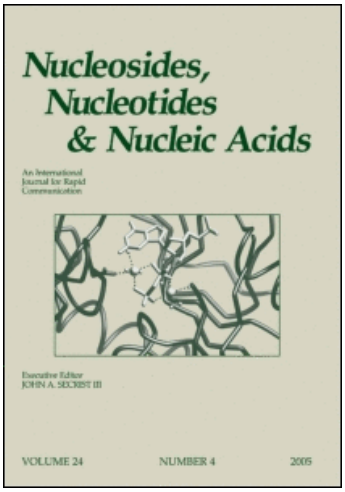

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Novel Strategies in the Thiopurine Treatment of Inflammatory Bowel Disease

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NOVEL STRATEGIES IN THE THIOPURINE TREATMENT OF INFLAMMATORY BOWEL DISEASE

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□ *Thiopurine drugs are widely used as immunomodulatory and corticosteroid-sparing agents in inflammatory bowel disease. Despite being old drugs, a renewed research and clinical interest in their application has emerged during the last decade. The application of pharmacogenetic insights and metabolic monitoring, together with treatment strategies in combination with anti-TNFα-antibodies and possibilities to modulate their metabolism, has paved the way to a "modern" use of the thiopurines. These aspects are briefly overviewed herein.*

Keywords Thiopurines; inflammatory bowel disease; Crohn's disease; ulcerative colitis; treatment strategies

INTRODUCTION

Inflammatory bowel disease (IBD)—Crohn's disease and ulcerative colitis—are chronic inflammatory disorders affecting the gastrointestinal tract. Their aetiology is not fully known, and in general, the cause is considered to be a combination of genetic and environmental factors leading to an exaggerated immunological response in the bowel. The disorders most often present with a relapsing-remitting course with a mixture of abdominal and systemic symptoms.

The life-time risk for developing IBD has been estimated to reach 1% in Western countries. Approximately 2.2 million people are affected in Europe. The age at diagnosis is usually 20–35 years, and since the diseases are

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TABLE 1 Treatment options in patients with inflammatory bowel disease

Corticosteroids
Aminosalicylates–mesalazine, balsalazide, sulfasalazine
Antibiotics
Immunomodulators–thiopurines, methotrexate
Anti-TNFα-antibodies–infliximab, adalimumab, certolizumab pegol–"biologics"
Anti-adhesion-molecules–"biologics"
Nutritional support
Surgery–fistulising, stricturing or medically intractable disease

chronic, it follows that an individual patient will need medical treatment and surveillance during decades.

Several treatment options are available (Table 1). Corticosteroids are used both in Crohn's disease and ulcerative colitis as first-line therapy for flares, and, aminosalicylates mainly for maintenance treatment of ulcerative colitis, since evidence for their effect in Crohn's disease is weak. Antibiotics are used for complicating fistulising Crohn's disease. These therapies are inefficient or limited by adverse drug reactions in about half of the patients, and therefore, at least in specialized centers, about 50% of IBD patients are qualified for long-term immunomodulation. The thiopurine drugs are the mainstay in this respect and they are increasingly and more liberally used in IBD (see review[1]).

During the last decade anti-TNFα-antibodies—infliximab, adalimumab, certolizumab pegol—have come into widespread use, especially in patients unresponsive or intolerant to previous therapies. Their single use, or in combination with other immunomodulators, have been debated, especially in patients qualified for maintenance treatment with the anti-TNFα agents. Focus has also been placed on the economic burden to an individual patient and to the society, since anti-TNFα antibodies are far more expensive than "traditional" immunomodulators.

Inherent to all therapy is the need to individualize and optimize treatment regimes, that is, to predict the response and the risks for development of adverse events in individual patients. Of special interest is the possibility to characterize treatment failures and to weigh efficacy and adverse events against costs.

THIOPURINES AND INFLAMMATORY BOWEL DISEASE

Azathioprine and 6-mercaptopurine are considered to be equally effective even if no formal head-to-head comparison has been carried out. Their use has been limited by the occurrence of adverse events in up to 30% of patients.

