

## **Thiopurines Treatment for Inflammatory Bowel Disease in Current Clinical Practice**

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### **Abstract**

Thiopurines, or purine analogues, are immunomodulators used in the treatment of malignancies, rheumatic diseases, dermatologic conditions, inflammatory bowel disease and in solid organ transplantation. These agents include azathioprine (AZA), mercaptopurine (6-MP) and thioguanine (6-TG). Thiopurines are converted by the enzyme thiopurine methyltransferase (TPMT) into metabolites. Measurement of TPMT activity may help identify patients at risk for excessive toxicity, most often myelosuppression, after receiving standard doses of thiopurine medications. Measurement of metabolites (metabolite markers) may help tailor individualized drug therapy.

Azathioprine, which is a prodrug of 6-mercaptopurine (6-MP), is considered an effective immunosuppressive treatment of inflammatory bowel disease, particularly in patients with steroid-resistant disease. For example, in the course of 1 year, 50% of patients with Crohn's disease will require steroids for its treatment; of these, 50% will either be steroid resistant or steroid dependent, and thus candidates for immunosuppressive therapy. Azathioprine therapy eliminates the need for corticosteroids in about 75% of patients; azathioprine is also considered an effective therapy for fistulizing disease.

**Results:** A recent randomized clinical trial of children with Crohn's disease suggests that compared to prednisone alone, the inclusion of azathioprine with prednisone at the time of initial diagnosis is associated with improved maintenance of remission, while simultaneously decreasing the dose of prednisone.

However, the use of azathioprine is limited by both its long onset of action (3–4 months) and drug toxicities, which include hepatotoxicity, bone marrow suppression, pancreatitis and allergic reactions. Long-term drug use has been associated with neoplasia.

**Objective:** The purpose of this comprehensive review is to suggest guidelines for the application of azathioprine and 6-mercaptopurine in the treatment of inflammatory bowel disease.

**Keywords;** Thiopurines, Purine Analogues, Immunomodulators, Crohn's Disease, Ulcerative Colitis, Inflammatory Bowel Disease, IBD.

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## **Introduction**

Inflammatory Bowel Disease (IBD) represents a group of idiopathic, chronic, inflammatory intestinal conditions. Its two main disease categories are: Crohn's Disease (CD) and Ulcerative Colitis (UC), which feature both overlapping and distinct clinical and pathological features.<sup>1</sup> Maximizing the efficacy of IBD-directed therapies while minimizing their toxicity remains the principal objective in developing management strategies for all IBD patients.<sup>2</sup> Thiopurines are widely used in the treatment of Inflammatory Bowel Disease (IBD). Azathioprine and mercaptopurine, two commonly utilized immunosuppressive agents, are not indicated for use as induction therapy in patients with mild to moderately active IBD due to their relatively slow onset of action ( $\geq 3$  months).<sup>3</sup> However, these agents have been found to be effective as a maintenance therapy following corticosteroid-induced remission of patients with mild to moderate IBD.<sup>4</sup> Azathioprine 2.5 mg/kg was more effective than placebo at maintaining remission for up to 15 months. Following 15 months of therapy, the proportion of azathioprine-treated patients in remission was 42% compared with 7% of placebo-treated patients ( $P = 0.001$ ).<sup>5</sup> These agents are currently reserved for patients who are steroid-dependent or have complications such as fistulizing disease because of their potential for serious toxicity. Furthermore, withdrawal of azathioprine maintenance therapy leads to a greater rate of relapse than continuation of azathioprine.<sup>6</sup>

Azathioprine therapy may also be advantageous for weaning IBD patients off conventional corticosteroid therapy. Seventy-five percent of azathioprine-treated patients were able to reduce or discontinue steroid usage compared with 36% of placebo-treated patients; a finding which was further supported by meta-analyses.<sup>7</sup>

A concern of long-term maintenance therapy is the potential for adverse side effects. Approximately 2% to 8% of patients report mercaptopurine-induced toxicity events including

pancreatitis, bone marrow depression, allergic reactions and infectious complications.<sup>8</sup> Others have also reported pancreatitis, allergy, and opportunistic infection, plus additional adverse events such as leucopenia and neutropenia. Furthermore, there is an increase in the rate of lymphoma during the treatment with azathioprine.<sup>9</sup>

## **Metabolism of Azathioprine and 6-Mercaptopurine**

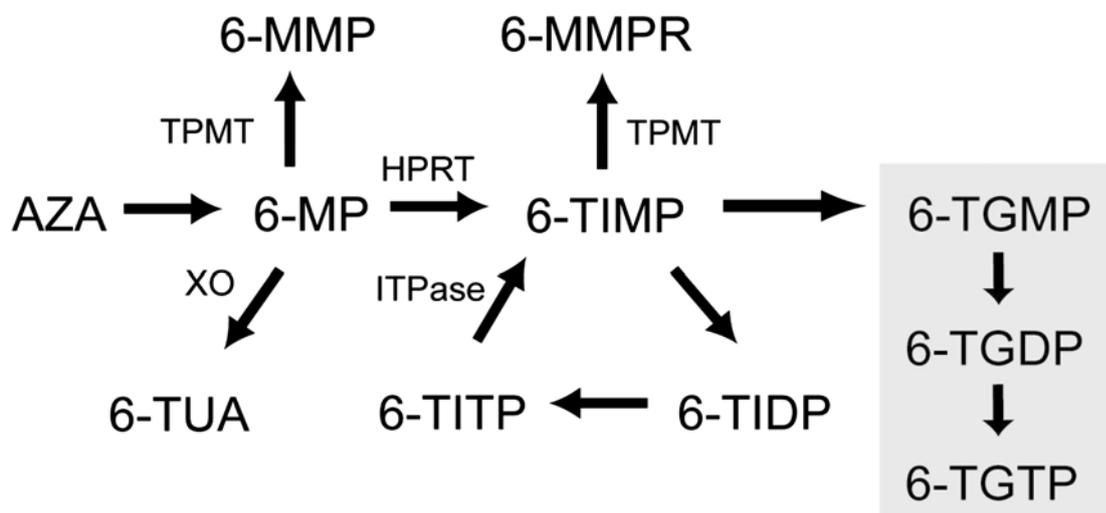
Both AZA and 6-MP need to undergo extensive metabolic transformations (Figure 1) before they can exert their immunosuppressive activity, because neither drug has intrinsic activity.<sup>10</sup>

