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Review Article

Review Article

Thiopurines in Inflammatory Bowel Disease: New Findings and Perspectives

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Abstract

Thiopurines, available as azathioprine, mercaptopurine, and thioguanine, are immunomodulating agents primarily used to maintain corticosteroid-free remission in patients with inflammatory bowel disease. To provide a state-of-the-art overview of thiopurine treatment in inflammatory bowel disease, this clinical review critically summarises the available literature, as assessed by several experts in the field of thiopurine treatment and research in inflammatory bowel disease.

Key Words: Thiopurines; azathioprine; mercaptopurine; thioguanine; inflammatory bowel disease; Crohn's disease; ulcerative colitis; therapeutic drug monitoring

1. Introduction

Thiopurines are antimetabolites and immunomodulators first described by Gertrude Elion and George Hitchings in the 1950s, originally developed for the treatment of childhood leukaemia, for which they received the Nobel prize in 1988.[1] The first patient with inflammatory bowel disease [IBD] was treated with thiopurines in 1962.[2] Nowadays, thiopurines are important immunomodulating agents to maintain remission in patients with IBD.[3,4] The thiopurines used in IBD are azathioprine [1.5–2.5 mg/kg], mercaptopurine [1.0–1.5 mg/kg], and thioguanine [0.2–0.3 mg/kg]. Although generally effective, up to half of patients discontinue treatment with thiopurines within the first 2 years, due to adverse drug events or failure of therapy.[5] Various treatment strategies, such as split-dose administration, dose adjustment based on thiopurine metabolite monitoring, or co-administration with allopurinol, may increase effectiveness or reduce toxicity of therapy.[6–8] In this practical review for gastroenterologists, we critically summarise the available literature regarding pharmacology, mode of action, effectiveness, optimisation strategies, toxicity, and cancer risk of thiopurine therapy.

2. Pharmacology

Numerous enzymes are involved in the multistep metabolism of thiopurines.[9] These metabolic transformations are essential to generate the pharmacologically active metabolites, as thiopurines (azathioprine [AZA], mercaptopurine [MP], and thioguanine [TG])

themselves have no known intrinsic immunosuppressive potential. Three competing enzymes (thiopurine S-methyltransferase [TPMT], hypoxanthine-guanine phosphoribosyl transferase [HGPRT], and xanthine oxidase [XO]) determine, in part, the bioavailability of the critical metabolites 6-thioguaninenucleotides [6-TGN] and 6-methylmercaptopurine [6-MMP][9] [Figure 1].

After ingestion, followed by highly variable absorption, AZA is converted under the influence of glutathione S-transferases into 6-mercaptopurine [6-MP; approximately 90%] and S-methyl-4-nitro-5-thioimidazole [approximately 10%].[10] Via HGPRT, 6-MP is metabolised into 6-thioinosine-monophosphate, which in turn is converted by inosine monophosphate dehydrogenase [IMPDH] and guanosine monophosphate synthetase [GMPS] into 6-thioguanine monophosphate [6-TGMP]. This metabolite is further phosphorylated into 6-thioguanine diphosphate [6-TGDP] and 6-thioguanine triphosphate [6-TGTP], and these three forms give the total pool of 6-TGN. The metabolism of TG is less complex, as the 6-TGN are directly generated via the enzyme HGPRT. Recent experimental data even indicate that the conversion of TG to 6-TGN is not strictly dependent on host HGPRT but might also be accomplished by gut microbiota.[11]

In addition, 6-MP [being the metabolite of AZA or ingested as drug itself] may be methylated by the key enzyme TPMT, leading to the formation of 6-MMP.[12] The metabolite 6-thioinosine-monophosphate can also be metabolised into the 6-methylmercaptopurine ribonucleotides [6-MMPR]. When TPMT activity is low or even absent, grossly elevated 6-TGN levels are expected. When TPMT activity is normal or high, elevated methylated thiopurine product levels are present at the cost of 6-TGN formation. The activity of TPMT in a Caucasian population is distributed in a trimodal manner: low/absent [0.3%], intermediate [10%], and normal/high [90%] activity.[13] The TPMT genotype is the primary determinant of TPMT activity [phenotype].[14] Thiopurines may be incorporated into DNA via 6-thio-deoxyGTP which is a substrate for nucleoside diphosphate-linked moiety X-type motif 15 encoded by the *NUDT15* gene.[15] The activity of this nudix hydrolase is modulated by genetic polymorphism, and deficient enzyme activity leads to excessive incorporation of thioguanine into DNA.[16]

The molecular weight of AZA consists of approximately 50% of 6-MP and, taking the 90% conversion to 6-MP into account, a conversion factor of 2.08 in weight is frequently used when calculating equivalent oral dosages of AZA and MP for clinical purposes [e.g. 1 mg/kg of MP is equivalent to 2.08 mg/kg of AZA].[17]

3. Mode of Action

The mode of action of thiopurines in the context of chronic gut inflammation remained enigmatic for a long period of time. Only during the past 15 years has our understanding of intracellular target molecules and signalling pathways started to improve.[11,18] During replication of DNA in cells, a small proportion of 6-TGN is incorporated into DNA instead of guanine. This stimulates the mismatch repair [MMR] system that works incompletely and leads to cell death instead of cell recovery.[19]

