of evidence* 2+)
- Patients with absent TPMT activity (TPMT null) treated with conventional azathioprine doses are at very high risk of profound neutropenia and should in general not be prescribed azathioprine (*Strength of recommendation A; level of evidence* 1+)
- Side-effects such as dose-dependent nausea may be minimized by building up to the recommended maintenance dose over the first few weeks of therapy (*Strength of recommendation D; level of evidence* 4)

10.2 Contraindications

There are few absolute contraindications to the use of azathioprine, but those listed in the manufacturer's data sheet[119] are: hypersensitivity to azathioprine/6-MP; severe infections; severely impaired hepatic or bone marrow function; pancreatitis; live vaccines (section 10.71); pregnancy unless benefits outweigh risks (section 10.62); and lactation (section 10.63).

In hypersensitive patients, desensitization to azathioprine and 6-MP has been successfully attempted,[123] but this cannot be recommended as its safety is unproven. Pregnancy is a relative contraindication (see section 10.62) and women taking azathioprine are advised to not breastfeed their infants, although more recent data suggest this may be safe (see section 10.63). It is not usually recommended that azathioprine is initiated or continued in patients with known malignancy, as immunosuppression may increase the risk of disease progression.

There are also several relative contraindications to azathioprine use that are not included in the SPC. Discussion of the following issues is covered in subsequent sections of these guidelines: (i) renal impairment (section 10.51); (ii) viral hepatitis (section 10.53); (iii) human immunodeficiency virus (HIV) infection (section 10.54); (iv) previous varicella zoster

virus exposure (section 10.55); (v) premalignancy (section 10.56).

10.3 Baseline blood tests

FBC with differential white cell count, renal function and liver blood tests including transaminases (alanine aminotransferase or aspartate aminotransferase) should be determined as a baseline.

10.4 Baseline thiopurine methyltransferase activity

A growing body of evidence supports the assessment of TPMT activity prior to starting azathioprine. U.K. dermatologists have played a leading role in advocating this test as good clinical practice, but its uptake has varied among other disciplines and countries. A U.K. survey in 2006 found that although nearly all dermatologists, gastroenterologists and rheumatologists prescribe azathioprine, the respective rates of TPMT testing were 90%, 60% and 47%.[124] A similar survey among gastroenterologists in three states of the U.S.A. found that although only 35% reported measuring TPMT levels, 46% used adjunctive metabolite (TGN) monitoring to assist management[125] (see section 13.0). It is widely cited that TPMT activity may be induced by thiopurines[58] and that consequently measurement of TPMT in patients currently receiving azathioprine is not advised, as values may be falsely elevated. However, some studies have failed to demonstrate any induction of TPMT by concomitant thiopurine use,[48,126,127] but have shown repeat TPMT measurements in individuals on therapy to be highly variable.

Genotyping or phenotyping techniques can be used to determine TPMT status, but genotyping is not routinely used.[124] TPMT phenotyping is based on RBC enzymatic activity and this can be affected by recent blood transfusion. TPMT activity exhibits a trimodal distribution;[3] this reflects the three different allele combinations. Results are usually reported in three ranges corresponding to the presumed TPMT genotype. (i) Homozygous mutant (*TPMT* null), i.e. two copies of the variant allele (described as absent/very low TPMT activity, depending on the laboratory). (ii) Heterozygous, i.e. one copy of a variant allele (most commonly described as intermediate enzyme activity; this terminology is adhered to throughout these guidelines). It should be noted that some laboratories may describe this range as 'low' TPMT activity. This may result in unnecessary confusion, as clinicians could erroneously assume the patient is in the *TPMT* null, 'absent/very low' TPMT activity group. (iii) Homozygous wild-type, i.e. two functional copies of the active gene (described as high/normal enzyme activity, depending on the reporting laboratory).

Phenotyping is the preferred method of assessment, with genotyping reserved for patients with borderline results and those in whom a blood transfusion has recently been carried out[128] (see section 12.3).

Recommendations: baseline TPMT activity

- TPMT activity should be checked in all patients prior to receiving azathioprine (*Strength of recommendation A; level of evidence* 1+)
- Clinicians should ensure they take into account differences in TPMT activity reporting practices across the U.K., in order to be certain of the likely genotypic group of their patients (*Strength of recommendation D; level of evidence* 4)
- TPMT genotyping is only required for patients with indeterminate phenotype (i.e. borderline values) or those who have had a recent blood transfusion (*Strength of recommendation D; level of evidence* 4)

10.5 Other baseline considerations

10.51 Renal impairment

The British National Formulary (BNF) advises that dose reduction may be needed in renal impairment.[129] However, the manufacturer's data sheet states that controlled studies do not show enhanced toxicity in the presence of renal insufficiency.[119] Nevertheless, it is recommended that the dosages used should be at the lower end of the normal range and that FBC should be carefully monitored. The dosage should be further reduced if haematological toxicity occurs. Azathioprine dose does not need to be altered in those undergoing haemodialysis.[130]

10.52 Hepatic disease

The SPC advises cautious azathioprine administration in patients with hepatic dysfunction, with regular monitoring of blood count and liver blood tests.[119] In such patients, drug metabolism may be impaired, and the azathioprine dosage should therefore be reduced if hepatic or haematological toxicity occurs.