Approximately 90% of absorbed AZA undergoes a rapid nonenzymatic conversion in the liver to yield 6-MP and 1-methyl-4-nitro-5-thioimidazole. The remaining 10% of AZA, which is also cleaved nonenzymatically, yields other products, including hypoxanthine and S-methyl-4-nitro-5-thioimidazole. These nonenzymatic conversions are aided by glutathione or other sulfhydryl-containing proteins. The absorption of AZA is incomplete and variable, both interindividually and intraindividually and as a result the bioavailability of this drug is highly variable (16-72%).<sup>11</sup> The molecular weight of 6-MP is 55% of the molecular weight of AZA; therefore, a conversion factor of 2.08 is necessary to convert 6-MP dosage to an equivalent dosage of AZA, assuming 100% oral bioavailability.<sup>12</sup>

The plasma half-life of 6-MP is very short, being in the range 1-2 h. 6-MP is metabolized by three competing enzyme systems: xanthine oxidase, thiopurine S-methyltransferase (TPMT) and hypoxanthine phosphoribosyltransferase (HPRT). Xanthine oxidase catalyzes the conversion of 6-MP to the pharmacologically inactive metabolite 6-thiouric acid (6-TUA), whereas the enzyme TPMT methylates 6-MP to form 6-methylmercaptopurine (6-MMP).<sup>13</sup> The HPRT enzyme is responsible for the conversion of 6-MP into 6-thioinosine 5'-monophosphate (6-TIMP).

**Figure (1): Metabolism of thiopurines.**

**Abbreviations:** 6-MMP, 6-methylmercaptapurine; 6-MMPR, 6-methylmercaptapurine ribonucleotide; 6-MP, 6-mercaptopurine; 6-MTG, 6-methylthioguanine; 6-TGDP, 6-thioguanine 5'-diphosphate; 6-TG, 6-thioguanine; 6-TGMP, 6-thioguanosine 5'-monophosphate; 6-TGNs, 6-thioguanine nucleotides; 6-TGTP, 6-thioguanosine 5'-triphosphate; 6-TIDP, 6-thioinosine 5'-diphosphate; 6-TIMP, 6-thioinosine 5'-monophosphate; 6-TITP, 6-thioinosine 5'-triphosphate; 6-TUA, 6-thiouric acid; AZA, azathioprine; HPRT, hypoxanthine phosphoribosyltransferase; ITPase, inosine triphosphate pyro.



6-TIMP can be metabolized by inosine monophosphate dehydrogenase (IMPD) into 6-thioxanthosine 5'-monophosphate (6-TXMP), which in turn can be metabolized via guanosine monophosphate synthetase (GMPS) into 6-thioguanosine 5'-monophosphate (6-TGMP).<sup>14</sup> 6-TGMP can be converted into 6-thioguanosine 5'-diphosphate (6-TGDP) and 6-thioguanosine 5'-triphosphate (6-TGTP) by two kinases. Within the 6-thioguanine nucleotide (6-TGN) pool, 6-TGTP (median proportion 80%) and 6-TGDP (median proportion 16%) are the main metabolites, whereas only traces of 6-TGMP are present. Levels of 6-TGN are, therefore, markedly correlated with concentrations of 6-TGTP and 6-TGDP. 6-TGNs have a average half-life of approximately 5 days (range 3-13 days), and they can all be methylated by TPMT.<sup>15</sup> 6-TIMP can be methylated by TPMT to form 6-methyl thioinosine 5'-monophosphate (6-MTIMP), 6-methyl thioinosine 5'-diphosphate (6-MTIDP) and 6-methyl thioinosine 5'-triphosphate (6-MTITP).<sup>16</sup> The last three metabolites listed are the so-called 6-methylmercaptapurine ribonucleotides (6-MMPRs). 6-TIMP can also be

phosphorylated by monophosphate kinase to form 6-thioinosine 5'-diphosphate (6-TIDP), then by diphosphate kinase to produce 6-thioinosine 5'-triphosphate (6-TITP), and ultimately back to 6-TIMP following an enzymatic reaction catalyzed by inosine triphosphate pyrophosphatase (ITPase). The metabolism of 6-TG is less complex than that of 6-MP and AZA.<sup>17</sup> The absorption of orally administered 6-TG is incomplete and highly variable, leading to a bioavailability of 14-46%. In patients with leukemia, plasma concentrations of 6-TG generally become undetectable after 6 h owing to its rapid intracellular transport.<sup>18</sup>

The metabolic transformations of 6-TG occur via three short pathways. In one pathway, the enzyme HPRT converts 6-TG directly to 6-TGMP, then subsequently 6-TGDP and 6-TGTP are produced by kinase activity. There is wide interindividual variation, not only in total 6-TGN concentrations, but also in 6-TGMP, 6-TGDP and 6-TGTP concentrations, which cannot be explained on the basis of different 6-TG doses per kilogram body weight or different levels of TPMT activity.<sup>19</sup>

The main metabolites within the total nucleotide pool of 6-TG are 6-TGDP and 6-TGTP. In the second pathway, the enzyme TPMT methylates 6-TG to form 6-methylthioguanine (6-MTG). Via the third pathway, 6-TG can be converted by the enzyme guanase to produce 6-thioxanthine, which can be further degraded into 6-thiouric acid (6-TUA) by xanthine oxidase.<sup>20</sup>

### **6-Thioguanine Nucleotide Therapeutic Range**

Most laboratories use a therapeutic range for 6-TGN concentration of 235-450 pmol/ $8 \times 10^8$  RBC. In particular, there are compelling data suggesting that a thiopurine dose increase to achieve 6-TGN concentrations  $>235$  pmol/ $8 \times 10^8$  RBC resulting in clinical remission in a significant proportion of patients. The evidence supporting an upper limit of 450 pmol/ $8 \times 10^8$  RBC is not so strong. The cut-off point is based on the observed increase in risk of myelotoxicity above 450 pmol/ $8 \times 10^8$  RBC and on the results of studies showing that the proportion of patients in remission does not increase significantly with 6-TGN concentrations  $>450$  pmol/ $8 \times 10^8$  RBC.<sup>21</sup>