The main immunosuppressive ability of thiopurines in IBD results from the binding of 6-TGTP to the small GTPase Rac1.[18,20,21] Shifting between an activated GTP-bound form and an inactive GDP-bound status, Rac1 represents an important intracellular mediator that crucially impacts on T cell fate.[22] Rac1 activation via GDP/GTP exchange in T cells is catalysed by the guanine nucleotide exchange factor Vav1.[22] At a biochemical level, 6-TGTP forms a disulphide adduct with the side-chain Cys_{18} of the Rac1 GXXXXGK[S7T]C motif, which is located at the Rac1 nucleotide binding site.[23]

Equivalent to physiologically occurring GTP-bound Rac1, 6-TGTP-bound Rac1 is biologically active, but will be converted into inactive 6-thio-GDP-bound Rac1 by GAP proteins.[20,23] As 6-TGDP is covalently linked to Rac1, Vav1 is unable to catalyse the 6-TGDP/6-TGTP exchange on Rac1, which subsequently results in a cellular accumulation of inactive 6-TGDP-bound Rac1.[20,23] The thiopurine-mediated blockade of Rac1 signalling is able to efficiently suppress pro-inflammatory T cell responses via at least two key mechanisms: induction of T cell apoptosis and impairment of the complex formation between T cells and antigen-presenting cells [APC].[18,20]

As an efficient co-stimulation of T cells results in the initiation of an anti-apoptotic signalling cascade which involves Vav1-catalysed activation of Rac1 and finally results in an increased expression of the anti-apoptotic protein Bcl-x_L, the thiopurine-triggered lack of

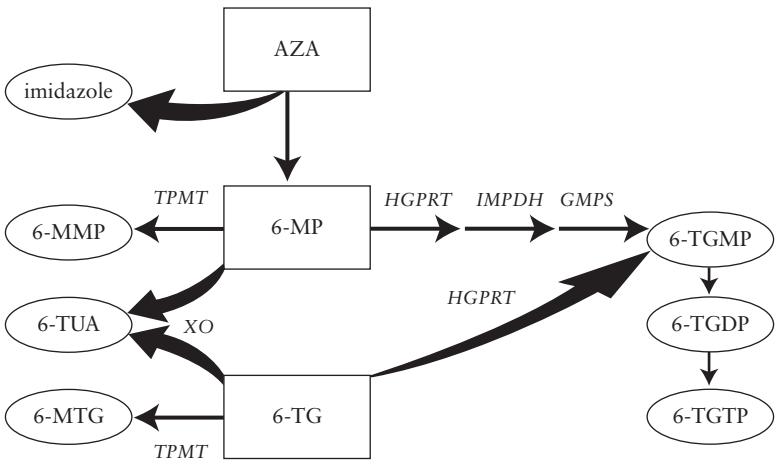

Figure 1. Simplified overview of thiopurine metabolism. Azathioprine [AZA] is converted into 6-mercaptopurine by separating an imidazole-group. 6-Mercaptopurine [6-MP] is enzymatically converted into 6-methylmercaptopurine [6-MMP] by thiopurine-S-methyltransferase [TPMT] and into 6-thiouracic acid [6-TUA] by xanthine oxidase [XO]. The remaining portion of 6-MP is converted into the biochemically active end-metabolites 6-thioguaninenucleotides (6-TGN, consisting of 6-thioguanine monophosphate [6-TGMP], 6-thioguanine diphosphate [6-TGDP], and 6-thioguanine triphosphate [6-TGTP]) by the so-called purine salvage pathway of hypoxanthine-guanine phosphoribosyl transferase [HGPRT], inosine monophosphate dehydrogenase [IMPDH], and guanosine monophosphate synthetase [GMPS]. 6-Thioguanine [6-TG] is metabolised by TPMT into 6-methylthioguanine [6-MTG] and into 6-TUA by XO. The remaining portion of thioguanine is directly converted into 6-TGN by HGPRT. Adapted from van Asseldonk *et al. Curr Drug Metabolism* 2009;**9**:981-97.[9]

activated Rac1 results in a decreased Bcl-x_L expression and subsequently promotes apoptosis of co-stimulated T cells.[18]

Well in line with this mode of action, IBD patients who clinically responded to thiopurine therapy could be characterised by decreased levels of activated Rac1 in blood leukocytes and increased numbers of apoptotic lamina propria T cells in the gut.[18] The capacity to diminish T cell-APC complex formation is based on the fact that activated Rac1 induces the dephosphorylation of ezrin-radixin-moesin [ERM] proteins in T cells, which strongly supports T cell-APC conjugation.[24] Accordingly, T cells exposed to thiopurines showed decreased levels of dephosphorylated ERM proteins and a reduced capacity to form complexes with APCs.[20]

Taking into account that Rac1 is involved in the intracellular signalling machinery not exclusively of T cells, but also of many other immune and non-immune cells,[25] a thiopurine-mediated impact on other cellular players within the pathogenesis of IBD appears to be obvious. Indeed, Rac1-dependent pro-inflammatory iNOS expression in activated macrophages could successfully be decreased by thiopurine treatment.[26] Furthermore, by decreasing Rac1 activity in endothelial and intestinal epithelial cells, thiopurines are able to modulate the function of important biological barriers.[26,27] Thiopurine-exposed endothelial cells could be characterised by an altered expression profile of pro-inflammatory cytokines, and turned out to be impaired in their ability to form membrane protrusions as important docking structures for migration of leukocytes into inflamed tissue.[27] Beside a Rac1-dependent effect of thiopurines on the proliferation and IL-8 expression of intestinal epithelial cells,[26] a very recent publication even describes the capacity of TG to promote autophagy in gut epithelium and thereby strengthen the epithelial defence mechanisms against bacterial invasion.[11] Accordingly, murine studies were able to demonstrate beneficial thiopurine-mediated effects in experimental colitis even in the absence of adaptive immune cells.[11] Figure 2 shows the mode of action of thiopurines.