10.53 Viral hepatitis

Approximately one-third of the world's population has serological evidence of past or present infection with hepatitis B virus (HBV).[131] Individuals positive for HBV surface antigen (HBsAg) are at risk of a flare in disease if given immunosuppressant drugs including azathioprine.[132,133] Furthermore, the development of acute liver failure in previously well carriers of HBV is well recognized after withdrawal of immunosuppressive therapy.[132–134] Measurement of transaminases does not reliably detect all infected individuals, as these may be intermittently normal during the immune tolerant phase of infection. Consequently, the European Association for the Study of the Liver (EASL) recommends that all candidates for immunosuppressant treatment should be screened for HBsAg and anti-HBV core antibodies (anti-HBc) prior to the initiation of treatment. Similarly, baseline screening for hepatitis C virus (HCV) should be considered in all patients. EASL also recommend vaccination against HBV in those who are seronegative. There-

fore, this approach should be part of the baseline screening for treatment with azathioprine, and a hepatitis-risk history should also be taken. Prior to initiation of azathioprine, all serologically positive cases should be discussed with the local team experienced in the management of HBV infection (hepatology or infectious diseases), and prophylactic antiviral therapy considered on a case-by-case basis, dependent on factors such as the risk of liver disease and HBV viral load [M. Prince (Manchester Royal Infirmary, Manchester, U.K.), personal communication].

10.54 Human immunodeficiency virus infection

There are no specific recommendations regarding generic screening for HIV prior to commencing azathioprine, but in those with risk factors, baseline HIV status should be established. Treated HIV infection is not necessarily a contraindication to the use of immunosuppressive agents; there is growing experience of the safe concurrent use of azathioprine and other immunosuppressants in HIV-positive organ transplant recipients whose disease is stable and has been treated with highly active antiretroviral therapy for at least 12 months.[135,136] Initiation of azathioprine in patients with HIV infection should only be undertaken after consulting those with special expertise in HIV medicine.

10.55 Previous varicella zoster virus exposure

All patients who may require treatment with azathioprine should be asked whether they have had chickenpox. For those who are uncertain about previous exposure, VZV serology should be checked. For the nonimmune, administration of VZV vaccine should ideally occur several weeks prior to commencement of azathioprine therapy, as the vaccine is live.[137] Importantly, the Department of Health 'Immunisation against infectious diseases – the Green Book'[137] also advises against administration of all live vaccines (including VZV) to patients receiving immunosuppressants such as azathioprine. This U.K. advice contrasts with the guidance from the U.S. Centers for Disease Control and Prevention,[138] which states that VZV vaccine may be given to persons with impaired humoral immunity, including those receiving azathioprine $\leq 3{\cdot}0$ mg kg^{-1} daily. However, a degree of impaired cellular immunity may also occur with azathioprine [G. Spickett (Royal Victoria Infirmary, Newcastle upon Tyne, U.K.), personal communication]. Consequently, these guidelines recommend adhering to the Green Book's advice, but difficult cases would be best discussed individually with an immunologist, particularly as administration of vaccine prior to azathioprine treatment may not always be practically possible, given that 'the disadvantages of delaying immunosuppression are often significant'.[137] In the rare situation when nonimmune individuals receive azathioprine and subsequently have 'significant exposure' to chicken pox or herpes zoster, then protocols defining this exposure and outlining the administration of VZV immunoglobulin and/or prophylactic aciclovir are detailed in the Green Book.[137]

10.56 Premalignancy

Patients receiving immunosuppressant drugs have an increased frequency of cervical intraepithelial neoplasia (CIN), and in these patients the risk of progression to invasive disease is higher and the success rate of treatment is lower.[139] There is some debate whether immunosuppressed patients should be screened more frequently, and in some European centres annual cytology combined with colposcopy is recommended.[139] Data from the renal transplant population (receiving multiple immunosuppressive agents) suggests a fivefold increase in the prevalence of abnormal cervical cytology (15%) above the normal population.[140] However, there is no information on risk in dermatology patients on single immunosuppressant drugs such as azathioprine. Nevertheless, it would be sound practice to ensure that, prior to receiving azathioprine, women have been concordant with the national cervical screening programme, and a pretreatment gynaecological review should be requested in those patients with previous CIN.

