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Current status of thiopurine analogues in the treatment in Crohn's disease

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Abstract

In the last decades, with the development of biological therapy, the treatment paradigms in patients with Crohn's disease have continuously evolved. Several studies focusing on the optimal use of both traditional immunosuppressants and biological therapy have been published, investigating conventional, accelerated step-up and top-down approaches. In addition, much emphasis has been placed in recent years on the determination of important predictive factors that could enable early patient stratification, which would lead to a tailored management strategy. In this review, the authors try to highlight new evidence on the optimal timing, benefits, and risks of immunosuppressants alone, or in combination, in patients with Crohn's disease.

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Key words: Crohn's disease; Immunosuppressives; Azathioprine; Thiopurine methyltransferase; Biologicals

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INTRODUCTION

Inflammatory bowel disease (IBD) is a multifactorial disease with probable genetic heterogeneity^[1]. In addition, several environmental risk factors (e.g., diet, smoking, measles or appendectomy) may contribute to its pathogenesis. During the past several decades, the incidence pattern of both forms of disease, Crohn's disease (CD) and ulcerative colitis, has changed significantly^[2], showing some common characteristics yet also quite distinct features between the two disorders.

The phenotypic classification of CD plays an important role in determining the treatment, and may assist in predicting the likely clinical course of disease. In 2005, the Montreal revision of the Vienna classification system was introduced^[3]. Using the Vienna classification system, it has been shown in clinic-based cohorts that there can be a significant change in disease behavior over time, whereas disease location remains relatively constant^[4]. Population-based studies have demonstrated that up to one-third of the patients had evidence of a stricturing or penetrating intestinal complication at diagnosis, and half of all patients experienced an intestinal complication within 20 years after diagnosis^[4,5]. Similarly, these complications occurred in more than 50% of children, after a median follow-up of 84 mo^[6]. Half of the adult patients required surgery within 10 years after diagnosis, while in children, 34% of patients required surgery within 5 years of diagnosis. The risk of postoperative recurrence was approximately 44%-55% after 10 years. These data suggest that Crohn's disease is a chronic progressive disease,

where effective intervention prior to the onset of bowel damage (stricture, fistula, abscess) is required in order to improve the outcome. Of note, however, not all patients with CD will show disease progression. Thus, recognizing patients at the highest risk of developing a disabling disease or complications at an early stage is crucial. In CD cohorts from referral centers, an initial need for steroids, an age below 40 years, the presence of perianal or stricturing disease and a significant weight loss were associated with the development of disabling disease^[7,8].

A systematic review published in 2004, which analyzed population-based studies in CD with a complete follow-up, failed to demonstrate a significant improvement in disease outcome during the past four decades^[9]. Of note, disease activity, occurrence of complications, and need for surgery did not significantly change during that period. For example, time to intestinal surgery did not change despite the more frequent use of immunosuppressants in CD patients from the end of the 1990s^[10]. According to the authors' conclusion, the timing of immunosuppressants use might have been inappropriate. Nevertheless, data support that azathioprine (AZA) allows not only for the maintenance of remission and weaning off steroids in approximately two-thirds of patients with steroid-dependent CD, but may lead to complete or near-complete mucosal healing and histological remission in a significant proportion of CD patients^[11].

More recently, Peyrin-Biroulet *et al.*^[12] published a systematic review on the natural history of CD in population-based cohorts. The authors conclude that the impact of changing treatment paradigms with the increasing use of immunosuppressants and biological agents on the natural history of CD is poorly understood. To investigate this question, two approaches may be appropriate; (1) to conduct a disease-modification trial using the newly proposed definition of "early Crohn's disease"^[13]; (2) to investigate the evolution of the disease phenotype and complications in population-based cohorts with unified patient management. The limitation of the first approach is that only the relatively short-term outcomes (e.g., clinical remission, endoscopic healing, short-term risk of hospitalization and/or surgery) can be investigated with adequate statistical power. In contrast, in the second setting, the follow-up is complete in every patient; however, patient management is more individualized and variable.

Treatment paradigms have been evolving in the last two decades, with the inclusion of biological therapy. In the last several years, numerous studies focusing on the optimal use of traditional immunosuppressants and biological therapy have been published. In addition, much emphasis has been placed in recent years on the determination of important predictive factors to identify patients at risk for disease progression as soon as possible, in order to enable a tailored management strategy^[14]. In this review, the authors try to highlight some of the new, available evidence on the benefits, timing, and risks of immunosuppressants alone, or in combination, in pa-

tients with CD.