Regarding the two main limitations of thiopurines in IBD therapy, namely its delayed onset of action and the risk of toxic or procarcinogenic side effects,[28] new insights into the mode of action have also improved our understanding of these phenomena. It is likely that the prolonged period between initiation of therapy and onset of clinical benefit is at least partly due to the fact that an efficient blockade of Rac1 signalling presupposes a sufficient cellular accumulation of inactive 6-TGDP-bound Rac1 proteins.[20] Moreover, data from an experimental mouse model strongly imply that only long-term thiopurine treatment is able to reduce the response of memory T cells to a repeatedly encountered antigen, whereas effector T cell differentiation remains unaffected after short-term treatment.[29] Although 6-TGN incorporation into cellular DNA was initially suggested to represent the main therapeutic mode of action of thiopurines,[30] more recent studies highlight the capacity of DNA-incorporated 6-TGN

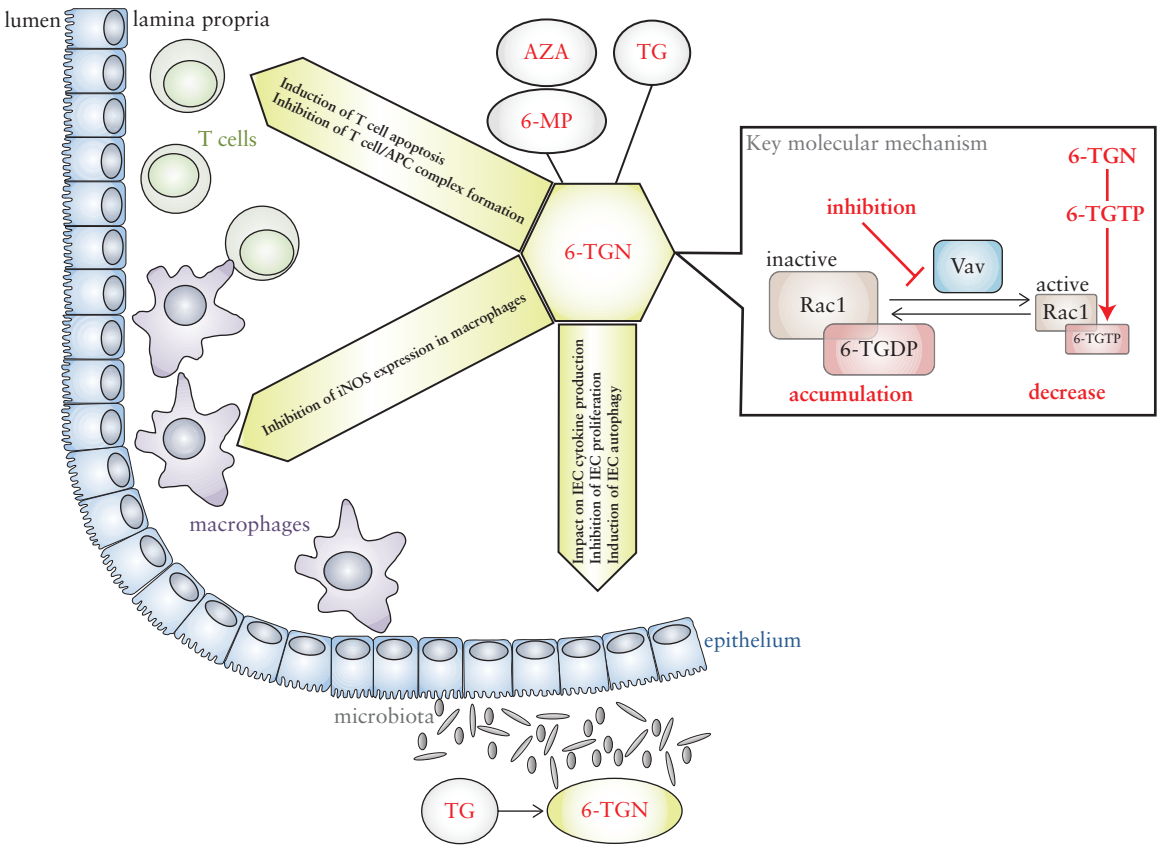

Figure 2. Mode of action of thiopurines. The therapeutic efficacy of thiopurines in the context of inflammatory bowel disease is based on their capacity to impact on adaptive immune cells [induction of T cell apoptosis; impairment of T cell/APC complex formation], innate immune cells [dampened pro-inflammatory function of macrophages], and non-immune cells [modulated function of biological barriers] within the inflamed intestine. On a molecular level, the ability of the active thiopurine metabolite 6-thioguanine-triphosphate [6-TGTP] to bind to the small GTPase Rac1 and subsequently block the Vav1-catalysed activation of Rac1 represents the key mechanism of action underlying the immunosuppressive function of azathioprine [AZA], mercaptopurine [6-MP], and thioguanine [TG]. APC, antigen-presenting cell; 6-TGN, 6-thioguanine-nucleotides; 6-TGDP, 6-thioguanine-diphosphate; IEC, intestinal epithelial cells; iNOS, inducible nitric oxide synthase; GAP, GTPase-activating protein.