Guidelines exist for the management of premalignant skin lesions in immunosuppressed solid-organ transplant recipients. A baseline dermatological examination is recommended in these patients prior to transplantation, and dysplastic keratoses should be treated before (or soon after) starting azathioprine therapy.[108] Similar guidelines do not exist for azathioprine use in other contexts, but given what is now known about the risks of NMSC with prolonged azathioprine monotherapy for IBD,[104] it would be wise to adopt a similar approach in any circumstance when long-term administration of the drug is likely.

10.6 Special groups

10.61 Male fertility

Several studies indicate that male patients receiving azathioprine father healthy children, and that azathioprine at standard doses does not appear to affect male fertility.[141,142]

10.62 Pregnancy

Both azathioprine and 6-MP cross the placenta and the U.S. Food and Drug Administration categorizes azathioprine as risk group D, indicating 'positive evidence of fetal risk is available, but the benefits may outweigh the risk in life-threatening or serious disease'. However, the literature is inconclusive on any teratogenic effects.[143] Most investigators have found azathioprine to be relatively safe in pregnancy and its use in transplant recipients is not associated with any increased risk of congenital defects, although this group is at increased risk of premature birth and small-for-dates babies.[144] One case report has even shown a healthy child born to parents who were both receiving thiopurines.[145] The general conclusion nevertheless is to limit use of azathioprine in pregnancy to those with severe disease, particularly if there is no safer alternative treatment.[143]

10.63 Lactation

The manufacturer's data sheet states that breastfeeding is contraindicated in women receiving azathioprine.[119] However, several studies have shown that the drug and its metabolites are either absent or present in negligible amounts in breast milk.[146,147] Although the World Health Organization (WHO) has previously recommended that the risks of azathioprine to the infant outweigh the benefits of breast milk, a recent review has suggested that the drug may be safe in this scenario.[143]

10.64 Children

A retrospective evaluation of 48 children has reported azathioprine to be a safe and effective treatment for children with atopic eczema when high-risk patients were excluded by TPMT measurement.[59] Higher doses than those used in adults were often required (2·5–3·5 mg kg^{-1}). Similarly, a study of children with IBD aged 6 years and under showed that higher dosages (> 3 mg kg^{-1} daily) were needed in order to achieve clinical remission.[148] The authors postulated that the underlying reason was decreased drug absorption.

In view of the concerns surrounding prolonged use of azathioprine and risk of malignancy, careful consideration should be exercised if long-term use of the drug is needed in children. In particular, as it is now known that the risk of photocarcinogenesis escalates with increasing duration of thiopurine treatment (see section 9.31), then advice on photoprotection is essential, and adherence to this should be assessed at follow-up visits.

10.65 Elderly

Care should be taken with use of azathioprine in the elderly; the SPC recommends that additional care should be taken with haematological monitoring and that doses used should be at the lower end of the recommended range.[119] Increasing age is associated with an increased risk of drug interactions due to polypharmacy and, in the solid-organ transplant population, increased vulnerability to immunosuppression-related infections.[149] A recent prospective study of azathioprine monotherapy for inflammatory diseases has also shown the elderly to have a significant higher incidence of all categories of side-effects.[4]

10.7 Drug interactions

A few drugs have important interactions with azathioprine and these are summarized in Table 3. There is also a large category of drugs which may interact but the evidence for this is less strong. This group encompasses drugs with the theoretical potential for interactions but with little or no supporting clinical data, or case reports of queried drug interactions in limited numbers of patients. Unfortunately, these categories are often described collectively and indiscriminately, with the result that some of the less definite associations are subsequently cited in a manner that suggests clinically important interactions occur (including in the SPC and BNF).

10.71 Definite interactions

Allopurinol and febuxostat

The most potentially serious azathioprine drug interaction occurs with xanthine oxidase inhibitors (data only available for allopurinol but in theory febuxostat should have a similar effect). Combined use carries a substantially increased risk of myelotoxicity.[150,151] However, co-prescription of these drugs with dose reduction and under strict monitoring may improve efficacy in the event of nonresponse to azathioprine alone. This issue is covered in detail in Section 13.4. Such an approach should be considered experimental at present, as there is insufficient evidence of safety or efficacy to advocate its use in dermatology patients.

Table 3 What drugs can interact with azathioprine?[a]

Drug	Risks/interactions
Allopurinol and febuxostat	Risk of severe, life-threatening myelotoxicity
Immunosuppressant drugs	Combination with other drugs such as cyclophosphamide, methotrexate and ciclosporin increases the risk of myelotoxicity
Drugs that can cause haematological ADRs	Caution is advised when considering concomitant use with drugs such as co-trimoxazole, trimethoprim and clozapine
Warfarin	Warfarin resistance is reported and warfarin dose may need to be increased. Close monitoring of anticoagulation is advised
Ribavirin	Severe pancytopenia has been reported. This drug inhibits IMPD, an enzyme in the purine salvage pathway
Live vaccines	Should not be prescribed to immunocompromised individuals
Aminosalicylates	Inhibit TPMT *in vitro* but the clinical importance of this is unknown. The drugs are often co-prescribed for IBD and increased monitoring of FBC is advised

[a]Section 10.7 provides a full discussion of these drugs and also considers possible interactions which are listed in other publications but are based on weak evidence only. These include succinylcholine, tubocurarine, furosemide, bendroflumethiazide, nonsteroidal anti-inflammatory drugs and angiotensin-converting enzyme inhibitors. ADR, adverse drug reaction; FBC, full blood count; IBD, inflammatory bowel disease; IMPD, inosine monophosphate dehydrogenase; TPMT, thiopurine methyltransferase.