In different studies of adolescent Crohn's disease patients, a significant correlation was shown between 6-TGN concentration and disease activity measured using a modified Harvey-Bradshaw index. They found that treatment efficacy correlated with 6-TGN concentrations  $>250$  pmol/ $8 \times 10^8$  Red Blood Cells (RBC) in patients with colonic and fistulating, but not ileocolonic, disease.<sup>22</sup>

6-Thioguanine nucleotide concentrations  $>450$  pmol/ $8 \times 10^8$  RBC are associated with increased risk of myelotoxicity, which will occur universally in TPMT deficient patients commenced on standard thiopurine doses. However, some patients have very high 6-TGN concentrations for long periods without developing myelotoxicity, and myelotoxicity may also occur with 6-TGN concentrations  $<450$  pmol/ $8 \times 10^8$  RBC. While some authors have suggested that patients commenced on a thiopurine drug for IBD should have routine 6-TGN monitoring to identify those with high 6-

TGN concentrations, the evidence for this is not strong. Patients commenced on 6-thioguanine for IBD have developed 6-TGN concentrations  $>1000$  pmol/ $8 \times 10^8$  RBC and yet appear not to have an increased risk of myelotoxicity.<sup>23,24</sup>

### **6-Methylmercaptopurine Concentration and Thiopurine Drug Toxicity**

As with erythrocyte 6-TGN concentration and myelotoxicity, the relationship between elevated erythrocyte 6-MMP concentration and elevated transaminases is not absolute. Patients without elevated 6-MMP concentrations may develop elevated transaminases and *vice versa*.<sup>25</sup> The clinical significance of elevated transaminases with thiopurine therapy is not well understood. While elevations in transaminases may be transitory, mild elevations of transaminases have been associated with nodular regenerative hyperplasia and portal hypertension in IBD patients taking 6-thioguanine. Mild elevations of transaminases should not be confused with progressive hyperbilirubinemia, which is rare but more serious.<sup>26</sup>

### **Thiopurines Mechanism of Action**

6-TGNs are considered the pharmacologically active end metabolites of thiopurines. Classic immunosuppressive theory specifically ascribes their mechanism of action to cytotoxic pathways. Owing to their structural similarity to endogenous purine bases, 6-TGNs can be incorporated into DNA or RNA as 'fraudulent' bases, which can result in strand breakage, inhibition of DNA repair mechanisms, inhibition of replication and interference with *de novo* synthesis of proteins and nucleic acids. 6-TGNs can also rival their endogenous counterparts (e.g. guanosine triphosphate) with respect to their crucial roles in intracellular messaging and energy carrying processes.<sup>27</sup> As a result of these actions, cellular growth and proliferation of T and B cells might be impeded, and this may cause immunosuppression.<sup>28</sup>

6-MMPR can also contribute to the immunosuppressive potential of thiopurines, as it

inhibits *de novo* purine synthesis. It has also been suggested that 6-TGNs can inhibit the inflammatory mediator, interferon  $\gamma$ . Genomewide expression profiling studies have indicated that AZA inhibits the stimulus-induced expression of several genes involved in the metabolism, signal transduction and immune functions of T cells. More recently, the activity of 6-TGTP was found to contribute significantly to the overall molecular immunosuppressive effect of thiopurine therapy in patients with IBD, especially on the mucosal immune system of the gut. This specific end metabolite binds to and inhibits the function of the small GTPase Rac1 on CD28-mediated co-stimulation in T cells, thereby inducing apoptosis. Rac1 is important for the inhibition of T-cell apoptosis. When 6-TGTP binds to Rac1 instead of GTP, the activation of target genes such as those encoding nuclear factor kappa B, mitogen-activated protein kinase and bcl-xL is suppressed, leading to mitochondria-mediated T-cell apoptosis. Moreover, 6-TGTP suppresses ezrin-radixin-moesin dependent conjugation of T cells and antigen presenting cells by modulating the Vav-Rac1 signaling pathway. *In vitro*, it has been established that the therapeutic levels of thiopurine metabolites generated from clinically relevant doses of AZA (2.5 mg/kg), 6-MP (1.5 mg/kg) and 6-TG (0.3 mg/kg) mainly induce anti-inflammatory, apoptosis-inducing effects rather than cytotoxic effects.<sup>29,30</sup>

### **Thiopurines Efficacy**

Thiopurines have proven efficacy in IBD. In clinical practice, in 68% of IBD-patients the initial therapeutic goal (i.e. mucosal healing, elimination of steroids, healing of internal fistulas or abscesses, pain relief,.. etc) is achieved and after initial response, efficacy is reasonably well sustained with remission rates of 95%, 69% and 55% after 1, 3 and 5 years, respectively.<sup>31</sup>

Both AZA and MP have proven efficacy in induction of remission in active Crohn's Disease (CD) with Odds Ratios (OR) up to 3.1 compared with placebo. The OR of response increases after

17 weeks, suggesting that there is a minimum length of time for trial of thiopurine therapy. Thereby, the thiopurines have a considerable steroid-sparing effect and the combination of AZA with prednisolone is superior to treatment with prednisolone alone.<sup>32</sup>

Azathioprine and MP seem to be effective in maintaining remission of quiescent CD patients that are steroid dependent or refractory or in CD-patients finished with surgical treatment. Moreover, in the prevention of exacerbations, the addition of MP to a regimen of steroids significantly lessens the need for prednisone. There is debate for how long thiopurines should be given to CD-patients in prolonged remission. Some question the use of potential toxic immunosuppressants for longer than 4 years, whereas, others demonstrate that their efficacy is reasonably well sustained over a period of 5 years.<sup>33</sup>

Azathioprine and MP in combination with other immunosuppressants like cyclosporine, tacrolimus, methotrexate, thalidomide or infliximab have proven efficacy in CD perianal fistulas with prolonged fistula closure in 30-40% of cases. Moreover, both thiopurines help to prevent the recurrence of CD after surgery, especially in high risk patients.<sup>34</sup>

Although less pronounced than in CD populations, both AZA and MP are effective in inducing remission in Ulcerative Colitis (UC) with remission rates up to 70%. However, scarce but conflicting data have been published, demonstrating that AZA had no effect at all in achieving remission. The role of thiopurines in maintaining remission of UC is less controversial. AZA maintenance treatment in UC is beneficial for at least 2 years after an initial response and significantly lowers the proportion of relapses. Moreover, combination therapy is more effective than treatment with steroids or 5-aminosalicylic acids (5-ASA) alone. Also, the combination of thiopurines and oral cyclosporine has proven to be safe and effective for maintenance of intravenous cyclosporine-induced remission of

severe steroid-refractory UC, although monotherapy with a thiopurines may be sufficient.<sup>35</sup>