to drive DNA damage, cytotoxicity, and mutagenicity.[31,32] Exposure of DNA 6-TGN to ultraviolet-A light is known to trigger the formation of reactive oxygen species [ROS] which subsequently cause mutagenic oxidative DNA damage, and might therefore mechanistically underlie the increased risk of thiopurine-treated patients for the development of skin cancer.[32] As a future perspective, the now achieved improved insights into the molecular mode of action of AZA/MP might pave the way for innovative strategies allowing us to overcome today's limitations of thiopurine therapy. The development of optimised thiopurine drugs characterised by an accelerated Rac1 blockade and a decreased level of 6-TGN incorporation into cellular DNA could potentially result in a more beneficial ratio between clinical efficacy and unwanted toxicity.[31]

4. Effectiveness of Thiopurines in Inflammatory Bowel Disease

4.1. Induction therapy in Crohn's disease

Following the original report by Present *et al.* that MP [1.5 mg/kg/day] was effective as induction therapy at 3 months and as maintenance therapy over 1 year, it was shown in several randomised placebo-controlled trials and meta-analyses, that both AZA and MP were effective for inducing remission in CD.[33,34] However, in a recent Cochrane review evaluating AZA/MP in CD, the pooled analysis failed to demonstrate a statistically significant difference between AZA/MP and placebo for induction of remission (relative risk [RR], 1.23; 95% confidence interval [CI], 0.97–1.55).[35]

Although recent data show no significant effect of the use of thiopurines for the induction of remission in CD, two major criticisms of the data should be taken into account. In most induction studies, no therapeutic drug monitoring [TDM] was performed, possibly leading to an underestimation of the effectiveness, as the concentration of 6-TGN has been associated with therapeutic response.

Furthermore, in the studies included in the Cochrane reviews of AZA/MP as induction therapy, clinical remission was assessed after 12–17 weeks of therapy, whereas it has been shown that median time to response with AZA in IBD is 4.5 months, with response times even up to 6 months in some patients.[36] Therefore, assessment at 12–17 weeks is likely too early and underestimates efficacy. Still, with such a long delay of action, thiopurines should not be considered as induction therapy.

4.2. Maintenance therapy in Crohn's disease

The most recent Cochrane review by Chande *et al.* on AZA/MP for maintenance of clinical remission in CD identified 15 studies that satisfied the inclusion criteria. A pooled analysis of the nine studies comparing AZA/MP with placebo showed a modest advantage in favour of AZA/MP for maintenance of remission [RR, 1.28; 95% CI, 1.15–1.43].[37] Again, one caveat is that thiopurine therapy was not optimised in these studies, which may have led to an underestimation of the maintenance effect of thiopurines.

4.3. Induction therapy in ulcerative colitis

In current guidelines, the use of thiopurines as monotherapy to induce remission in ulcerative colitis [UC] is not recommended. One of the few placebo-controlled trials [N = 80] comparing AZA with placebo showed that there was no benefit in using AZA in addition to corticosteroids for induction of remission in UC.[38] Moreover, a pooled analysis of the available literature on induction therapy in UC failed to demonstrate a positive effect of thiopurine monotherapy.[39]

4.4. Maintenance therapy in ulcerative colitis

The data on the efficacy of thiopurine monotherapy to maintain remission in UC are limited. Yet, thiopurines have been used widely in clinical practice for UC treatment for many decades now. In two randomised trials, a positive effect of thiopurines was demonstrated compared with either 5-ASA or placebo.[40,41] Furthermore, in a meta-analysis, the use of thiopurines to maintain remission in UC patients was beneficial over placebo.[42]

5. When to stop thiopurine therapy?

Whether patients achieving [deep endoscopic] remission may successfully stop thiopurine therapy is a challenging question in daily practice, but currently firm data are lacking. In two recent [systematic] reviews, it was shown that discontinuation of thiopurine therapy contributed to a higher proportion of patients experiencing clinical relapse in both CD and UC, with an average 1-year relapse percentage of 38% in CD and 53% in UC.[43,44] When thiopurine therapy is terminated and patients develop a relapse of disease, re-introduction of the original thiopurine is effective in about 75% of the patients.[45] In clinical practice, stopping thiopurines is a case-by-case discussion. Predictive factors for relapse should be taken into account, such as evidence of absence of disease activity (elevated C-reactive protein [CRP] level, increased leukocyte or neutrophil count, decreased haemoglobin level), lack of poor prognostic factors [ileal disease location, perianal disease, younger age], or a worse disease course or disease more difficult to control before de-escalation [shorter duration of remission, previous complications, and need for corticosteroids in the previous 50 months].[43]

6. Prevention of postoperative recurrence

The efficacy of thiopurines has been demonstrated in the prevention of postoperative recurrence in CD patients. In a meta-analysis, it was determined that the efficacy of purine analogues is superior to placebo for the prevention of clinical and endoscopic recurrence at 1 year (mean percentage differences, 95% CI: 13, 1.8–25%, p = 0.025, number needed to treat [NNT] = 7, and 23, 9–37%, p = 0.0016, NNT = 4, respectively).[46] Recently, it was shown by the TOPPIC study group that this beneficial effect might be even higher in patients who are smokers.[47]