Warfarin

A series of case reports have documented that azathioprine and 6-MP are associated with warfarin resistance.[152,153] The onset is rapid. A dose-dependent increase in warfarin therapy (at least 2·5-fold) and careful monitoring of coagulation is therefore required when these drugs are co-administered. The exact mechanism of the interaction is unknown.

Ribavirin

The antiviral drug ribavirin is an inhibitor of inosine monophosphate dehydrogenase (IMPD). Severe pancytopenia has been reported in a series of patients treated with azathioprine and ribavirin,[154,155] with bone marrow suppression reaching a nadir between 3 and 6 weeks after initiation of ribavirin for treatment of HCV.[154]

Other immunosuppressant and myelotoxic drugs

The risk of haematological adverse reactions is increased when azathioprine is used with any drug which has a potential myelotoxic effect. This includes both immunosuppressants such as cyclophosphamide, MTX, ciclosporin, anti-TNF agents and other biologics, and drugs which can cause unintended myelotoxicity. The list of these latter drugs is large; important examples are co-trimoxazole, trimethoprim and clozapine. Caution must be exercised with co-prescription of azathioprine and any of these drugs, and more frequent monitoring of the FBC is advised.

Vaccines

Severe complications may result from the administration of live-attenuated vaccines to immunocompromised individuals and such vaccines are therefore contraindicated in patients receiving azathioprine. Live vaccines available in the U.K. include bacillus Calmette–Guérin (BCG), varicella (see section 10.55), measles, mumps, rubella, yellow fever, oral polio and oral typhoid. Inactivated vaccines are safe in immunocompromised patients. All azathioprine-treated individuals should receive pneumococcal vaccine and yearly influenza vaccination. Studies in transplant recipients receiving azathioprine have shown this group to mount a similar antibody response to healthy controls.[156] Further information is available in the Department of Health 'Immunisation against infectious disease – the Green Book'.[137]

10.72 Possible interactions

Aminosalicylates

Aminosalicylates cause *in vitro* inhibition of TPMT but whether this translates to increased incidence of neutropenia in the clinical setting remains unclear. Gastroenterologists continue to co-prescribe these drugs and more cautious dosing and increased monitoring is advised.[157]

Other drugs (interactions unlikely)

The manufacturer's datasheet suggests that azathioprine may alter neuromuscular blockade by succinylcholine and tubocurarine, and advises that patients should inform their anaesthetist prior to receiving a general anaesthetic.[119] However, this is based on studies performed with supratherapeutic doses of azathioprine in animals[158,159] and there is no evidence to suggest a clinically important interaction in humans.

Furosemide,[160] bendroflumethiazide[161] and some non-steroidal anti-inflammatory drugs[162] have been found to cause a degree of inhibition of TPMT *ex vivo*, but these reports are of unclear clinical relevance. Anaemia was reported with azathioprine and angiotensin-converting enzyme inhibitors[163] but in a follow-up paper the same authors concluded that an interaction was not likely,[164] and no further reports have emerged since (Table 3).

10.8 Patient counselling

These guidelines attempt to establish an explicit link between evidence and recommendations for clinical usage. However, this may be difficult as decisions in clinical medicine are made in relation to single patients in a setting that may not relate to the context from which the guideline recommendation has been made. Nevertheless, the following general principles for azathioprine prescribing are in widespread usage by dermatologists and are generally accepted as good clinical practice.

10.81 Pretreatment discussion

Prior to prescribing azathioprine, dermatologists and nurses routinely discuss the drug with their patients, who are reliant on this guidance as their main source of information. It is therefore crucial that they are provided with a complete and balanced overview of the advantages and disadvantages of treatment. The prescriber should bear in mind that the prospect of commencing a long-term treatment which carries potential health risks may be an unnerving experience for any individual. It is essential, therefore, that the patient is both fully informed and takes part in the decision-making process. Initial discussion typically involves a broad-brush approach which attempts to give the patient some basic information about the immune system, provide a rationale for the use of azathioprine and place the drug in the context of other therapeutic options. Alternative immunosuppressant drugs which may be prescribed at a later date in the event of a poor therapeutic response or side-effects with azathioprine should also be discussed. The dermatologist must not overdramatize starting such treatment for fear of causing unnecessary alarm. Thus, a delicate line is adhered to that seeks to inform without causing anxiety.