Thioguanine (TG) may be a safer and more efficacious thiopurine in the subgroup of IBD patients resistant to AZA or MP therapy. Theoretically, the administration of TG also seems an attractive approach in patients with AZA or MP intolerance, because the number of possibly toxic metabolites is strongly reduced. In a study with 49 AZA or MP intolerant or resistant IBD patients, only 10% was forced to discontinue TG due to the occurrence of adverse events. 6-TGN concentrations were considerably higher on TG compared with the concentrations measured on AZA or MP therapy in all studies. Despite higher 6-TGN levels, no haematological toxicity occurred. Initial enthusiasm tempered when nodular regenerative hyperplasia (NRH) of the liver was reported in a substantial number of TG treated IBD patients. NRH is a serious complication also associated with AZA and MP and a frequent cause of portal hypertension. Currently, all use of TG in the IBD setting is strongly discouraged until more definite results on toxicity are available.<sup>36</sup>

### **Thiopurines Toxicity**

The incidence of dose-independent adverse effects (Idiosyncratic reactions) as a result of thiopurine therapy is in the range of 1.0-6.5%. These adverse effects are considered to be immune-mediated, and include rash, arthralgia, hepatitis, myalgia, flu-like symptoms, gastrointestinal complaints, fever and pancreatitis. Adverse effects occur most commonly within 4 weeks after initiation of AZA or 6-MP therapy. Such idiosyncratic reactions induced by AZA therapy can be partially overcome by switching to 6-MP therapy, thereby indicating that the imidazole moiety of AZA might be responsible for the adverse effects.<sup>37</sup> Surprisingly, diminished activity of the enzyme ITPase has also been associated with the induction of rash, flu-like symptoms and pancreatitis, as well as leukopenia. Potentially, patients with deficient ITPase might accumulate 6-TIMP during AZA or 6-MP therapy. The role of

ITPase activity in the development of adverse effects should, however, be assessed in studies that measure levels of metabolites such as 6-TIMP, 6-TIDP and/or 6-TITP, because the evidence currently available does not include this type of characterization.<sup>38</sup>

The incidence of 6-TG-induced dose independent adverse effects is approximately 20%. In a 1-year trial of 6-TG therapy that included a series of 95 patients, the adverse effects that led to withdrawal of 6-TG were gastrointestinal disturbances (8.4%), hepatotoxicity (4%), pancreaticotoxicity (1%), general malaise (4.2%) and allergic reaction (1%). The reoccurrence of AZA-induced or 6-MP-induced pancreatitis during 6-TG therapy is rare.<sup>39</sup>

### **Myelotoxicity**

The definition of drug-induced leucopenia varied markedly, but the cutoff point for the number of leukocytes was generally set at  $3-4 \times 10^9/L$ , and neutropenia when the absolute neutrophil count of less than  $1.5 \times 10^9/L$ . The reported frequency of myelotoxicity during AZA or 6-MP therapy is in the range 3-7%. Myelotoxicity is considered a dose-dependent adverse effect that can be caused by elevated concentrations of the pharmacologically active 6-TGNs. In a subpopulation of patients with IBD who also had leukopenia, a mean 6-TGN level in RBCs of  $490 \text{ pmol}/8 \times 10^8$  was observed.<sup>40</sup>

TPMT has a pivotal role in the bioavailability of 6-TGNs; therefore, diminished TPMT activity shifts 6-MP and 6-TIMP away from the formation of 6-MMP and 6-MMPR and towards increased production of 6-TGNs. Severe, and sometimes life-threatening, pancytopenia is likely to occur in patients who have an inherited TPMT deficiency;<sup>41</sup> however, variation in the activity of TPMT is not the only risk factor for the development of myelodepression, but can also be induced by factors such as viral infections, co-medications that affect thiopurine metabolism (e.g. 5-aminosalicylic acid [5-ASA], aspirin or furosemide) and medications that can induce myelodepression itself.<sup>42</sup>

The risk of myelotoxicity associated with 6-TG therapy seems to be equivalent to that associated with AZA or 6-MP therapy.<sup>43</sup> The incidence of leukopenia was 3.2% after 1 year of 20-40 mg of 6-TG therapy taken daily. Finding high 6-TGN levels in erythrocytes during 6-TG therapy was not indicative of the development of myelotoxicity. This observation suggests that the risk with different thiopurine drugs is probably too small to influence the choice of agent.<sup>44</sup>

Bone marrow toxicity from AZA/MP treatment may develop at any time after starting the AZA/MP therapy, as has been demonstrated in long-term (>20 yr) follow-up studies of AZA/MP treatment in IBD. The duration of AZA/MP treatment in patients with myelotoxicity ranged from 12 days to 27 year.<sup>45</sup> Nevertheless, thiopurine-induced myelotoxicity frequently occurs within the first weeks or months of treatment. Myelosuppression may occur suddenly or over a period of several months. However, most cases of severe leucopenia occurred abruptly (and early in the treatment). This observation suggests that the recommendation of performing analytical controls (for example, every 3 months) may have limited efficacy on the early detection of bone marrow toxicity.<sup>46</sup>

The AZA/MP-induced neutropenia is often asymptomatic or presents an insidious onset. The classic presentation in symptomatic patients is severe sore throat and fever. The warning signs in the form of symptoms do not occur until myelosuppression is advanced. Consequently, it seems prudent to decrease the dose or to stop the drug if moderate suppression of the white blood cells occurs; an intervention after the symptoms have occurred may be too late.<sup>47</sup> Infections are the only significant consequence of neutropenia. The cumulative incidence of infections among those patients suffering from AZA/MP-induced myelotoxicity, was 6.5%. The common sites of infection include the oral cavity and mucous membranes and the skin; and with severe, persistent neutropenia, systemic infections in the lungs and bloodstream may occur. Endogenous bacterial flora is the most common pathogen.