TABLE 2 Indications for thiopurine treatment in Crohn's disease

A. Induction of remission
Mild-to-moderate luminal disease
Together with biologics in moderate-to-severe disease
Perianal, fistulizing disease
Extensive disease >100 cm of bowel inflamed
B. Steroid-refractory and steroid-dependent disease
C. Early relapse within 3 months of a previous flare
D. Frequent relapses ≥2 per year
E. Maintenance of remission
F. Prevention of postoperative recurrence

In Crohn's disease, the thiopurines are effective for the induction of remission, as corticosteroid-sparing agents and for the maintenance of remission (Table 2). In meta-analyses the odds ratios for efficacy as compared to placebo are in the range of 2–4 and the numbers of patients needed-to-treat are 3–6.[2]

Furthermore, a striking benefit has been demonstrated in children when introducing 6-mercaptopurine together with prednisone within eight weeks of a diagnosis of Crohn's disease.[3] Relapse rates at 18 months were 9% for 6-mercaptopurine and 47% for placebo. Prevention of postoperative recurrence is another important indication even if the results here are less impressive; the benefit of thiopurines was 8–13% over comparators and the numbers of patients needed-to-treat were 8–13 (see meta-analysis in[4]).

The available evidence-based data are stronger for Crohn's disease than for ulcerative colitis. Despite this, the use of thiopurines in ulcerative colitis has become widespread, mainly for the maintenance of remission. In an early randomized withdrawal study, the 12-month remission rate was 65% in patients who continued azathioprine compared to 40% in the placebo group.[5] In a recent 6-month study of steroid-dependent patients with ulcerative colitis, azathioprine was significantly more effective than 5-aminosalicylic acid in inducing clinical and endoscopic remission and avoiding steroid treatment-58% versus 21%.[6]

Adverse events are commonly encountered in patients under thiopurine treatment, the majority (70%) within the first three months (Figure 1). A risk for myelotoxicity persists throughout the entire treatment period with 25% of cases appearing beyond the first year.[7] However, more than 50% of azathioprine intolerant patients tolerate 6-mercaptopurine long-term (Figure 2); tolerance was obtained in more than two-thirds of patients who had experienced arthralgia/myalgia, hepatotoxicity or myelotoxicity when on azathioprine.[8] Patients that tolerated 6-mercaptopurine had higher TPMT acitivity and had less often undergone bowel resection than those who also were intolerant to 6-mercaptopurine.

The use of 6-thioguanine has been proposed in patients intolerant or unresponsive to azathioprine or 6-mercaptopurine. Due to a more

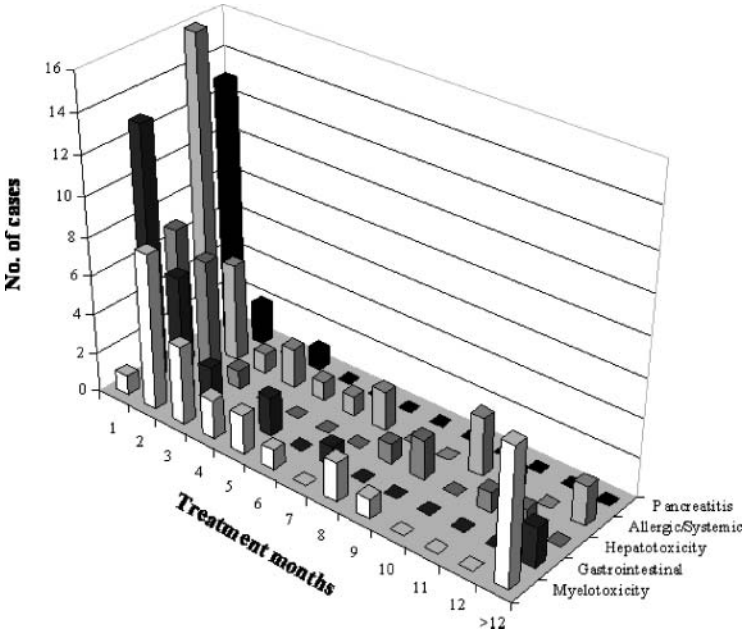


FIGURE 1 Time to emergence of adverse events during thiopurine therapy in 364 patients with inflammatory bowel disease. The majority of adverse events (70%) occurred within the first three months, while 25% of cases with myelotoxicity appeared beyond 12 months. Adapted from.[7]