Finally, as the issues discussed are novel and complex for most patients, it is essential to provide a patient information leaflet (PIL) after the initial discussion; this should be documented in the patient's medical notes. Written information

must describe all aspects of azathioprine treatment in clear and unambiguous lay terms. Often, the patient will not remember details of the discussion and a PIL will allow information to be considered and discussed with family if necessary. Decision-making may require careful thought over a period of time, sometimes with more than one consultation to discuss the pros and cons with the dermatologist. The need for a period of consideration is aided by the fact that the onset of a therapeutic response with azathioprine is usually slow, and it is seldom necessary to prescribe this drug in haste. This permits prescribing characterized by shared patient–physician decision-making that is well informed and thoughtful.

Before starting azathioprine, patients are typically focused on trying to gauge the potential risks and benefits of this drug. A detailed knowledge of the hazards of azathioprine treatment (see section 9.0) allows the dermatologist to discuss these risks and to set them in context. Ultimately, it is often hard for patients to decide on the best course of action; the well-informed dermatologist with excellent communication skills and sufficient time to cover all angles is a precious resource to patients when faced with this difficult choice.

10.82 Discussion of cancer risk with patients

The issue of carcinogenesis and thiopurine administration is considered in detail in section 9.31. This topic is often difficult to discuss with patients without causing undue concern; many will have chronic, severe inflammatory diseases that need immunosuppressive therapy in order to improve quality of life: in the majority these benefits will far outweigh the small risk of cancer. The clinician must then tread a fine line between assessing the need for azathioprine treatment and providing a balanced appraisal of cancer risk. Otherwise, this could deter some patients with debilitating conditions from having an effective and safe treatment. There are two important areas that require discussion: first the risk of photocarcinogenesis (section 9.31), and second the evidence relating to internal malignancy risk (section 9.31).

10.83 Withdrawal of previous advice (pancreatitis)

The previous version of this guideline advised that clinicians should warn patients about the risk of developing pancreatitis with azathioprine, but there is now evidence that there is negligible risk in dermatology patients, and consequently there is no obligation for dermatologists to discuss this extremely rare side-effect with patients (see section 9.12).

Clinic checklist for use prior to prescribing azathioprine

1 Explain that the onset of therapeutic benefit with azathioprine is slow and may not be apparent for 2–3 months. Patient expectations need to be realistic
2 Emphasize the need for toxicity monitoring with regular blood tests. Patients unable to comply should not be given the drug
3 Explain if usage is for a licensed or unlicensed indication. For unlicensed indications give a clear explanation of prescribing precedent
4 Advise patients to seek urgent medical attention if they develop signs or symptoms of azathioprine hypersensitivity, bone marrow suppression or liver impairment. Specifically warn patient about:
   (a) High fever/severe flu-like illness
   (b) Unexplained bruising
   (c) New-onset jaundice
5 Ensure there are no contraindications to azathioprine use (section 10.2)
6 Check results of baseline investigations (section 10.3):
   (a) FBC
   (b) Urea and electrolytes
   (c) Liver blood tests
   (d) TPMT activity (rarely also genotype – sections 10.4 and 12.3)
   (e) Hepatitis B and C serology (section 10.53)
   (f) HIV serology, especially in high-risk groups (section 10.54)
   (g) VZV serology (if no history of varicella)
7 Give special consideration to the following:
   (a) Children and the elderly (sections 10.64 and 10.65)
   (b) Hepatic and renal impairment (sections 10.51 and 10.52)
   (c) Premalignancy, i.e. CIN and actinic keratoses (section 10.56)
   (d) Breastfeeding (section 10.63)
   (e) VZV nonimmune: immunization required (section 10.71)
   (f) HBV nonimmune: consider immunization in at-risk groups (section 10.53)
   (g) Positive HIV serology (section 10.54)
8 Advise on the need for pneumococcal vaccine and a yearly influenza vaccination (section 10.71)
9 Discuss the possible increased risk of malignancy with long-term use (section 9.31)
10 Give advice on sunscreens and sun avoidance
11 Caution regarding avoidance of pregnancy (section 10.62)
12 Warn about potential drug interactions (also detailed in the PIL)
13 When possible, formulate a plan for duration and eventual withdrawal of therapy
14 Supply with a PIL (if not previously) and record provision in case notes

11.0 How should azathioprine treatment be monitored?

11.1 Follow-up visits

At each visit, the dermatologist should seek to assess efficacy of treatment and other possible treatment-related problems. Patients should be asked how they feel the drug is working, and if adverse events are reported it is helpful to know if these are sufficiently compensated for by a positive therapeutic response. The dermatologist should enquire about the patient's general health and other possible comorbidities that might be relevant to azathioprine usage. Each appointment also presents

an opportunity for azathioprine dose adjustment depending on adverse events and efficacy. Patients also appreciate discussion of their blood test results; even if these are normal, being kept up to date provides valuable reassurance.