Surprisingly, many of the infectious complications in patients treated with AZA/MP occur in the absence of leukopenia. Furthermore, it still has to be analyzed in a prospective randomized fashion if the presence of leukopenia leads to a higher rate of infectious complications.<sup>48</sup> Nevertheless, a relationship seems to exist between the degree of neutropenia and the infection risk. It holds particularly well in situations in which neutrophil production is affected at the stem cell or myeloblast stage of maturation and/or in which the marrow is hypocellular. In comparison, the degree of neutropenia does not correlate as well with a propensity to infection in neutropenic patients.<sup>49</sup>

Neutropenia is often categorized as mild, moderate, or severe based upon the level of absolute neutrophil count. Mild neutropenia corresponds to an absolute neutrophil count between  $1.0$  and  $1.5 \times 10^9/L$ , moderate between  $0.5$  and  $1.0 \times 10^9/L$ , and severe less than  $0.5 \times 10^9/L$ . The risk of infection begins to increase at an absolute neutrophil count below  $1 \times 10^9/L$ .<sup>50</sup>

The propensity to infection in neutropenic patients is related to the absolute neutrophil count and the duration of neutropenia. In patients with a neutrophil count  $>1,500 \times 10^9/L$ , no significant risk of infection exists, and fever can be managed on an outpatient basis. In patients with a neutrophil count between  $500$  and  $1,000 \times 10^9/L$ , some risk of infection has been described, and fever can occasionally be managed on an outpatient basis.<sup>51</sup> When the number of neutrophils is lower than  $500$ , a significant risk of infection exists, and therefore, fever should always be managed on an inpatient basis with parenteral antibiotics (although even these patients usually have few or no clinical signs of infection precisely due to neutropenia). The administration of recombinant G-CSF can correct neutropenia and reduce infectious morbidity in infected patients. The risk of death among IBD patients who had developed myelotoxicity is approximately 1%.<sup>52</sup>

In cases of mild leukopenia, it has been reported that leucopenia has been resolved spontaneously

without change in dose, so AZA/MP dosage may be kept unchanged with close follow-up. The appropriate cutoff values for leukocyte or neutrophil count which indicate that the dose of AZA/MP should be lowered or the drug be stopped are still unknown. Some “conservative” authors have suggested to lower the AZA/MP dose (for example, a 50% reduction) when leukocyte count is  $<4 \times 10^9/L$ .<sup>53</sup> Others have advised this same attitude when this figure is  $<3 \times 10^9/L$ . However, the risk of myelotoxicity is more closely related with neutropenia than with the total leukocyte count. Neutropenia is defined as an absolute neutrophil count of less than  $1.5 \times 10^9/L$ ; so this figure seems to be a more appropriate cutoff for deciding dose modification. In cases of mild neutropenia, which corresponds to an absolute neutrophil count between 1.0 and  $1.5 \times 10^9/L$ , dose reduction (for example, to 50%) may be sufficient to resolve leukopenia.<sup>54</sup> Nevertheless, some authors have observed that after AZA/MP dose has been reduced to 50%, the dose may be safely increased again to 100% once leukocyte values have normalized. Obviously, in cases of leucopenia relapse, the dose should be reduced permanently and individualized with great care. As the risk of infection begins to increase at an absolute neutrophil count below  $1 \times 10^9/L$ , it seems prudent to stop AZA/MP administration (and not only decrease the dose) in patients with lower values. Nevertheless, it has been suggested that even if the AZA/MP treatment has been withdrawn, if leukocyte count normalizes after stopping the treatment, it may be restarted (for example, with 50% of the initial dose); obviously, in cases of relapse, AZA/MP should be permanently stopped.<sup>55</sup>

Numerous authors have reported a favorable evolution of bone marrow toxicity after withdrawal of the AZA/MP treatment. However, exceptional cases have also been reported in which the patient has died of infection despite withdrawal of the drug. The time necessary for normalization of the leukocyte count after AZA/MP withdrawal may be variable, ranging from 1 week to several months.<sup>56</sup>

As thiopurine-induced myelotoxicity occurs more often within the first months of treatment, more frequent analytical controls are generally recommended during the first months after starting the AZA/MP treatment.<sup>57</sup> The current recommendations suggest that when initiating therapy with either MP or AZA, complete blood count measurement with differential is advisable at least every other week as long as the drug dose is being adjusted; thereafter, this measurement should be performed as clinically appropriate at least once every 3 months. For the long term, the most recommended schedule advises leukocyte count every 3 months. Clinicians should be alert to a sudden myelosuppression, which can occur despite close monitoring; and patients should be educated to pay attention to unusual symptoms or signs of infection.<sup>58</sup>

### **Hepatotoxicity**

Different types of abnormal liver test results are reported as signs of hepatotoxicity. The pathogenesis of these allergic-like reactions is unknown; however, the incidence of hepatotoxicity (defined as elevated levels of one or more liver enzymes) associated with AZA or 6-MP use seems to vary between 0% and 17%. This marked variation in the reported incidence of hepatotoxicity can be largely explained by differences in the definitions of hepatotoxicity applied, the number of patients studied, the duration of follow-up and the type of study design used. Thiopurine-induced hepatotoxicity can be dose-independent or dose-dependent. The onset of dose-independent hepatotoxicity is usually within weeks after initiation of drug therapy.<sup>59</sup>

In general, abnormalities in liver enzyme levels disappear after cessation of thiopurine therapy. The level of 6-MMPR during AZA or 6-MP therapy has been associated with hepatotoxicity. The role of 6-MMPR in the induction of liver enzyme abnormalities remains a matter of ongoing discussion, because signs of hepatotoxicity are also observed in patients who have low levels of 6-MMPR, which means that the sensitivity and specificity of 6-MMPR levels

for predicting thiopurine-induced liver enzyme abnormalities are poor.<sup>60</sup>

