Combination therapy with allopurinol and low-dose thiopurine (azathioprine and mercaptopurine) has been described as an alternative immunosuppressive strategy in adult inflammatory bowel disease patients. Currently, this combination treatment is used in clinical practice to optimize ineffective or non-tolerated weight-based thiopurine monotherapy. In the setting of persistent disease or thiopurine intolerances in combination with an aberrant (‘skewed’) thiopurine metabolism (6-MMP/6-TGN ≥ 20) allopurinol–thiopurine combination therapy can be a safe and effective approach. Here we will discuss the efficacy and safety of allopurinol–thiopurine combination therapy in inflammatory bowel disease patients. Furthermore, we will review the mechanism of action, recommendations for the use of combination therapy in daily clinical practice and points of interest for future research.

Keywords: allopurinol • azathioprine • inflammatory bowel disease • mercaptopurine

In 1951 mercaptopurine (MP) was the first thiopurine derivate developed by Gertrude Elion for the treatment of leukemia. Subsequently, she designed allopurinol in order to improve the effectiveness of MP in leukemic patients [1]. Although combination therapy with MP and allopurinol was acknowledged to enhance efficacy, more toxicity was observed with concomitant treatment, particularly myelotoxicity. Therefore, in the subsequent decades, allopurinol was mainly used as monotherapy for the treatment of gout and ‘tumor lysis’ syndromes. In the early 1990s, the combination of a thiopurine derivate (azathioprine [AZA]) and allopurinol (25 mg on alternate days) regained interest; initially in the treatment of renal allograft survival. In order to reduce the risk of myelotoxicity, the dose of AZA was decreased (1.3 mg/kg instead of 1.8 mg/kg) [2]. The addition of allopurinol to AZA, in combination with cyclosporine and prednisolone, reduced the frequency of acute rejection of renal allografts. Twelve years later, allopurinol–thiopurine combination therapy was described as successful immunosuppressive maintenance treatment by Sparrow and colleagues in adult inflammatory bowel disease (IBD) patients with preferential 6-methyl-mercaptopurine (6-MMPR) production compared to 6-thioguanine nucleotides (6-TGN) and at the time ascribed to high thiopurine S-methyltransferase (TPMT) activity [3]. Currently, allopurinol–thiopurine combination treatment is used in clinical practice to optimize maintenance therapy in IBD patients. Although the understanding of the clinical pharmacology of this combination treatment has increased, the exact mechanism of (inter)action remains elusive. In this article, we will discuss efficacy and safety of allopurinol–thiopurine combination therapy in IBD. Additionally, we will review the proposed mechanisms of action, recommendations for the use of combination therapy in daily clinical practice and points of interest for future research.

Thiopurine metabolism

Thiopurine metabolism is complex and involves many enzymes with pharmacogenetically determined functional activity involved in a network of interacting pathways.
to generate ‘true’ purine derivatives and degrade ‘false’ purine derivatives (not useable for DNA generation). After the conversion of AZA into 6MP, the first step in bioactivation of 6MP is mediated by hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) to 6-thioinosine monophosphate (6TIMP). Moreover, 6MP can also be methylated by TPMT into 6-methyl mercaptopurine (6-MMP) or oxidized by xanthine oxidase (XO) into inactive 6-thiouric acid. In addition, 6MP can be inactivated by aldehyde oxidase, into 2-hydroxy-6-mercaptopurine. 6TIMP can further be metabolized in three different ways. 6TIMP is a substrate for TPMT, which results in the formation of 6-methyl mercaptopurine ribonucleotides (6-MMPR), which include 6-methyl thioinosine monophosphate (6meTIMP), 6-methyl thioinosine diphosphate and 6-methyl thioinosine triphosphate. Moreover, 6TIMP can be phosphorylated via 6-thioinosine diphosphate to 6-thioinosine triphosphate, which can be converted back to 6TIMP by inosine triphosphate pyrophosphohydrolase. In addition, inosine-5-monophosphate dehydrogenase converts 6TIMP into 6-thioxanthosine monophosphate. 6-Thioxanthosine monophosphate is further converted by guanosine monophosphate synthetase into 6-thioguanine monophosphate, which is further phosphorylated by kinases to 6-thioguanine diphosphate and 6-thioguanine triphosphate (6TGTP). Together these nucleotides form 6-TGN of which 6TGTP is especially pharmacologically active (Figure 1).