Patients should be reminded at each follow-up appointment of what to look out for in terms of possible side-effects. They should also be encouraged to keep the PIL accessible and to occasionally re-read this. This is particularly important for stable patients receiving long-term treatment, as this group may be more likely to forget key information. Finally, the dermatologist should repeat advice about photoprotection, emphasizing the importance of compliance during the summer months and while on sunny holidays.

11.2 Toxicity monitoring

This is the responsibility of the dermatologist prescribing azathioprine. With mutual agreement, responsibility is sometimes shared with the patient's general practitioner according to protocols agreed locally.

11.21 Frequency

The BNF suggests that monitoring for azathioprine toxicity should be performed weekly for the first month of azathioprine treatment and monthly thereafter.[129] The azathioprine datasheet recommends the more cautious approach of an initial 8 weeks of weekly monitoring.[119] Once the patient is stable on a fixed dosage, the frequency of monitoring can be reduced to a minimum of at least once every 3 months.

11.22 Monitoring for hepatotoxicity

An optimal monitoring schedule for liver blood tests remains to be determined, but it is clear that they should be checked more frequently during the first few months of treatment, as this is the period during which hepatotoxicity is most likely to occur. However, as some forms of liver injury may develop after several years, regular monitoring for the duration of treatment is essential. If liver test abnormalities persist despite azathioprine withdrawal or dose reduction, it is important to bear in mind alternative potential causes of hepatitis, particularly those related to immunosuppression such as Epstein–Barr virus infection.

11.23 Monitoring for myelosuppression

Regular checking of FBC is essential during long-term treatment with azathioprine. Macrocytosis is a common finding and can be used to assess patient noncompliance. Leucopenia is the most common haematological adverse event. Other rarer haematological side-effects include anaemia, thrombocytopenia and, rarely, pancytopenia. With the exception of macrocytosis, occurrence of any of these haematological side-effects should be carefully monitored and the dose of azathioprine adjusted accordingly.

Recommendations: toxicity monitoring

(*Strength of recommendation D; level of evidence 4*)

- Regular monitoring of liver blood tests and FBC are required for the duration of therapy
- Once a patient is stable on a fixed dose of azathioprine monitoring should occur at least 3 monthly
- Prior to stabilization, monitoring bloods should be performed more frequently

12.0 Health economics

12.1 Cost of drug

Azathioprine is relatively inexpensive compared with other immunosuppressive drugs used by dermatologists such as ciclosporin and mycophenolate mofetil. The U.K. price for 100 azathioprine 50 mg tablets is currently (BNF 61, 2011) £7·99; thus at 100–200 mg daily, the daily cost of this drug would be £0·32–£0·64. In contrast, the U.K. price for a 30-capsule pack of ciclosporin 100 mg is currently £69·11 (BNF 61, 2011); thus, at 300–500 mg daily the daily cost of this drug would be £6·91–£11·52. Additionally, the U.K. price for a 50-tablet pack of mycophenolate mofetil 500 mg is currently £82·26 (BNF 61, 2011); thus, at 1·5–3 g daily the daily cost of this drug would be £4·94–£9·87. Expressed in relative terms, the daily cost of ciclosporin or mycophenolate mofetil in the doses used in dermatology is up to 20 times as great as the daily cost of azathioprine.[129]

12.2 Cost of thiopurine methyltransferase testing

Thirty years after the initial publication by Weinshilboum and Sladek[3] on the genetics of TPMT inheritance, the measurement of red cell TPMT activity is now a routine test in the U.K.[165] (details of biochemistry laboratories offering TPMT measurement are given in Appendix 2 at the end of this guideline). Due to the high volume of demand, and high throughput in the laboratories, the cost for this assay is so low that there is no longer a credible argument to be made against testing on the basis of cost. Furthermore, the turnaround time (i.e. delay between ordering the test and receiving the result) is stated to be 24 h in the U.K. biochemistry department with the highest throughput of TPMT assays. The current charge for TPMT testing is around £30; this cost may fall with further refinements to testing methodology [J. Berg (City Hospital, Birmingham, U.K.), personal communication]. The current cost of TPMT screening in the U.K. is low compared with other countries, which is partly explained by economies of scale that have resulted from widespread uptake of this assay by prescribing doctors.