NEW DATA AND NEW STRATEGIES ON THE USE OF THIOPURINE ANALOGUES: ALONE OR IN COMBINATION?

Efficacy of conventional immunosuppressants

In CD, the efficacy of immunosuppressive therapy with purine analogues has been established in controlled trials, which assessed the role of AZA/6-mercaptopurine (6-MP), both as induction agents and as steroid-sparing agents in a withdrawal study^[15,16]. The study reported by Present *et al.*^[15] in 1980, was the first to demonstrate with certainty the efficacy of 6-MP in the induction of remission in CD. By using a dose of 1.5 mg/kg per day, 67% of patients responded to therapy as compared to only 8% of patients who received placebo. However, not all controlled trials report such a positive clinical response to thiopurines in the induction of disease remission in CD. The notion of the delayed onset of action of 6-MP also stems from the study by Present *et al.*^[15], which reported that the mean time to response was 3.1 mo, with 89% of responders doing so within 4 mo of initiating therapy. However, it should be noted that the first clinical evaluation in this study was not performed until the 12-wk mark. Thus, it is likely that a proportion of patients were already responding before the first assessment. It is important to note that if therapy is started relatively late in the disease course, when the anatomical damage is irreversible, these medications will not prevent the occurrence of complications. Until recently, immunosuppressants were introduced relatively late during the disease course, mainly in steroid-dependent/resistant or postoperative patients^[17]. Thiopurines were started in the majority of patients years after the diagnosis. Even so, in clinical cohorts, the efficacy of thiopurine therapy was defined as optimal in approximately 47% of the patients. Similar results were published by the Oxford clinic^[18], where the mean remission rate was 45% and the proportion of patients remaining in remission at one-, three-, and five-years was 95%, 69% and 55%, respectively. In general, it is recommended that thiopurines be added to the therapeutic regime in patients failing to wean off corticosteroids during their first attempt at tapering the dose or alternatively after a second attempt.

The most convincing data to support a benefit from early use of AZA, however, come from the pediatric literature^[19], where in a randomized controlled trial involving 55 children, the early use of 6-MP was associated with a significantly lower relapse rate (only 9% compared with 47% in controls ($P = 0.007$)). Moreover, the duration of steroid use was shorter ($P < 0.001$) and the cumulative steroid dose was lower at 6, 12 and 18 mo ($P < 0.01$). The benefit of an early aggressive treatment was also demonstrated in another pediatric study^[20], where 80.5% of children with newly diagnosed moderate-to-severe CD were treated with immunomodulators within

the first year. Early immunomodulator use was associated with reduced corticosteroid exposure and fewer hospitalizations per patient. Candy *et al*^[21] similarly showed that AZA offers a therapeutic advantage over placebo (47% *vs* 7% remission rate at 15 mo; $P < 0.001$) in the maintenance of remission in CD patients. Both studies showed no difference in the proportion of patients who had achieved remission at 12 wk, since corticosteroids served as the induction therapy for both groups. These results highlight the steroid-sparing benefits of thiopurines and suggest that the short-term use of corticosteroids for the induction of remission can serve as a bridge to the more long-term maintenance of a steroid-free remission with thiopurines.

The benefit of thiopurines was also demonstrated in cohort studies. In the pediatric setting, since the year 2000, the more systematic introduction of AZA at the time of diagnosis led to a 2-fold longer first remission period^[22]. Similarly, the long-term beneficial effect of early AZA treatment was demonstrated in an adult referral cohort study from Hungary, where early AZA treatment was independently associated with a decreased risk for disease behavior change and resective surgery. It also prevented the deleterious effects of smoking^[23,24]. Similarly, a lower risk of surgery (HR: 0.41; 95% CI: 0.21-0.81) in non-penetrating non-stricturing CD patients with an immunomodulator use lasting more than 6 mo was also reported from the United States^[25]. An important clinical question is of course, patient adherence to treatment. A wide range of non-adherence was reported in Germany, for patients taking AZA and in long-term remission, ranging from 7.1% to 74.3%^[26]. Limited data are available with regards to factors predicting effectiveness and failure of long-term thiopurine use in IBD patients. There is evidence to suggest that 6-methylmercaptopurine (6-MMP) concentration and the 6-MMP/6-thioguanine nucleotides (6-TGN) ratio may be associated with therapeutic failure^[27]. In patients with suboptimal response on AZA and high 6-MMP levels, the addition of allopurinol was effective and safe in optimizing 6-TGN production, leading to improved disease activity scores, reduced corticosteroid requirements, and normalization of liver enzymes, but careful monitoring for adverse effects and profiling of thiopurine metabolites is essential^[28].