Sinusoidal dilatation, nodular regenerative hyperplasia (NRH), fibrosis, peliosis hepatis and Venous Occlusive Disease (VOD) are considered to be signs of dose-dependent hepatotoxicity. These histopathological changes often appear after months to years of thiopurine therapy. The reversibility of these histological liver disturbances is unknown. These hepatotoxicity associated conditions are thought to be induced by disorders of the liver vasculature. Sinusoidal dilatation is assumed to be an early and essentially reversible, less-severe form of VOD, while VOD is characterized by additional involvement of the central venules. In the case of NRH, vascular flow impairment can lead to diffuse hepatocyte hyperplasia and nodule formation.<sup>61</sup> NRH can ultimately lead to noncirrhotic portal hypertension with splenomegaly. Male sex and stricturing disease behavior were associated with NRH in patients treated with AZA. NRH is rare, but it is probably underestimated. The prevalence of asymptomatic forms of NRH is high. NRH lesions might be reversible when they have been caused by relatively low dosages of thiopurines. Thus, diagnosis of NRH at an early stage seems critical. The use of 6-TG has been clearly associated with the development of NRH. The 6-TG dosage administered in most studies has varied between 40 mg and 80 mg, resulting in 6-TGN levels in RBCs above  $1,000 \text{ pmol}/8 \times 10^8$ . In these series, signs of NRH were observed in 18-62% of liver biopsy samples. In a group of 28 patients with IBD who were administered low-dose 6-TG (approximately 20 mg per day; mean 6-TGN level in RBCs of  $564 \text{ pmol}/8 \times 10^8$ ) for a period of at least 30 consecutive months, no cases of NRH were observed. The induction of this histological liver abnormality might be dependent on the dose of 6-TG or the level of 6-TGN.<sup>62</sup>

### **Malignancies**

Concerns have been raised about the possible induction of malignancies, such as lymphomas

and skin cancer, by thiopurine therapy. Increased frequencies of malignancies have been reported in other patient populations exposed to thiopurines, but not in patients with IBD.<sup>63</sup> One study demonstrated that patients with IBD had a four fold increased risk of developing a lymphoma during thiopurine therapy; however, the authors stated that the increased risk could be a result of the thiopurine administration, the severity of disease or a combination of both. Authors of a decision analysis concluded that the potential risk of developing a lymphoma as a result of thiopurine therapy are clearly outweighed by the benefits of the therapy.<sup>64</sup>

### **Thiopurines Interactions**

A number of coadministered drugs may potentially influence thiopurine metabolism and consequently has to be taken into account.

*In vitro* studies have demonstrated 5-aminosalicylic acid (5-ASA) compounds (i.e. balsalazide, olsalazine and sulphasalazine) to be potent TPMT inhibitors. *In vivo*, 6-TGN concentrations are significantly higher when thiopurines are combined with 5-ASA. The observed higher 6-TGN levels can not solely be explained by TPMT inhibition and other yet unknown interaction mechanisms may exist. Co-medication with 5-ASA could, potentially, increase a patient's risk of developing leukopenia owing to the generation of elevated 6-TGN levels.<sup>65</sup> Other frequently prescribed TPMT inhibitors include acetylsalicylic acid and furosemide.<sup>66</sup>

Allopurinol potently inhibits XO and a dose-reduction to 25-33% of standard daily dosages of AZA or MP is recommended when combined to prevent serious myelotoxicity. Interestingly, it has been demonstrated that co-medication with allopurinol in patients with nonresponsive IBD can potentiate AZA therapy by increasing 6-TGN levels.<sup>67</sup>

Mycophenolate inhibits IMPD, theoretically reducing the conversion of 6-TIMP to 6-TXMP and 6-TGNs consequently.<sup>68</sup>

### **Thiopurines in Pregnant IBD Patient**

6-Mercaptopurine (6MP) and its prodrug azathioprine (AZA) are pregnancy category D drugs. Animal studies have demonstrated teratogenicity with increased frequencies of cleft palate, open-eye and skeletal anomalies seen in mice exposed to AZA and cleft palate, skeletal anomalies and urogenital anomalies seen in rats.<sup>69</sup> Transplacental and transamniotic transmission of AZA and its metabolites from the mother to the fetus can occur. The oral bioavailability of AZA (47%) and 6MP (16%) is low and the early fetal liver lacks the enzyme inosinate pyrophosphorylase needed to convert AZA to 6MP. Both features may protect the fetus from toxic drug exposure during the crucial period of organogenesis.<sup>70</sup> The largest evidence on safety comes from transplantation studies, where rates of anomalies ranged from 0%-11.8% and no evidence of recurrent patterns of congenital anomalies emerged.<sup>71</sup>

In IBD, multiple case series have not noted an increase in congenital anomalies. One study, however, did report a higher incidence of fetal loss in women with IBD with prior treatment on 6MP compared to those who never had 6MP exposure.<sup>72</sup> Finally, the largest single study to date studied women who called a teratogen information service. Compared were 189 women exposed to AZA during pregnancy to 230 women who did not take any teratogenic medications during pregnancy. The rate of major malformations did not differ between groups with 6 neonates in each; for AZA the rate was 3.5% and for the control group rate it was 3.0%. The AZA group had more cases of prematurity (21.4% versus 5.2% [ $P= 0.001$ ]) and LBW (23% versus 6.0% [ $P= 0.001$ ]) as well, but this most likely reflects their underlying disease state, which was not controlled.<sup>73</sup>

### **Thiopurines for the Prevention of Postoperative Recurrence in Crohn's Disease**

In the overall analysis, purine analogs were more effective than were control arms in the prevention of clinical recurrence at 1 year ( $P =$

0.021). At 2 years, the mean difference and CI 95 % were 13 and 2- 24 %, respectively ( $P = 0.018$ ).<sup>74</sup>

Purine analogs were more effective than were control arms in preventing severe endoscopic recurrence (2-4 according to Rutgeerts' score) at 1 year ( $P = 0.026$ ). In the overall analysis, thiopurines were not effective for preventing very severe (3-4) postoperative recurrence at 1 year ( $P = 0.13$ ). Efficacy at 2 years could not be evaluated because only one study was available for this end point. In sensitivity analysis after exclusion of 5-ASA arms, i.e., including only placebo arms with or without antibiotic induction therapy, the efficacy of purine analogs was observed for the prevention of severe ( $P = 0.0016$ ) but not very severe ( $P = 0.094$ ) endoscopic recurrence at 1 year.<sup>75</sup>

### **The Effects of Thiopurine Therapy on Quality of Life in IBD Patients**

There is a significant improvement in all indications of the quality of Life when IBD patients treated with thiopurines.<sup>76</sup> This fact could be explained by several reasons. The healing of lesions and disappearance of symptoms induced by the azathioprine, as well as the prevention of postoperative recurrence possibly will be the causes of the improvement in the HRQoL.<sup>77</sup> Future investigations should assess the long-term impact on HRQoL of new scenarios in which thiopurines are currently used, such as early introduction in both UC and CD. Another important issue that should be addressed in future investigations is the implications on HRQoL and in the natural history of azathioprine-induced mucosal healing.