12.3 Thiopurine methyltransferase genotyping

Genotyping as a tool to assess risk of bone marrow toxicity is not routinely available and is very unlikely to replace functional assessment of TPMT activity. However, in some laboratories, genotyping in addition to TPMT assay is already routinely performed for patients with borderline/absent TPMT activity in order to clarify toxicity risk. The TPMT enzyme assay is superior to TPMT genotyping in predicting TPMT deficiency.[165,166] To date, 29 different variant *TPMT* alleles have been described:[165] using expression systems, 16 of these alleles have been shown to result in deficient TPMT activity. Of these, three *TPMT* polymorphisms are responsible for 80–95% of deficient TPMT activity.[165] Thus, genotyping for these three *TPMT* polymorphisms could predict deficient TPMT activity in about 90% of patients; importantly, it would miss TPMT deficiency in about 10% of patients. Thus, genotyping has emerged as a supportive and complementary test to the TPMT enzyme assay; when used in this way on selected samples, phenotyping and genotyping overcome the limitations of both tests.[165,166]

12.4 Cost-effectiveness of thiopurine methyltransferase testing

A recent systematic review of the health economics of azathioprine-related TPMT screening identified seven relevant studies.[167] These had many shortcomings, but the review concluded that attempts to identify TPMT deficiency prior to prescribing azathioprine had a modest cost that overall was essentially cost neutral. Unfortunately, despite the availability of the TPMT enzyme assay, new cases of azathioprine-induced pancytopenia in patients where baseline TPMT status was not established continue to be reported.[168] These cases emphasize the risk to life and high cost of the intensive supportive care needed for patients with severe and prolonged myelosuppression.[168] Collectively, these cases appear to make a watertight case for routine pretreatment TPMT measurement, and as TPMT testing in the U.K. is becoming an increasingly inexpensive test, previous health economic arguments are now of limited relevance. This is further highlighted by a case of severe neutropenia (*TPMT* null) that developed in the non-screened arm of the Department of Health-funded TARGET study[4] (see section 8.21) which was set up to address the utility of pharmacogenetic testing in the NHS.

13.0 Future directions

13.1 Personal genomic screening

Personal genomic screening is a relatively new type of genetic testing characterized by multiple statistical comparisons to assess for risk of a medical event. Such screening uses data from genome-wide association studies to predict a person's disease risk, or for drugs, adverse-event risk, using multiple genetic markers simultaneously. This is a rapidly evolving field and is likely, in the future, to be relevant to predicting the risk of adverse events with azathioprine; as described earlier in this guideline, some of these risks are dependent on genotype. If personal genomic screening for risk of adverse events with azathioprine becomes available, the accuracy and usefulness of such testing would need to be independently assessed before such testing became widely adopted.

13.2 Other pharmacogenetic factors

Several studies have linked polymorphisms in inosine triphosphate pyrophosphatase (ITPase; Fig. 1), an enzyme involved in the thiopurine catabolism, with adverse events[169,170] including hypersensitivity symptoms[65,171] and dropout from therapy.[172] However, other studies have failed to show a link.[173–176] Although the significance of ITPase polymorphisms as yet remains unclear, it seems possible that there is a relationship with toxicity; some studies may have been underpowered or an association may have been obscured, for example by misclassification of patients with dose-dependent nausea as drug hypersensitivity. However, as the relevance of this literature remains uncertain, ITPase status is not currently measured in clinical practice. Recently, a polymorphism in aldehyde oxidase, an enzyme which acts on several thiopurine intermediates, was shown to predict lack of response to azathioprine in a prospective cohort of 192 patients with IBD.[177] The reproducibility and clinical relevance of this preliminary finding will require additional confirmation.

13.3 Therapeutic drug monitoring

There is currently insufficient evidence in dermatological patients to support the use of TGN monitoring to guide azathioprine dosing and potentially optimize efficacy. However, this practice is now being increasingly used in the management of IBD in the clinic (see section 8.22), particularly in the U.S.A.,[125] and TGN assay is both inexpensive and available in the U.K. (see Appendix 2). Monitoring TGNs during azathioprine therapy may have several benefits including: (i) optimization of dose for TPMT heterozygotes; (ii) identifying the potential to increase dose in clinical nonresponders; and (iii) identifying nonresponders with TGNs above the threshold value fairly early during azathioprine therapy to avoid continued and unnecessary immunosuppressant exposure.[71] Although data for dermatology patients are limited, studies in IBD have identified a therapeutic range of 230–260 (lower limit) and 450 pmol per 8×10^8 RBCs (upper limit), which may serve as useful therapeutic targets in future dermatological practice. Some studies have shown that levels of other metabolites (see Fig. 1) such as MeMPR (section 9.23) and methylthioinosine monophosphate (MeTIMP) may also been linked to toxicity[98,99,126] and response (MeTIMP),[178] but the reproducibility of these findings or applicability to the clinical setting is yet to be determined.