The efficacy of thiopurine analogues for the induction of maintenance was also proven in recent reviews by the COCHRANE group^[29,30]. The odds ratio (OR) of a response to AZA or 6-MP therapy compared with placebo in active CD was 2.43 (95% CI: 1.62-3.64), 54% in AZA-treated patients and 34% in the placebo arms. This corresponded with a number needed to treat (NNT) equaling about five. When the two trials using 6-MP in active disease were excluded from the analysis, the OR was 2.06 (95% CI: 1.25-3.39). Treatment for longer than 17 wk resulted in an OR of 2.61 (95% CI: 1.69-4.03); however, a significant benefit was not observed for a shorter treatment period. A steroid-sparing effect was seen with an OR of 3.69 (95% CI: 2.12-6.42),

corresponding to a NNT of about three, in order to observe steroid-sparing in one patient. Similarly, AZA was effective in maintaining remission in the seven trials with AZA and one with 6-MP. AZA and 6-MP had a positive effect on maintaining remission (OR: 2.32; 95% CI: 1.55-3.49) with a NNT of six. The OR for the maintenance of remission with 6-MP was 3.32 (95% CI: 1.40-7.87) with a NNT of four. Higher doses of AZA improved response (AZA 1 mg/kg, OR: 1.20; 2 mg/kg, OR: 3.01; 2.5 mg/kg, OR: 4.13). A steroid-sparing effect with AZA was noted, with an OR of 5.22 (95% CI: 1.06-25.68) and a NNT of three. The Cochrane analysis reported a response rate of 55% with thiopurine therapy *vs* 29% for placebo, a pooled OR of 4.58 (95% CI: 0.49-42.82) also favored fistula healing. It should be noted that there was only a small number of patients evaluable for this analysis, and with the confidence interval crossing 1 this result is statistically insignificant.

In clinical practice, it is still uncertain if and when immunosuppressive therapy should be interrupted in patients in long-term (4-6 years) remission on thiopurines. In a recent withdrawal study by the GETAID group^[31], the authors have provided evidence for the benefit of long-term AZA therapy beyond 5 years in patients with prolonged clinical remission. The cumulative probabilities of relapse at 1, 3, and 5 years were 14.0%, 52.8%, and 62.7%, respectively. A C-reactive protein (CRP) concentration of 20 mg/L or greater (risk: 58.6), a hemoglobin level of less than 12 g/dL (risk: 4.8), and a neutrophil count 4×10^9 /L or greater (risk, 3.2) were independently associated with an increased risk of relapse. Among the 32 relapsing patients, 23 were retreated by AZA alone, with all but one leading to a successful remission.

Finally, in adults, a recently published clinical strategy trial from Belgium and the Netherlands^[32] randomized 133 patients with active CD, naïve to both steroids and AZA, to either a conventional step-up strategy [with full courses of steroids (prednisolone or budesonide) and introduction of AZA when the patients experienced a flare-up after tapering off or became dependent on steroids] or top-down (infliximab induction therapy and AZA at the first presentation). From week 6, AZA was continued as monotherapy, thus, a long-term combination was not administered. Up to the one-year mark after the initiation of therapy, steroid-free remission was more frequent in the early combined immunosuppressive group (61.5% *vs* 42.2%, difference: 19.4%, 95% CI: 2.4-36.3, $P < 0.05$). The median time to relapse was also longer in the early combined immunosuppressive - the "top-down" group [329.0 d, interquartile range (IQR) 91.0-∞ *vs* 174.5 d, IQR 78.5-274.0, $P < 0.03$]. In contrast, the difference was not significant after 52 wk. This open-label trial was liable to the intrinsic observer bias. Furthermore, patients in the conventional group had to fail two courses of steroids before the start of the immunosuppressant, which added a delay of appropriate treatment in at least one-third of the patients. None-

theless, a significant difference was found concerning complete ulcer healing during endoscopy, with 73.1% of evaluated patients (19/26) in the early combined immunosuppressants group *vs* 30.4% (7/23) in the controls, in a subgroup of patients who underwent ileocolonoscopy at week 104. In addition, the majority of patients (15/17) with mucosal healing in the early combined immunosuppressive group, after two years of therapy, remained in remission off steroids and did not need further infliximab (IFX) therapy in the subsequent two years of follow-up^[33]. The authors concluded that CD can be effectively treated without steroids, if patients are offered an early combined therapy of immunosuppressants. An interesting secondary result of the study was that approximately 10%-20% of patients required IFX after the induction period.