The immuno-suppressive mechanism of low-dose thiopurine therapy is largely based on inducing T-cell apoptosis by inhibition of the small GTPase Rac 1 by 6TGTP, as described 10 years ago [4].

Clinical relevance of thiopurine metabolism

The thiopurines, AZA and MP, play an important role as first-line immunosuppressive therapy in maintaining remission in IBD patients. [5,6] However, up to 60% of patients on weight based (AZA 2.0–2.5 mg/kg and MP 1.0–1.5 mg/kg) thiopurines eventually discontinue this therapy, mostly due to the development of adverse events such as allergic reactions and hepatotoxicity or because of a lack of efficacy [7]. These clinical events have been correlated to certain cut-off values of the metabolites 6-MMPR and 6-TGN. High 6-MMPR concentrations (> 5700 pmol/8 × 10⁸ RBC) have been associated with adverse events; in particular, ‘hepatotoxicity’ but also myelosuppression, whereas 6-TGN concentrations above 235 pmol/8 × 10⁸ RBC were correlated with therapeutic efficacy and, in high concentrations (> 450 pmol/8 × 10⁸ RBC) with myelosuppression [8,9]. Indeed, measuring the thiopurine metabolites (6-TGN and 6-MMPR), has been shown to be clinically useful for optimizing thiopurine therapy in IBD patients with inadequately controlled disease or ‘hepatotoxicity’ [10,11]. Based on the results of thiopurine metabolite measurement, several scenarios may occur. If both metabolites are undetectable it is likely that the patient is not taking the medication and adherence to therapy should be discussed. In patients with low levels of both 6-TGN and 6-MMPR the dose of AZA/MP should be increased in order to achieve therapeutic 6-TGN levels. If both 6-TGN and 6-MMP are levels are above the recommended limits, or if 6-TGN levels are considered ‘therapeutic’, it is unlikely that thiopurine therapy is sufficient to maintain clinical remission and this choice of therapy should be reconsidered. There is also a group of patients (15–20%) that produce high 6-MMPR concentrations instead of 6-TGN formation [10,12]. This so called ‘skewed’
metabolism or ‘hypermethylators’ is probably the most important aberrant thiopurine metabolism leading to lack of efficacy. Although high TPMT enzyme activity would be expected in this subgroup of patients, it is remarkable that high 6-MMPR concentrations could not be predicted by high TPMT in vitro enzyme activity [13]. Increasing the dose of the thiopurine in this group of patients usually results in an excessive increase in 6-MMPR levels rather than an increase in the pharmacologically active 6-TGN levels [14]. These patients are at risk for the development of both adverse events and inefficacy of thiopurine therapy. Two definitions for a ‘skewed’ metabolism were used in previous literature (6-MMPR/6-TGN ≥ 11 or 6-MMPR/6-TGN ≥ 20) [14–16]. It is these patients with a skewed thiopurine metabolism which can benefit from switching therapy to low-dose thiopurine in combination with allopurinol. Most recent publications recommend using ratios ≥ 20 regarding allopurinol combination therapy, since high ratios are reported to be clinically relevant in the largest published series so far, but no prospective study has specifically studied a clinically relevant cut-off value for 6MMP/6TGN ratio [17,18].