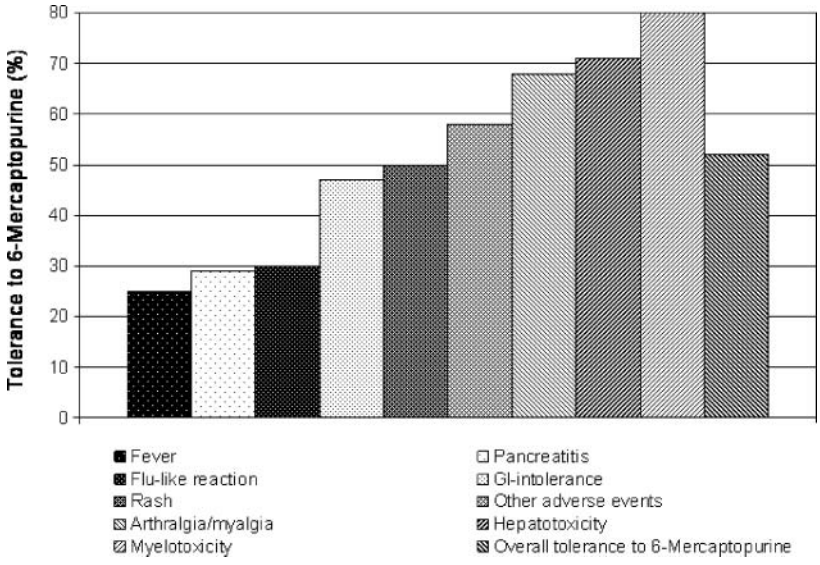


FIGURE 2 Long-term tolerance to 6-mercaptopurine in 135 azathioprine intolerant patients with inflammatory bowel disease. The percentage numbers refer to the proportion of patients with each respective adverse event on azathioprine that tolerated 6-mercaptopurine. Adapted from.[8]

straight-forward metabolism with almost no formation of methylated metabolites, adverse events have been expected to be rare. However, in a highly selected group of patients with Crohn's disease intolerant to azathioprine, 6-mercaptopurine and other immunomodulators, a benefit of 6-thioguanine was demonstrated in only a small fraction of patients.[9]

Due to serious drug-induced liver injury,[10] especially nodular regenerative hyperplasia, 6-thioguanine has, in most centers, been abandoned. It is possible that a low-dose regimen in highly selected patients under close monitoring of liver function, including MRI and liver biopsy, is defendable.[11]

COMBINATION THERAPY WITH ANTI-TNFα ANTIBODIES

Steroid-dependent patients represent a considerable proportion of patients with Crohn's disease. Thiopurines alone are effective but the combination of thiopurines with anti-TNFα-blockade may be superior to the use of either drug. Previous studies have indicated that the main contribution of thiopurines (or methotrexate) in combination therapies were to reduce the formation of autoantibodies to the anti-TNFα antibody.

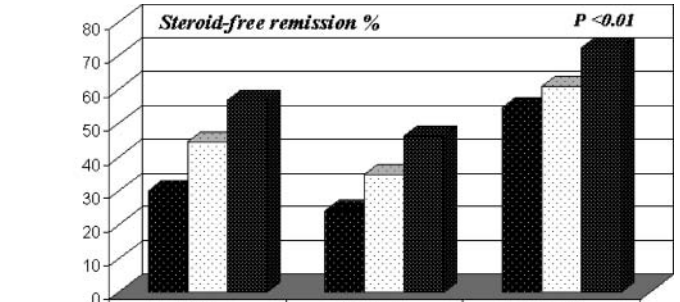
In steroid-dependent patients, a combination of three infusions of infliximab over 6 weeks together with maintenance thiopurine treatment was highly effective, especially in thiopurine-naïve patients. In this group, steroid-free clinical remission at 12 weeks was obtained in 83% of patients compared to 41% in the group that received placebo infusions plus thiopurines; at 52 weeks the proportions were 52% and 32%, respectively.[12] Combination therapy was also more effective in patients already on thiopurines, although to a lesser degree.

In the pivotal SONIC-study, patients with active Crohn's disease who were naïve both to thiopurines and to infliximab were randomized to long-term therapy with either azathioprine, infliximab or a combination of both. Impressive preliminary results for steroid-free remission at 50 weeks, both in the intention-to-treat group and in the per-protocol group (Figure 3) have recently been presented, again with an overall benefit for the combined treatment.[13]

Very few reliable data exist on the combination of thiopurines with other immunomodulators in IBD patients.