7. Thiopurines as disease modifier

Thiopurines have a long-lasting effect and may contribute to the change of the natural history of CD. In the study by Magro *et al.*, the hazard ratio [HR] for disease progression, such as phenotype change, was lower for monotherapy with AZA [HR: 0.15, $p < 0.001$] when administered before phenotype change. The time delay between CD diagnosis and start of therapy with AZA is also predictive for disease progression, with longer delays being associated with a higher likelihood of disease progression with change of phenotype.[48]

Surgery rate may be considered as another objective parameter for disease progression. A recently published meta-analysis on surgery rates in IBD patients using thiopurines, shows that the pooled HR is 0.59 [95% CI, 0.48–0.73] for the efficacy of thiopurines in reducing surgery.[49] Early use of thiopurines is associated with a reduction in risk of first surgery of 39% [HR, 0.61; 95%, CI, 0.41–0.91] in CD patients.[50]

8. Combination Therapy with Azathioprine and Infliximab

In both CD and UC, the combination of AZA and infliximab [IFX] is more effective than either drug administered alone. The SONIC trial showed superiority of combination therapy to both AZA and IFX monotherapy. At Week 26, 56.8% [96/169] of patients receiving IFX/AZA combination therapy achieved corticosteroid-free remission compared with 44.4% [75/169] of patients who received IFX alone [$p = 0.02$] and 30.0% [51/170] of patients who received AZA alone [$p < 0.001$]. Furthermore, mucosal healing at 26 weeks had occurred in 47 of 107 patients [43.9%] receiving combination therapy, as compared with 28 of 93 patients [30.1%] receiving infliximab [$p = 0.06$] and 18 of 109 patients [16.5%] receiving azathioprine [$p < 0.001$ for the comparison with combination therapy and $p = 0.02$ for the comparison with infliximab.[51] Furthermore, both patients on AZA monotherapy and AZA/IFX combination with a change in mean corpuscular volume [MCV] over 7 fl [indicating higher thiopurine exposure] displayed significantly higher response rates to therapy, thus underlining the probable beneficial effect of TDM in these patient groups [$p = 0.005$ and $p = 0.02$, respectively].[52] Recent data suggest that the contribution of AZA to superiority of combination therapy with IFX is at least partially linked to its impact on pharmacokinetics of IFX [antibody formation] rather than to a synergistic effect.[51] Similarly, anti-tumour necrosis factor-α–naive patients with moderate to severe UC treated with IFX plus AZA were more likely to achieve corticosteroid-free remission at 16 weeks than those receiving either monotherapy. Combination therapy led to significantly better mucosal healing than AZA monotherapy.[53] There is an additional role for thiopurine therapy in patients experiencing loss of response to biologic therapy, as the addition of an immunomodulator can reverse the antidrug antibody formation, leading to a regained response in these patients.[54,55]

9. Thioguanine

Thioguanine has been considered as an alternative treatment option in IBD patients who have failed conventional thiopurines due to intolerance or ineffectiveness. In a systematic review, a total of 353 (Crohn's disease [CD]: 225; UC: 119; IBD unclassified [-U]: 9) patients were treated with TG with a starting dose of 20–40 mg daily.[56] The dosing was adjusted per individual to 10–80 mg/day, based on either the development of adverse events or effectiveness of therapy. Thioguanine was administered for an estimated 268 treatment years. The clinical response rates were up to 22% and 79% for induction and maintenance therapy, respectively.

10. Optimising Thiopurine Therapy

If a decision to use a thiopurine is made, then optimising the dose and type of therapy forms an important part of best practice and accords with the modern principles of precision medicine. Regular weight-based doses lead to the generation of higher levels of 6-TGN than lower dosing strategies. Hence, measurement of 6-TGN level on treatment provides a reasonable guide to optimal dose. Although monitoring 6-TGN confirms adherence at the same time, dosing to achieve a 6-TGN above 235 pmol/8 × 10^8 red blood cells [RBC] makes clinical efficacy more likely.[57,58] Although the 6-TGN threshold level varies between authors, this approach is supported by a number of cohort studies and by meta-analysis.[7] The approach applies particularly to monotherapy. In combination therapy, a lower dose of thiopurine may be comparable to higher doses in maintaining efficacy by reducing antidrug antibodies and preventing loss of response to biologics, but this needs further analysis.[59]

Optimised thiopurine therapy is a classic example of precision medicine. Pre-treatment testing of TPMT avoids the risk of severe myelotoxicity in the one in 300 patients with zero TPMT activity [homozygous or compound TPMT heterozygosity], and is recommended in most guidelines. TPMT testing also permits dose reduction to 50% in the 10% of patients with heterozygous TPMT deficiency and dose reduction to 10% in the patients with zero activity.[60] Although TPMT3*C and TPMT*3A are the most common variants associated with TPMT deficiency, the TPMT gene is highly polymorphic and, for this reason, phenotyping by TPMT enzyme assay before the start of thiopurine therapy is preferred to genotyping for common variants only.[61]

Once established on a TPMT-guided dosing schedule, metabolite measurement at 4 weeks provides the best opportunity to ensure adherence and optimise the dose early, and hence shorten the average time to therapeutic benefit [considered to be around 16 weeks from initiation but up to 6 months in some cases]. However, many prefer to check the 6-TGN profile at 12–16 weeks or, for example, at 6 months, when assessing response.[62] Furthermore, determination of thiopurine metabolites 1 week after the initiation of therapy seems to predict the chance of developing leukopenia or hepatotoxicity.[63,64] Each provides an opportunity to optimise therapy.