Mechanism of action of allopurinol & low-dose thiopurine

Sparrow and colleagues described that following allopurinol–thiopurine combination therapy in IBD patients, the pharmacologically active 6-TGN concentrations substantially increased (mean 185 to 385 pmol/8 × 10⁸ RBC), and that concurrently 6-MMPR concentrations dramatically decreased (mean 10,380 to 1732 pmol/8 × 10⁸ RBC) [3]. The exact pharmacokinetic mechanism of action of combination therapy is not fully clarified, but several hypotheses have been put forward. Allopurinol inhibits the activity of the XO enzyme and this would theoretically result in less 6MP converted by XO leaving more 6MP available for conversion into 6-TGN. Indeed, a recent case report described the use of a new XO inhibitor, febuxostat, in combination with low-dose thiopurines [19]. This novel combination also effectively reduced 6-MMPR levels combined with increased 6-TGN concentrations. However, XO inhibition also leaves more 6MP available for conversion into 6-MMPR but higher 6-MMPR levels are not routinely observed with combination therapy, indicating that increased 6MP conversion is insufficient to explain the mechanism behind the parallel observed reduction of 6-MMPR levels following initiation of allopurinol. The easiest explanation, that allopurinol would inhibit TPMT was found not to be the case [3]. Instead, oxypurinol, an active metabolite of allopurinol was recently shown by Blaker and colleagues to directly inhibit TPMT, contributing to decreasing 6-MMPR levels. In addition, patients on allopurinol–thiopurine combination therapy also have high levels of urinary and serum thioxanthine which also may inhibit TPMT [12]. Moreover, other metabolites of allopurinol, such as oxypurinolriboside monophosphate and 2-hydroxy-6-mercaptopurine, are known to be TPMT inhibitors [20]. Another important enzyme in the thiopurine metabolism is HGPRT. In a previous prospective study regarding enzyme activities in allopurinol–thiopurine treatment it was demonstrated that an increase in HGPRT activity after 12 weeks of thiopurine therapy is present [21]. HGPRT is the first enzyme responsible for the generation of 6-TGN, increased activity of this enzyme may partly explain the increase of 6-TGN levels as HGPRT activity and 6-TGN levels are reported to correlate [22]. The decrease in 6-MMPR levels could not be clarified by this increase as HGPRT has been shown to be also involved in the formation of 6-MMPR and thus a 6-MMPR increase would be expected. In summary, several theories have been proposed to explain the mechanism of inverting the 6-TGN/6-MMPR ratio following allopurinol–thiopurine combination therapy (Figure 2). Probably, more than one enzyme contribute to the reported changes in thiopurine metabolites favoring beneficial clinical outcome.

Clinical effect of allopurinol–thiopurine combination therapy

In 2005, the first clinical study was published regarding combination treatment with allopurinol and a thiopu-
rime derivate in IBD patients with a skewed thiouprine metabolism. Fifteen steroid-dependent, mainly Crohn’s disease patients, were treated with 100 mg allopurinol in combination with 25–50% of the original dose of AZA or MP. The authors reported a statistically significant increase of 6-TGN levels (mean 186 to 385 pmol/8 × 10^8 RBC) and a decrease in 6-MMP levels (of 10380 to 1732 pmol/8 × 10^8 RBC) after 2–4 weeks of combination therapy. The majority of patients showed a clinic response after the introduction of allopurinol–thiopurine therapy, although formal disease assessment was not performed. A significant decrease in leukocyte count was also described after initiation of combination therapy, which often resolved after thiopurine dose reduction [3]. Two years later, a subsequent study regarding allopurinol–thiopurine combination therapy evaluated both the efficacy of thiopurine therapy but also the resolution of hepatotoxicity in IBD patients with a skewed thiopurine metabolism. Clinical disease activity scores (Harvey Bradshaw for CD and Mayo score for UC) improved and the mean dose of corticosteroids could be reduced from 18 to 2 mg and even discontinued in half of the patients, after initiation of combination therapy. Moreover, in 85% of the thirteen patients who developed ‘hepatotoxicity’ during thiopurine monotherapy, combination therapy led to a normalized of alanine aminotransferase and aspartate aminotransferase levels [23]. In addition, also nonhepatic adverse events (nausea, fatigue, myalgia) during standard dosing thiopurine monotherapy showed resolution following the initiation of combination therapy [24]. In 72% of the patients with nonhepatic adverse events and in 88% of the patients with hepatotoxicity clinical remission was achieved after initiation of combination therapy. Hepatotoxicity improved in 94% of the patients. Only 16% of patients who previously discontinued thiopurine therapy due to nonhepatic adverse events had to discontinue combination therapy due to adverse events. Of note, patients with both a ‘normal’ thiopurine and a ‘skewed’ thiopurine metabolism were included in the latter study. Also in the group of patients with a known ‘normal’ thiopurine metabolism (6-MMPR/6-TGN < 20), combination therapy was effective in reducing toxicity and inducing clinical remission although the group size was limited (80%, n = 5) [24]. Recently, the same group presented an abstract regarding the efficacy and safety of combination therapy in IBD patients with insufficient clinical response or adverse events during thiopurine monotherapy without measurement of thiopurine metabolites (n = 138) and in thiopurine naïve patients (n = 88). Both groups demonstrated high rates of clinical response: 74% in the patients with an inadequate response to monotherapy AZA and 84% in the thiopurine naïve IBD patients. The latter observations could indicate that combination therapy may also benefit patients with an inadequate response to thiopurine monotherapy but also in thiopurine naïve patients [24,25].