### **Most Important Practical, Unanswered Questions in the Use of Thiopurines in Inflammatory Bowel Disease**

The following 8 questions are a literature search and draft answers identified by the International Steering Committee of the IBD ahead 2010, 4<sup>th</sup> annual exchange on the advances in Inflammatory Bowel Disease.

**Question 1:** How early should immunosuppressives be introduced in the management of Crohn's disease and which regimen should be used?

Initiation of thiopurines early in the disease course (at diagnosis) should be considered in patients at high risk of complicated disease. Thiopurines are indicated in immunosuppressive-naïve patients starting systemic steroids or infliximab in order to achieve steroid-sparing effects or added benefit. Purine analogues are indicated in post-operative prophylaxis immediately after surgical resection of ileocolonic disease. Evidence for the use of purine analogues as first-line therapy in perianal fistulating Crohn's disease is limited.<sup>78-80</sup>

**Question 2:** What is the best dosing strategy for thiopurines in Crohn's disease, in terms of: starting and maximum doses, duration, dose escalation/de-escalation (when? rate?), which immunosuppressive first?

The most effective doses appear to be 2-3 mg/kg for azathioprine and 1-1.5 mg/kg for 6-mercaptopurine administered orally, based on reported clinical trials. There is no evidence to support dose de-escalation. Azathioprine is used as a first-line immunosuppressive, as higher response rates were obtained with it compared with 6-mercaptopurine. Recommended initial dose strategies are either a gradual dose increase starting with 50 mg of azathioprine (25 mg of 6-mercaptopurine) or full dose therapy with prior determination of thiopurine methyltransferase activity/genotype. Azathioprine or 6-mercaptopurine treatment should be maintained for several years due to a high relapse rate in patients with Crohn's disease when these drugs are discontinued.<sup>81-83</sup>

**Question 3:** How should the efficacy of thiopurines treatment be monitored clinically and biologically? What is the definition of treatment failure? When should the effect of treatment be evaluated? Should mucosal healing be assessed to monitor the efficacy of treatment?

Remission of signs and symptoms is the most widely clinically accepted endpoint for treatment efficacy. The Crohn's Disease Activity Index and Harvey Bradshaw Index are accepted tools for quantification of efficacy in clinical trials. However, their use outside this environment is limited by their cumbersome nature. Indirect biomarkers of treatment efficacy include elevated serum C-reactive protein (correlates well with disease relapse and mucosal healing) and faecal calprotectin below the cut-off level of the individual test (predictive of mucosal healing and reduced relapse in Crohn's disease). The use of azathioprine metabolites may help management decisions and more accurately identify non-responders. For treatment with thiopurines, clinical response should be assessed after 3 months. However, if mucosal healing is to be assessed, this should be performed between 6 and 12 months. Patients failing to respond symptomatically after adequate therapy with thiopurines or methotrexate for at least 3-6 months constitute a treatment failure.<sup>84,85</sup>

Achievement of mucosal healing in Crohn's disease leads to prolonged steroid-free remission, fewer abdominal surgeries and may reduce hospitalizations. There is good evidence to suggest that azathioprine is effective at healing the colonic mucosa completely. Early combined immunosuppressive therapy in moderately active Crohn's disease is superior to standard therapy in establishing mucosal healing. There is currently insufficient evidence to recommend the routine assessment of mucosal healing in the absence of a clinical indication. Non-invasive markers such as C-reactive protein and in particular faecal calprotectin may offer realistic alternatives to endoscopy for the assessment of mucosal healing.<sup>86,87</sup>

**Question 4:** If azathioprine and a biologic are given in combination, should any of the treatment be stopped? Which treatment should be stopped to achieve the smallest reduction in efficacy? When should that treatment be stopped?

In patients with moderately active Crohn's disease naïve to thiopurines therapy, the combination of thiopurines with infliximab improves rates of steroid-free remission up to 1 year after commencement of therapy. In patients refractory to thiopurines therapy, continuation of that therapy in conjunction with a biologic offers no clinical benefit up to 2 years.<sup>88,89</sup>

If the thiopurines is to be continued in conjunction with a biologic, then the immunosuppressive may be discontinued after 6 months. However, this decision must be individualized. It is unclear if patients on a long-term combination of azathioprine and a biologic have an increased risk of opportunistic infection or malignancy. There is a small potential risk of hepatosplenic T-cell lymphoma in young males with Crohn's disease being treated with a combination of azathioprine and infliximab.<sup>90-91</sup>

**Question 5:** If the thiopurines does not work, what should then the approach be? Increase the dosage? Add steroids? Change the immunosuppressive? Move to a biologic?

Anti-TNF agents should be the first consideration in patients who have been on thiopurines and have lost response. Adding steroids may be necessary in the short term, but they are not recommended for long-term use (patients should be weaned off steroids). In the setting of intolerance or side effects to purine metabolite immunosuppressives, other immunosuppressives may be considered. Alternative immunosuppressives include 6-mercaptopurine, methotrexate, tacrolimus and 6-thioguanine (in limited settings only). Optimization of thiopurine therapy should always be considered if underdosing is suspected on a dose/weight basis.<sup>92-94</sup>

**Question 6:** If a patient experiences flare-ups when receiving thiopurines, should corticosteroids be added?

Patients failing immunosuppressive therapy can be started on corticosteroids to help induce remission when transitioning to another

immunosuppressive or biologic agent. When started, corticosteroid dose should be rapidly tapered over a period of weeks to avoid long-term exposure. Given their significant side-effect profile, use of corticosteroids should be limited or avoided where possible.<sup>95-97</sup>

**Question 7:** What are the risks of cancers (all kinds) and infections associated with the short-, mid- and long-term use of thiopurines?

Although the overall cancer risk does not seem to be increased in patients on immunosuppressives, thiopurines increase the risk of lymphoproliferative disorders in IBD patients. Thiopurines are associated with an increased risk of infection. The risk of infection in patients with IBD increases with the number of anti-inflammatory agents that are used concomitantly. The concomitant use of thiopurines agents and biologics should be minimized, especially in adolescents and young adults.<sup>98-100</sup>

**Question 8:** What is the optimal safety monitoring (clinical, laboratory, radiological) of patients receiving thiopurines? How often?