An additional benefit of 6-TGN profiling is the detection of patients [around 15–20%] with preferential methylation of 6-MP, away from generation of 6-TGN towards 6-MMP. This is important for two reasons. First, the preferential methylation may prevent generation of a high enough 6-TGN level, hence preventing efficacy; and second, around 50% of those with significant hypermethylation [e.g. 6-MMP > 5700 pmol/8 × 10^8 RBC] will develop hepatotoxicity.[65] Importantly, this can be circumvented by co-prescription of allopurinol with AZA, MP dose-reduced to 25–33% of the monotherapy dose target, or a switch to TG.[8] On this regimen, 6-MMP levels reduce dramatically, often to near zero, and 6-TGN levels can once again be used to optimise dose to the same therapeutic range as monotherapy.[66] Allopurinol can occasionally cause toxicity, for which reason the same caution regarding blood monitoring is needed as for monotherapy with thiopurines. Combination therapy successfully restores the opportunity for the best clinical outcome expected from monotherapy.[67] Split-dose therapy may lead to a reduction in 6-MMP levels but without a concomitant increase in 6-TGN levels.[6] In patients with low 6-TGN levels despite optimal dosing, the addition of mesalazine to thiopurine therapy might lead to an increase in 6-TGN concentration, without increasing 6-MMP levels.[68,69]

TPMT deficiency is relatively rare in South and East Asian populations. However in these ethnic backgrounds, genetic variation in the NUDT15 gene is strongly associated with leukopenia and unusually alopecia, which is independent of 6-TGN levels.[70] Genotyping for the NUDT15 p.R139C variant in these populations is recommended. The p.G17-V18del variant is present in Caucasian populations, albeit at low allele frequencies of less than 1%, and may be a cause of unexplained neutropenia.[15] Thiopurines should be avoided in patients with homozygous NUDT15 variant genotypes or NUDT15 / TPMT variant diplotypes.

The concept of optimised thiopurine therapy also includes the types of actions taken with the different types of toxicity. In patients with IBD, drug withdrawal due to adverse effects occurs in up to 50% of cases.[5] Hence, ameliorating adverse effects gives an important opportunity for optimising therapeutic outcome. Hence, for

nausea on AZA, a switch to an equivalent dose of MP [around 50% dose reduction] can often prevent this, demonstrating the likely influence of the imidazole moiety of AZA in the aetiology of nausea.[71] Causes of hepatotoxicity are heterogeneous in patients on thiopurines. However, in most cases, whether due to hypermethylation or, for example, hypersensitivity, combination therapy with allopurinol is effective in reversing the toxicity.[72] For hypersensitivity, this is most likely a direct independent effect of allopurinol on oxidative stress in the liver.

11. Toxicity of thiopurine therapy

Thiopurine therapy has been associated with many different presentations of toxicity, some of which are immunosuppression-related, some are dose-related and some are idiosyncratic [i.e. compound-related and individual-specific].

11.1. Idiosyncratic adverse events

Specific thiopurine-related toxicity includes general malaise and gastrointestinal complaints, which are primarily responsible for early discontinuation of therapy. The most observed presentation of an adverse event due to thiopurines comprises a mixture of nausea, loss of appetite, and abdominal discomfort or pain, in combination with general malaise. Less frequently, toxicity may present as a syndrome of elevated erythrocyte sedimentation rate or C-reactive protein, combined with high fever, often misjudged as an infection.

Pancreatitis is also a recognised thiopurine-related 'immune-allergic' reaction, usually occurring in the first 8 weeks of treatment.[35,42,73] Although in many reports hyperamylasaemia or hyperlipasemia has been documented as being sufficient for the diagnosis of pancreatitis, it is mainly the clinically defined acute pancreatitis that is of concern. This is a relatively infrequent finding [2.6%], and if observed when using the conventional derivatives AZA or MP, most patients tolerate the derivative TG when treatment is switched.[74]

Arthralgia, often of small joints such as the interphalangeal joints, may present at any time during thiopurine use, with the presentation including pain and stiffness without inflammation. As arthralgia is a common finding in IBD patients, the differential diagnosis may be challenging, especially when a stop-and-rechallenge strategy is not clinically feasible or is undesirable.[75]

Finally, rash, in the form of small nodular exanthema or pruritic, flaking skin, particularly when exposed to sunlight, may be so unpleasant that therapy must be discontinued.[5]

11.2. Dose-related toxicity

Hepatotoxicity [i.e. elevation of one or more liver enzymes] may be an idiosyncratic reaction, but is often related to the methylated metabolites of conventional thiopurines, and thus is seldom seen with TG.[57] Most debated, however, is the insidious occurrence of nodular regenerative hyperplasia of the liver [NRH], occasionally causing non-cirrhotic portal hypertension. NRH, which is associated with a wide array of drugs [e.g. platina-based chemotherapy, anti-retroviral therapy, thiopurines], is mostly defined on histopathological criteria. Not only are histopathological criteria problematic due to high disagreement between and within observers, but also association with clinical outcome is erratic.[76,77] Extensive histopathological data are scarce, but when available, these indicate that NRH is associated with age, IBD, and all thiopurine derivatives [in percentages around 5%], probably in a dose-related manner [Table 1].[77–81] Thioguanine or the combination of either MP or AZA with allopurinol does not seem to increase this relative risk in a clinically significant way.[77,82] When clinical symptoms are overt, these may subside, although not always, when treatment is discontinued.[83]

Table 1. Incidence of [histopathologically-proven] nodular regenerative hyperplasia.