13.4 Potential benefits of combined allopurinol and azathioprine therapy

The therapeutic indications for allopurinol listed in the SPC include gout and other hyperuricaemic conditions;[119] interestingly, the drug was originally developed to enhance the therapeutic effects of thiopurine drugs.[179] Inhibition of xanthine oxidase and thereby reduction of azathioprine catabolism to inactive thiouric acid increases metabolism via the activation pathway to TGNs (Fig. 1). However, in routine clinical practice, the combination of azathioprine and allopurinol is relatively contraindicated and the previous version of this guideline advised against concomitant use due to the risk of TGN-related toxicity. Recently however, allopurinol has been successfully combined with thiopurine drugs in nonresponsive patients with IBD, in whom preferential metabolism to MeMPR rather than TGN has been identified.[98] Several studies have demonstrated that allopurinol (100 mg daily) combined with thiopurine drugs (at 25–50% of the conventional dose) results in increased TGN levels and an improved therapeutic response, although the mechanism of this action remains to be elucidated.[180,181]

The concept of combination azathioprine/allopurinol therapy has also been extended to patients with thiopurine-induced hepatotoxicity associated with high MeMPR levels[98,182,183] (see section 9.23). These individuals have been shown preferentially to shunt metabolism away from the formation of TGN to MeMPR,[98] an effect which seems to be reduced by the addition of allopurinol.

There is, however, insufficient experience with these approaches to recommend routine usage in clinical practice, and caution is advised if combination therapy is to be used in dermatology patients. Close monitoring for haematological toxicity would obviously be needed.

Recommendations

- There is good evidence from nondermatological diseases linking TGN levels to toxicity and therapeutic response (*Strength of recommendation A; level of evidence 1+*)
- Measurement of metabolites including TGN should be included in future research studies of azathioprine, in order to assess their usefulness in optimizing dosimetry in the clinical setting (*Strength of recommendation D; level of evidence 4*)

14.0 Recommended audit points

Clinicians prescribing azathioprine should use audit to evaluate their care against predefined standards. Possible topics include:

1 Compliance with pretreatment assessment for patients starting azathioprine, including baseline assessment of TPMT enzyme activity and the provision of written patient information.
2 Compliance with monitoring recommendations (at least 3-monthly when stable but weekly for the first 1–2 months of therapy).
3 Monitoring of the provision of sun-awareness advice to patients on long-term azathioprine (including patients with IBD and solid-organ transplants).

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Appendix 1

Levels of evidence

Level of evidence	Type of evidence
1++	High-quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+	Well-conducted meta-analyses, systematic reviews of RCTs, or RCTs with a low risk of bias
1−	Meta-analyses, systematic reviews of RCTs, or RCTs with a high risk of bias[a]
2++	High-quality systematic reviews of case–control or cohort studies High-quality case–control or cohort studies with a very low risk of confounding, bias or chance and a high probability that the relationship is causal
2+	Well-conducted case–control or cohort studies with a low risk of confounding, bias or chance and a moderate probability that the relationship is causal
2−	Case–control or cohort studies with a high risk of confounding, bias or chance and a significant risk that the relationship is not causal[a]
3	Nonanalytical studies (for example, case reports, case series)
4	Expert opinion, formal consensus

[a]Studies with a level of evidence '−' should not be used as a basis for making a recommendation. RCT, randomized controlled trial.

Strength of recommendation

Class	Evidence
A	• At least one meta-analysis, systematic review, or RCT rated as 1++, and directly applicable to the target population, **or** • A systematic review of RCTs or a body of evidence consisting principally of studies rated as 1+, directly applicable to the target population and demonstrating overall consistency of results • Evidence drawn from a NICE technology appraisal
B	• A body of evidence including studies rated as 2++, directly applicable to the target population and demonstrating overall consistency of results, **or** • Extrapolated evidence from studies rated as 1++ or 1+
C	• A body of evidence including studies rated as 2+, directly applicable to the target population and demonstrating overall consistency of results, **or** • Extrapolated evidence from studies rated as 2++
D	• Evidence level 3 or 4, **or** • Extrapolated evidence from studies rated as 2+, **or** • Formal consensus
D (GPP)	• A good practice point (GPP) is a recommendation for best practice based on the experience of the guideline development group

RCT, randomized controlled trial; NICE, National Institute for Health and Clinical Excellence.

Appendix 2

Laboratories offering thiopurine methyltransferase and thioguanine nucleotide measurement

Measurement of thiopurine methyltransferase (TPMT) activity requires a complex assay for which high sample throughput is desirable for economic viability. Different centres may use different units so reference ranges corresponding to absent/very low, intermediate and normal/high levels should always be clarified with the reporting laboratory. Although there are several NHS laboratories providing local TPMT services, the majority of TPMT testing in the U.K. is performed by two national services. Both laboratories also offer thioguanine nucleotide (TGN) measurement:

Purine Research Laboratory
4th Floor, North Wing, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

Clinical Biochemistry
City Hospital, Dudley Road, Birmingham B18 7QH, U.K.

For historical reasons, due to an ongoing trial in leukaemia, all U.K. patients with acute lymphoblastic leukaemia are tested at Sheffield.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Literature search strategies.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.