Immunosuppressive therapy is associated with myelosuppression. Patients with low thiopurine methyltransferase (TPMT) activity are at increased risk of developing severe myelosuppression. However, 73% of patients with severe bone marrow suppression do not carry a TPMT mutation. As TPMT analysis may predict 90% of life-threatening episodes and 60% of severe and moderate episodes of neutropenia, measuring TPMT activity prior to starting thiopurines is a cost-effective way to identify patients at high risk of severe haematological complications. All patients receiving thiopurines need regular monitoring of the full blood count and liver tests. Measurement of full blood count and liver function tests are advisable before and within 4 weeks of starting therapy, then monthly to every 3 months. Nodular regenerative hyperplasia is a rare but potentially severe complication of azathioprine in patients with IBD. Clinicians should be aware of this complication and should monitor liver function

tests and platelet counts closely.<sup>101-103</sup>

**Conclusions**

Thiopurines are not suitable as first-line treatment in inflammatory bowel disease owing to the delay in obtaining clinical efficacy. In the clinical use of these agents, physicians must maintain a subtle balance between the therapeutic benefits and potential risks or side-effects, and patients must accept the need for regular monitoring after a thorough education in potential toxicities.

In Crohn's disease, AZA and 6MP are indicated in chronic active disease that fails to respond to glucocorticoids, or when the prednisolone dosage cannot be reduced below 10-15 mg, particularly if adverse effects are problematic. The drugs should be used to maintain remission, but only in patients with previous extensive or troublesome chronic active disease. Furthermore, AZA and 6MP can be administered with advantage to Crohn's disease patients with fistulous complications.

In ulcerative colitis, chronic unresponsive glucocorticoid- dependent disease and frequent relapses (more than three in 2 years) are, in our opinion, indications for AZA or 6MP treatment. In older patients with longstanding total colitis (10 years or more of pancolonic ulcerative colitis), concerns about the disease-related risk of colorectal cancer may outweigh the benefits of

drug therapy and therefore result in the need for total colectomy, whereas AZA or 6MP may have a major role in younger patients with more recent onset of disease who wish to avoid surgery. Left-sided disease more commonly fails to respond fully to glucocorticoids or mesalazine preparations, and purine analogues are particularly useful in this subgroup. Obviously, maintenance therapy should be restricted to patients who have achieved satisfactory remission using these drugs.

For steroid-dependent patients, AZA and 6MP appear to be beneficial in both Crohn's disease and ulcerative colitis and, for the maintenance of remission, the use of AZA or 6MP should be considered in both diseases. If effective, they can be continued for a minimum of 4-5 years, although this period may be much longer.

It may be worthwhile to introduce therapeutic drug monitoring by investigating 6-thioguanine nucleotides during standard oral AZA therapy so that more insight into the therapeutic window may be obtained. However, TMPT genotyping does not appear to satisfactorily predict the development of myelosuppression. Finally, it would be useful to design new oral AZA formulations, which deliver the active metabolites directly to the inflamed intestine, in order to reduce the toxicity or adverse effects and at the same time improve the therapeutic ratio. A summary of the recommendations from this comprehensive review is given in Table (1).

**Table (1): Summary of Recommendations.**

<b>Maintenance Dosage</b>	<i>AZA (2.5 mg/kg daily); 6MP (1.5 mg/kg daily)</i>
<b>Monitoring</b>	<i>Initially blood tests every week (haemoglobin, white blood cell count, platelets, alanine aminotransferase, amylase if abdominal pain occurs); thereafter monthly or every second month for the duration of therapy.</i>
<b>Effect</b>	<i>Efficacy of AZA or 6MP may be expected after 3 months of treatment.</i>
<b>Drug Interaction</b>	<i>In allopurinol-treated patients, half dosage of AZA or 6MP should be applied.</i>
<b>Indications</b>	<i>AZA or 6MP may be administered on equal indications in both Crohn's disease and ulcerative colitis with frequent flare-ups, and in those who experience relapse of symptoms on tapering off glucocorticoid.</i>
<b>Duration</b>	<i>If tolerated and effective, AZA or 6MP can be continued for 4-5 years.</i>
<b>Pregnancy</b>	<i>No teratogenic effects of AZA or 6MP have been reported, including pregnant women with rheumatic disorders or kidney transplantation. However, breast-feeding is contraindicated in women taking AZA or 6MP. A slightly increased risk of spontaneous abortion or congenital abnormalities is seen in offspring of males with inflammatory bowel disease on AZA or 6MP.</i>

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## علاج التهاب القولون التقرحي باستخدام أدوية الثيوبورين

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### الملخص

**الهدف:** بيان التطبيق السريري لاستخدام أدوية الثيوبورين في معالجة مرض التهاب القولون التقرحي. تنسق ادوية الثيوبورين المناعة، وتستخدم لمعالجة السرطانات وأمراض المفاصل وبعض الأمراض الجلدية والتهاب القولون التقرحي، وكذلك في حالة نقل الأعضاء. هذه المجموعة من الأدوية تضم ازابوبرين وميركاتوبورين والثيوفوانين. ويمكن أن يكون قياس ما ينتج من أيض هذه الأدوية ذا فائدة لتحديد كمية الدواء اللازم وكذلك اجتناب التأثيرات الجانبية.

**النتائج:** عندما يدخل ازابوبرين الجسم يتحول إلى ميركاتوبورين ومن ثم إلى الثيوفوانين، وأي من هذه المركبات يستخدم لمعالجة التهاب القولون التقرحي بشكل فعال، وخاصة لدى من يقاومون الستيرويدات. كذلك تستخدم أدوية الثيوبورين لمعالجة مضاعفات التهاب القولون التقرحي مثل الناسور. أما المشاكل المتعلقة بالتعامل مع هذه الأدوية فهي: طول المدة لبداية عملها (3-4 شهور) وتأثيراتها الجانبية. الهدف من هذه المقالة هو بيان التطبيق السريري لاستخدام ادوية الثيوبورين في معالجة مرض التهاب القولون التقرحي.

**الكلمات الدالة:** الثيوبورين، متناظرة البيورين، المناعة، مرض كرون، التهاب القولون التقرحي، التهاب الأمعاء.