In 2013, our research groups reported a study regarding the long-term effectiveness and tolerability of allopurinol–thiopurine combination therapy in IBD patients. Seventy-seven IBD patients (mainly CD) were included. All patients had a skewed thiopurine metabolism and initiated allopurinol combination therapy due to therapy resistance or adverse events (mainly hepatotoxicity) during thiopurine monotherapy. Overall, combination therapy was well tolerated as only six patients discontinued this combination due to adverse events, mainly nausea. Sixteen percent developed leukopenia (WBC count ≤ 3.5 × 10^3/ml), which normalized in all patients after appropriate dose adjustments and no septic sequelae were observed. At the end of follow-up 61% of patients were in steroid free remission [17]. Another group described the use of allopurinol–thiopurine combination therapy in a large single center cohort study of 109 (mostly CD) IBD patients. Indication for initiation of allopurinol combination therapy in this study were hepatotoxicity, nonhepatic adverse reactions, inadequate clinical response to thiopurine monotherapy in combination with a skewed metabolism or, high TPMT activity (35 pmol/h/mgHb) and a skewed metabolism without a loss of response or toxicity. Overall, 82% of the patients receiving combination therapy successfully overcame the problem encountered on thiopurine monotherapy and at 1 year 81% of patients were in clinical remission. Only 13 adverse reactions (all mild) were reported during combination therapy; mainly gastrointestinal complaints but no myelotoxicity. Combination therapy was tolerated by 86% of the patients with nonhepatic adverse reactions and liver function tests normalized in 80% of the patients with hepatotoxicity during prior thiopurine monotherapy [18].

**Allopurinol–thiopurine combination therapy in daily clinical practice**

IBD patients with a skewed thiopurine metabolism (6-MMPR/6-TGN ≥ 20) who develop adverse events or remain without clinical benefit of weight based (2.0–2.5 mg/kg for AZA and 1.0–1.5 mg/kg for MP) thiopurine therapy can benefit from switching treatment to allopurinol in combination with low-dose conventional thiopurine. Although formal dose finding studies are lacking, the dose of the thiopurine should be decreased to 25–33% of the intended monotherapy dose (mainly 25 mg MP or 50 mg AZA) in order to prevent bone marrow depression. Allopurinol is usually added at a dose of 100 mg daily. It is mandatory for safety reasons to monitor complete blood count and liver test variables at week 1, 2, 4, 8 and 12 of combination therapy. Blood tests included hemoglobin,
hematocrit, platelet count, leukocyte count, C-reactive protein, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and γ-glutamyltranspeptidase concentrations. Thiopurine metabolites can be measured after 4–8 weeks of combination therapy to further optimize the dose of thiopurines to achieve the range of 6-TGN concentrations (230–400 pmol/ $8 \times 10^8$ RBC) without exceeding the upper limit that increases the risk for leucopenia [9]. Evidence for increasing clinical efficacy by routinely optimizing 6-TNG levels following the introduction of combination therapy is not yet available. Myelosuppression is an important and serious risk with combination therapy and a mild decrease in leukocyte count is described in up to 20% of the patients [17,18,23]. Also, nausea, fatigue and malaise are described adverse events and a few cases of dermatological abnormalities like Stevens–Johnson syndrome and toxic epidermal necrolysis secondary to allopurinol warrant careful monitoring [17,18,23].

Another, more controversial, therapeutic option for these skewed metabolizers is tioguanine [26]. Tioguanine is a non-conventional thiopurine which does not lead to the formation of the toxic metabolite 6-MMPR during the conversion into the pharmacological active 6-TGN. Although this therapy has shown therapeutic efficacy, an association with true hepatotoxicity, in particular veno-occlusive disease and nodular regenerative hyperplasia have been reported [27–29]. Although this is allegedly a dose related phenomenon, a liver biopsy is recommended in all patients treated with tioguanine after 1 and 3 years [30]. It is of interest that recent studies of combination therapy with thiopurines and infliximab have not taken into account patients with ‘skewed’ metabolism who might have been candidates for optimization of 6-TGN metabolites. In contrast to immediately switching to biologic therapy, optimizing thiopurine therapy by initiating allopurinol combination therapy may be effective, is reasonably safe and may contribute to a reduction of healthcare costs.