APPLICATION OF TPMT AND METABOLITE MEASUREMENTS

Azathioprine and 6-mercaptopurine are inactive prodrugs and subject to a complex metabolism that generates several active metabolites. Thioguanine nucleotides (6-TGN) are synthesized via hypoxanthine (guanine) phosphoribosyltransferase, inosine 5′-monophosphate dehydrogenase (IMPDH) and guanosine 5′-monophosphate synthetase (Figure 4).



	W26	W50 ALL	W50 PP
■ AZA + PLA	30	24,1	54,7
□ IFX + PLA	44,4	34,9	60,8
■ AZA + IFX	56,8	46,2	72,2

FIGURE 3 Preliminary one-year results of the Sonic study comparing infliximab and infliximab plus azathioprine to azathioprine in patients with Crohn's disease naïve to both drugs (n = 508). AZA = azathioprine, PLA = placebo, IFX = infliximab, W50 ALL = results for the intention-to-treat population at 50 weeks, W50 PP = results for the per-protocol population at 50 weeks. Adapted from.[13]

Routine measurement of 6-TGN includes codetermination of thioguanine mono-, di-, and triphosphates and corresponds to 69–77% of the sum obtained from separate determination of these metabolites (Figure 5).[14,15] In patients under thiopurine treatment, thioguanine triphosphate (6-TGTP) constitutes the largest fraction of 6-TGN irrespective of which of the

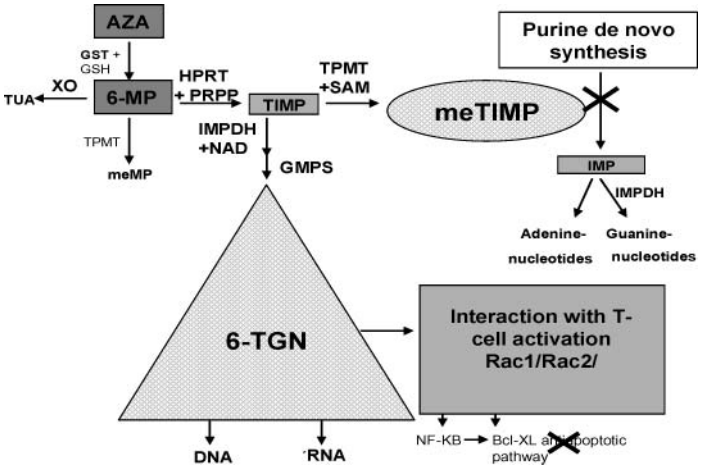


FIGURE 4 Metabolic scheme for the complex metabolism of thiopurines. Courtesy of Sofie Haglund, Linköping University. (Abbreviations: 6-MP, 6-mercaptopurine; AZA, azathioprine; 6-TGN, 6-thioguanine nucleotides; meTIMP, methyl thioinosine monophosphate; XO, xanthine oxidase; TUA, 6-thiouric acid; GST, gluthatione S-transferase; GSH, gluthatione; HPRT, hypoxanthine (guanine) phosphoribosyltransferase; PRPP, phospho-ribosyl-pyrophosphate; TIMP, thioinosine monophosphate; TPMT, thiopurine methyltransferase; SAM, S-adenosyl methionine; IMPDH, inosine 5′-monophosphate dehydrogenase; NAD, nicotinamide adenine dinucleotide; GMPS, guanosine 5′-monophosphate synthetase; IMP, inosine monophosphate.)