Population	Incidence of NRH	Reference
Background population	2.6%	[78]
IBD patients	6%	[80]
AZA/MP therapy	1.3%	[81]
AZA/MP + allopurinol combination therapy	4.8%	[82]
TG therapy, 80mg/day	18–62%	[79]
TG therapy, 40mg/day	27%	[79]
TG therapy, 20mg/day	0–6%	[77, 79]

NRH, nodular regenerative hyperplasia; AZA, azathioprine; MP, mercaptopurine; TG, thioguanine; IBD, inflammatory bowel disease.

Bone marrow toxicity may present as suppression of red and white cell lines, as well as platelet generation, and is only partly associated with dysfunctional TPMT allele variants.[84] Leukopenia occurs as neutropenia or lymphopenia; however, the difference in pathogenesis of suppression of these two cell types remains obscure. Bone marrow suppression, with leukopenia in particular, is obviously a dose-related phenomenon, reflected by the difference in indication and pharmacodynamics of oncological or anti-inflammatory dosages of thiopurines.[18,21,85] Monitoring of 6-TGN concentrations may indicate overdosage, which may result from dysfunctional TPMT variants, exceptional bioavailability of thiopurines, or straightforward overdosing. Less well recognised is the occurrence of thiopurine-related leukopenia when co-infected with viral disease,[86] or when extreme concentrations of methylated metabolites are present due to a so-called skewed metabolism with high 6-MMP, resulting in an antimetabolic effect.[85] In the case of thrombocytopenia, one has to exclude splenomegaly, potentially due to non-cirrhotic portal hypertension, as part of the presentation of NRH of the liver.

11.3. Immune suppression-related

Immune-suppression is believed to be the pivotal mechanism of action of thiopurines, primarily by interfering with a main cellular inflammation checkpoint [i.e. Rac1].[21] In consequence the risk for infection is increased, comprising bacterial, parasitic, and viral infection. Some of these seem particularly associated with thiopurines, e.g. warts from human papilloma virus and Epstein-Barr virus. The former is particularly embarrassing for patients, sometimes necessitating therapy switch, whereas the latter increases the risk for lymphoma.[87]

11.4. Pregnancy

According to the most recent literature reviews concerning this topic, the use of conventional thiopurines during conception or pregnancy is not associated with a higher risk of preterm birth or congenital disorders.[88,89] Evidence regarding the use of thioguanine or combination therapy with allopurinol is scarce, for which reason further prospective trials are warranted to confirm the safety of these therapies in pregnant woman.[90,91]

12. Thiopurine Therapy-related Cancer

The relationship between cancer and thiopurines in IBD patients is complex. Due to their anti-inflammatory activity, thiopurines can decrease the incidence of inflammation-related colorectal cancers [CRC].[92] On the other hand, thiopurines have an inherent mutagenic potential and so possible carcinogenic effects.[93] Thiopurine exposure has been described to cause several mutations in tumour suppressor genes, as PTCH [protein patched homologue], which can lead to skin neoplasms.[93] In the same way, clonal expansion of myeloid cells with a failed DNA mismatch repair system may also occur in patients treated with thiopurines, as thiopurines favour selective clonal expansion of mismatch repair-defective cells, causing the development of acute myeloid leukaemia.[94]

Literature is consistent in the increased risk of non-melanoma skin cancer [NMSC] in IBD patients. Long *et al*. found an incidence rate ratio for NMSC of 1.64 [95% CI, 1.51–1.78] among IBD patients compared with controls, especially in patients receiving thiopurines.[95] Singh *et al*. showed an increased risk for basal cell carcinoma, compared with controls, with a hazard ratio [HR] of 1.20 [95% CI, 1.03–1.40].[96] In the CESAME cohort study, an excess of NMSC was observed in the overall IBD population (standardised incidence ratio [SIR] 2.89; 95% CI, 1.98–4.08) and this was more pronounced with age over 65 years,[97] whereas the risk of melanoma among the overall IBD population was similar to that observed in the general population [SIR 0.64; 95% CI, 0.17–1.63].[98] A meta-analysis of eight studies involving 60 351 patients was published in 2014, showing a pooled adjusted HR for developing NMSC after exposure to thiopurines in patients with IBD of 2.28 [95% CI, 1.50–3.45].[99] For the prevention of NMSC, the European guidelines advise regular dermatological screening and protection against UV radiation lifelong, especially in sunny countries.[100]