### Allopurinol–thiopurine combination therapy & pregnancy

Conventional thiopurines are considered relatively safe during pregnancy in IBD patients [31]. However, evidence on the safety profile of combination therapy of allopurinol and low-dose thiopurine during pregnancy is limited to case reports and series. Recently, a study regarding 31

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**Executive summary**

**Background**
- Allopurinol was initially designed to improve the effectiveness of 6-mercaptopurine and is currently used to optimize thiopurine maintenance therapy in inflammatory bowel disease.

**Thiopurine metabolism**
- The thiopurine metabolism is complex and involves many enzymes with pharmacogenetically-determined functional activity.

**Clinical relevance of thiopurine metabolism**
- Efficacy and toxicity of thiopurine therapy correlate with levels of the thiopurine metabolites 6 methylmercaptopurine and 6-thioguanine nucleotides.
- A “skewed” metabolism (high 6-MMPR and low 6-TGN levels, ratio $>$20) is associated with lack of efficacy and this ratio can be optimized with low dose thiopurine in combination with allopurinol.

**Mechanism of action of allopurinol and low dose thiopurine**
- The mechanism underlying the (inter)action of allopurinol and low dose thiopurine remains poorly understood.

**Clinical effect of allopurinol-thiopurine combination therapy**
- Levels of 6-TGN rise and levels of 6-MMPR decrease after initiation of combination therapy of allopurinol and low dose thiopurine.
- Combination therapy of allopurinol and low dose thiopurine is effective in inducing clinical remission and is well tolerated in IBD patients with a “skewed” metabolism.
- Hepatotoxicity during monotherapy thiopurine improved in up to 94% of IBD patients with a “skewed” metabolism after initiation of allopurinol and low dose thiopurine.

**Allopurinol-thiopurine combination therapy in daily clinical practice**
- If patients combine disease activity or adverse events with a skewed thiopurine metabolism (high 6-MMP, low 6-TGN levels, 6-MMP/6-TGN $>$ 20), a switch to allopurinol-thiopurine combination therapy is to be considered.
- Daily 100 mg allopurinol should be combined with 25–33% of the intended thiopurine monotherapy dose.
- It is mandatory to monitor complete blood count and liver enzymes on a regular basis in order to detect potential bone marrow depression and hepatotoxicity.

**Allopurinol-thiopurine combination therapy and pregnancy.**
- Clinical experience with the use of combination therapy during pregnancy is limited and this topic warrants careful consideration on an individual basis.
pregnancies with allopurinol treatment for other diagnosis than IBD described an overall rate of major malformations of 3.7%, no different than ‘expected’. One child had multiple malformations [32]. Moreover, one case report described multiple malformations as a potential teratogenic effect of allopurinol, while in other studies in pregnant women and neonates no adverse reactions were observed [33,34]. A few case reports of IBD patients who were treated with combination therapy of allopurinol and low-dose thiopurine during the entire pregnancy showed no association with an adverse pregnancy outcome [35,36]. Moreover, placental transfer and intra-uterine exposure to thiopurine metabolites in patients treated with combination therapy was comparable to patients using thiopurine monotherapy [36]. Thus, our knowledge on the use of combination therapy during pregnancy is limited and warrants careful consideration on an individual basis.

Conclusions & future perspective
Although thiopurine therapy is considered first-line immunosuppressive therapy in IBD patients, lack of efficacy or adverse events happen frequently and, in this case, therapeutic drug monitoring is recommended as a starting point to aim for optimizing thiopurine therapy.

If patients combine disease activity or adverse events with a skewed thiopurine metabolism (high 6-MMP, low 6-TGN levels, 6-MMP/6-TGN ≥ 20), a switch to allopurinol–thiopurine combination therapy is to be considered. Clinical benefits include steroid sparing, higher remission rates and reduced adverse events and larger controlled trials are needed to confirm these outcomes. The underlying mechanism of action for allopurinol in improving thiopurine metabolism and therapy remains poorly understood. Additionally, thioguanine holds promise as alternative therapy, although benefit–risk ratio is yet insufficiently studied. Future mechanistic studies might aid in understanding the complex thiopurine metabolism.

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References
33 Kaandorp JJ, Benders MJ, Rademaker CM et al. Antenatal allopurinol for reduction of birth asphyxia induced brain damage (ALLO-Trial); a randomized double blind placebo controlled multicenter study. BMC Pregnancy Childbirth 10(8), (2010).