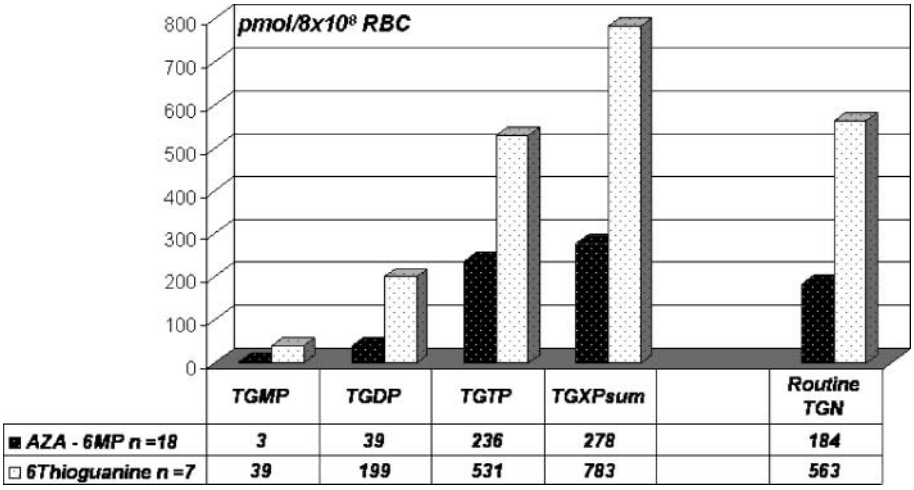


FIGURE 5 Comparison of measurements in red blood cells of fractionated TGN metabolites and routine TGN assays in patients under treatment with azathioprine/6-mercaptopurine or 6-thioguanine. The 18 patients on azathioprine or 6-mercaptopurine were treated with a daily mean dose corresponding to 147 mg azathioprine and the seven patients on 6-thioguanine were treated with 20 mg daily corresponding to 0.20–0.36 mg/kg body weight. TGMP, TGDP, and TGTP refers to thioguanine mono-, di- and triphosphates, respectively. Adapted from.[14,15]

thiopurine drugs the patient is treated.[14–16] Besides incorporation of 6-TGN in nucleic acid, the thiopurine responsiveness is mediated through the formation of 6-TGTP acting via Rac1-blockade to induce apoptosis in activated lymphocytes (Figure 4).[17]

In the presence of high 6-TGN levels, patients with high levels of active, Rac1-binding 6-TGTP and low levels of 6-TGDP have been shown to be more likely to respond than patients with low levels of 6-TGTP and high levels of inactive 6-TGDP.[16] These observations make it likely that measuring fractionated TGN metabolites may provide a better estimate of clinical efficacy in IBD than the present-day 6-TGN assays employed.

A "therapeutic interval" of phosphorylated metabolites (6-TGN) has been proposed to reflect clinical effect with higher 6-TGN levels in patients responding to treatment.[18,19] A best probability of response at 6-TGN levels >235 pmol/8 $\times$ 10^8 RBC with an odds ratio of 5.0 has been forwarded;[18] however, many patients with stable disease have lower levels.

In a meta-analysis which included six studies with altogether 437 patients and made use of threshold 6-TGN values >230–250 for clinical effect (variations between studies) it was found that even if 62% of patients with values above the threshold were in remission, so were also 36% of those with values below the threshold.[20]

The methylated metabolite, methylthioinsoine 5′-monophosphate (meTIMP), synthetized from thioinosine monophosphate (TIMP) by methylation of the polymorphic enzyme thiopurine S-methyltransferase (TPMT)

causes immunosuppression by inhibition of the purine-de-novo biosynthesis as demonstrated in vitro.[1]

Aminosalicylates inhibit TPMT in vitro and it has therefore been proposed that active introduction of cotherapy would be beneficial in patients unresponsive to thiopurines. However, even if some clinical data show a moderate increase in TGN levels under concomitant treatment with aminosalicylates,[21] this strategy has not yet been proven to be of any real clinical benefit to patients.

In clinical practice, a pre-treatment determination of TPMT status is advisable to identify patients with decreased enzyme activity and at risk for treatment failure. We have shown that patients heterozygous for the TPMT gene are less likely than wild-type patients to tolerate a normal thiopurine dose under a standardized dose-increment protocole over three weeks.[22] In the same study we also observed that patients with adverse events displayed higher 6-TGN and meTIMP levels than patients without such events.

To sum up, measurement of thiopurine metabolites has it's place in special scenarios, such as non-compliance, lack of efficacy, when adverse events occur or when high doses of thiopurines are indicated. The value of routine measurements of metabolite formation in uncomplicated patients has not been convincingly proven to be cost-effective or to improve patient outcomes.