The relationship between uterine cervical cancer and thiopurines in IBD patients is conflicting.[100,101] However, a recent meta-analysis including eight studies and 77 116 IBD patients on any immunosuppression [thiopurines, steroids, biologics] showed a mild increase in the risk of cervical high-grade dysplasia/cancer in these patients compared with the general population without IBD (odds ratio [OR] 1.34; 95% CI, 1.23–1.46),[102] but the independent role of thiopurines via human papilloma virus [HPV] infection is still unclear. For cervical cancer screening in the general population, European guidelines recommend either cytology Papanicolaou [Pap] testing starting at 25–30 years and every 3 or 5 years, or HPV testing starting at 35 years and every 5 years.[103] In immunosuppressed patients, Pap testing should be performed twice in the first year after diagnosis and, if the results are normal, annually thereafter. In a recent meta-analysis, vaccination significantly reduced HPV16/18 infection and anogenital warts by more than 60%.[104] HPV vaccines can be safely administered to immunocompromised patients. Both European and US guidelines recommend quadrivalent vaccination [HPV type 6/11/16/18] at 0, 2, and 6 months, ideally in sexually naïve individuals below the age of 26 years.[105]

A concern regarding thiopurines in IBD patients is the risk of lymphoma. Data issued from the CESAME study showed that ongoing exposure to thiopurines increased the risk of lymphoproliferative disorders by 5.3-fold [2.01–13.90; $p = 0.0007$].[106] These data were confirmed in a recent meta-analysis.[107] Interestingly, the increased risk does not seem to persist after discontinuation of therapy. Two subgroups of patients have an even higher absolute risk of lymphoma per year on thiopurines: those above age 50 and men under 35 years. IBD patients also have a higher risk of intestinal lymphoma [SIR, 17.51; 95% CI, 6.43–38.11], and this risk is highest with thiopurine exposure [SIR, 49.5; 95% CI, 13.5–126.8].[108] Hepatosplenic T cell lymphoma is an infrequent but severe non-EBV-related lymphoproliferative disorder with about 200 cases reported in the literature worldwide. Of these, one-quarter occurred in IBD patients, all treated with thiopurines, either alone or in association with anti-tumour necrosis factor [TNF] therapy.[100] Males under 35 years comprised 90% of patents, supporting the recommendations of the European Crohn's and Colitis Organisation [ECCO] to avoid continuation of anti-TNFα with a thiopurine beyond 2 years in this subgroup of patients, when possible.[100] Finally, the CESAME cohort study found that the risk of myeloid disorder was not increased among the overall IBD population compared with the general population, but past exposure to thiopurines increased this risk 7-fold [SIR, 6.98; 95% CI, 1.44–20.36].[109]

Recent data were provided for kidney and bladder neoplasms in IBD patients. A new analysis of the CESAME study showed that the multivariate-adjusted HR of urinary tract cancer between IBD patients receiving thiopurines and those not receiving thiopurines was 2.82 [95% CI, 1.04–7.68; $p = 0.04$].[110] Increasing age [>65 years versus <50 years] was also a significant risk factor [HR, 13.26; 95% CI, 3.52–50.03].[110] Interestingly, smoking status was not recorded in this study, although smoking is known to act as an independent risk factor for urinary tract cancer in general and bladder cancer in particular.[111]

IBD patients with a history of cancer are at increased risk of developing any new or recurrent cancer, with a predominant incidence of new cancers [13.2/1000 patient-years], but there is no significant association between exposure to immunosuppressants and the risk of new or recurrent cancer in these patients.[112] These results

Table 2. Impact of thiopurines exposure on cancer risk among inflammatory bowel disease patients.

Author, year	Cancer type	Study design[a]	Sample size	Risk in current thiopurine users versus non users [95% CI]
Jess, 2014[92]	Colorectal cancer	Meta-analysis	84 789	OR 0.87 [0.71–1.06]
Ariyaratham, 2014[99]	Non-melanoma skin cancers	Meta-analysis	60 351	HR 2.25 [1.50–3.45]
Peyrin-Biroulet, 2012[98]	Melanomas	Cohort study	19 486	SIR 1.09 [0.13–3.94]
Jess, 2013[101]	Cervical cancer	Cohort study	1 515	SIR 2.47 [1.54–3.73]
Kotlyar, 2015[107]	Lymphomas	Meta-analysis	23 998[b]	SIR 5.71 [3.22–10.10]
Sokol, 2012[108]	Intestinal lymphomas	Cohort study	19 486	SIR 49.50 [13.49–126.80]
Lopez, 2014[109]	Myeloid disorders	Cohort study	19 486	SIR 6.98 [1.44–20.36]
Bourrier, 2016[110]	Urinary tract cancers	Cohort study	19 486	HR 2.82 [1.04–7.68]

OR, odds ratio; HR, hazard ratio; SIR, standardised incidence ratio.

[a]When a meta-analysis was available for a cancer type, we reported its results.

[b]Number of patients was not reported in one study.

were confirmed in a recent meta-analysis of 16 studies including 11 702 patients with an inflammatory chronic disease treated with an immunosuppressive therapy.[113] Similar rates of cancer recurrence were observed among individuals with previous cancer, who received or did not receive immune-modulator therapy.

In conclusion, thiopurine exposure in IBD patients is associated with an increased risk of NMSC, lymphoproliferative/myeloid disorders, and urinary tract cancer [Table 2], and a probably higher risk of cervical high-grade dysplasia/cancer. Interestingly, this effect is increased with age,[97,106,110] leading to reconsideration of the benefit-risk balance of thiopurines in elderly IBD patients.

13. Conclusion

Thiopurines are immunomodulators used frequently in the therapeutic armamentarium used for inflammatory bowel diseases. In this clinical review, we have provided information on pharmacology, mode of action, effectiveness, optimisation strategies, toxicity, and cancer risk of thiopurines.

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Conflict of Interest

None.

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