ABBERANT, "SKEWED" METABOLISM

Responders to thiopurine treatment are characterized by 6-TGN production and non-responders by unexpectedly high formation of methylated metabolites (meTIMP, MMPR) although similar base-line TPMT activity exists in the two groups.[23] The inability to produce 6-TGN could be associated with resistance to therapy[23,24] but also to adverse reactions since methylated metabolites have been associated with myelotoxicity[22] and liver injury.[23]

It has been discussed whether a "skewed" metabolic profile with high meTIMP and relatively low 6-TGN levels is due to a difference in disease pathogenesis or variations in drug metabolism. Ultrahigh TPMT activity may explain some, but not all cases since only 1–2% of a population display such high activity.[25] Other results suggest that an altered thiopurine metabolism *per se* is a contributing factor.[23]

TIMP is the metabolic precursor both to meTIMP via TPMT and to 6-TGN via several successive metabolic steps (Figure 4). As IMPDH competes with TPMT for their common substrate TIMP, it is possible that IMPDH activity can explain variations in the thiopurine metabolism. IMPDH is proposed to be the first enzyme in the metabolic sequence leading to the formation of 6-TGN and ultimately to 6-TGTP.

We investigated IMPDH activity in peripheral blood mononuclear cells from randomly selected IBD-patients and from blood donors and found a

TABLE 3 Issues related to thiopurine treatment of inflammatory bowel disease

1. Are azathioprine and 6-mercaptopurine equally effective?
2. Length of treatment? Limited time or for ever?
3. The place of allopurinol add-on to a low-dose thiopurine regimen
4. In combined treatment with thiopurines and anti-TNFα-antibodies: when to stop which drug?
5. Any place for the combination of thiopurines with other immunomodulators (methotrexate, mycophenolic acid, tacrolimus, etc)?
6. Does fractionated metabolites better reflect efficacy and risk for adverse events than present-day metabolite monitoring?
7. Cost-benefit analysis of routine metabolite monitoring.

wide range of activity in both groups. There was no significant difference in enzymatic activity between patients and blood donors, median 14.0 (range 7.0 to 21.7) versus 13.1 (range 4.7 to 24.2) nmol formed XMP/mg protein/h.[26] Evidence of genetic polymorphism was not observed. A negative correlation between IMPDH activity and meTIMP-level but no correlation to 6-TGN was found.[26] This lack of correlation was surprising, but might be due to IMPDH being measured in mononuclear cells whereas the thiopurine metabolite concentrations are measured in red blood cells for ease of availability.

In preliminary results from a study of IBD-patients with distinct metabolite patterns we could confirm previously obtained observations in the randomly selected patients, that is, absence of a correlation between IMPDH activity and 6-TGN formation. Furthermore, downregulation of IMPDH in vitro was associated with an increase in 6-TGMP irrespective of intracellular TPMT status.[27] These results indicate that other pharmacogenes have to be investigated to explain a skewed metabolite profile.

Patients with a "skewed" metabolic profile presents a therapeutic challenge. These patients may be eligible for a trial of low-dose thiopurine and coadministration of the xanthine-oxidase inhibitor allopurinol, which has been shown to reverse the metabolite pattern and to be effective in IBD without serious toxicity.[24,28] A low metabolite ratio meTIMP/6-TGN therefore seems to be associated with better drug efficacy.[24] At present, the underlying mechanism for a skewed metabolite pattern remains unknown.

In order to further optimize the use of thiopurines in IBD, several other issues need to be addressed, some examples of which are given in Table 3.

CONCLUSIONS

Thiopurines are very important immunomodulators in the treatment of inflammatory bowel disease. In clinical practice, a pre-treatment determination of TPMT status is advisable and allows the identification of patients at risk for the development of adverse events and facilitates direct dose adjustements.

A trial of 6-mercaptopurine should be considered in azathioprine intolerant patients since more than half of them tolerate a shift to 6-mercaptopurine. Treatment with thiopurines and infliximab seem to be more effective in active or steroid-dependant Crohn's disease than each drug separately.

Measurement of thiopurine metabolites has it's place in special scenarios, such as noncompliance, lack of efficacy, when adverse events occur or when high doses of thiopurines are indicated. Routine measurements of metabolite formation in uncomplicated patients has not been proven to be cost-effective or to improve patient outcomes.

Patients with a "skewed" metabolic profile with high meTIMP and relatively low 6-TGN levels represent a therapeutic challenge. This metabolic pattern is poorly understood but a trial of low-dose thiopurine and coadministration of allopurinol might be worthwhile.

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