Current use of thiopurines and anti-TNF blockers: Alone or in combination?

There is no consensus on the appropriateness of concomitant immunomodulators with anti-tumor necrosis factor (anti-TNF) therapy for CD. Some patients benefit from concomitant immunomodulators, but there are increasing concerns related to infections and the risk of lymphoma. Until recently, anti-TNF antibodies have usually been initiated as second or third line immunosuppressants in patients failing or dependent on steroids and/or AZA. In 2003, immunosuppressants were shown to inhibit the development of neutralizing anti-infliximab antibodies, when this drug was used in an episodic, on-flare strategy^[34]. Moreover, IFX serum levels were also significantly higher in patients with concomitant immunosuppressive therapy^[35]. Therefore, theoretically, combined therapy may have synergistic immunosuppressive effects resulting in increased efficacy, but it may also increase the long-term toxicity. However, in randomized controlled trials (discussed previously in detail) in CD patients with long disease duration, often after multiple surgical interventions, a synergistic effect was not observed. Concomitant immunosuppressive and/or steroid therapy was not more efficacious compared to the anti-TNF agent alone in patients on scheduled maintenance therapy^[36]. Thirty to seventy percent of patients in these trials received either of the drugs. In PRECISE 1, for example, 23% of patients on certolizumab with concomitant immunosuppressants *vs* 23% without immunosuppressants, showed a drop of more than 100 points at weeks 6 and 26 in the Crohn's disease activity index (CDAI). The numbers were identical for patients with and without concomitant steroid therapy^[37]. Similarly, in PRECISE 2, at week 26, 61% of patients receiving concomitant immunosuppressive agent and 64% without demonstrated a clinical response. A similar tendency was also reported for adalimumab in CHARM. Clinical remission rates with or without concomitant immunosuppression were not significantly different either at week 26 or 52 [37% *vs* 33% for adalimumab every other week (EOW) and 39% *vs* 50% for adalimumab every

week (EW)]. In addition, similar to certolizumab, the clinical efficacy was significantly different based on the disease duration. This tendency was also similar for IFX in the ACCENT I and II trials, although the rate of infusion reactions was lower (12.5%) in patients receiving concomitant immunosuppression compared to those without (22.0%), and the rate of formation of antibodies was higher^[37]. In contrast, reported IFX concentrations were not different over time. Although significance was not reported, in ACCENT I, the clinical response and remission rate in the 5 mg/kg group was reported as 54% and 38%, respectively, in patients with an immunosuppressant, and was 34% and 26% without immunosuppressant therapy.

Additionally, a recent, prospective, open-label study demonstrated that withdrawing immunosuppressants in patients with CD on a combined maintenance schedule of IFX and immunosuppressive therapy for at least six months did not affect efficacy over two years of follow-up, but tended to decrease IFX trough levels and CRP elevation^[38]. This indicates that the impact of withdrawing antimetabolites in patients treated with biologicals has no, or only limited, risk of loss of efficacy, although the impact on IFX trough levels warrants further long-term follow-up. Noteworthy, however, is that most patients had been failing AZA therapy before having entered the trial. As a final point, in a recent large Belgian cohort study^[39], concomitant AZA or methotrexate (MTX) therapy did not influence the outcome of IFX treatment during a median follow-up of five years. Importantly, 49.7% of patients were on AZA and 9.4% on MTX at the time of anti-TNF induction therapy. 34.1% of those on AZA at baseline stopped its use after a median of 15 mo; however, in 41.3% of these patients MTX was started later during the follow-up, based on the clinical indication. Moreover, 26% of the patients needed one intervention (increasing the dose to 10 mg/kg or decreasing the interval) during IFX maintenance therapy, while 10% and 14% needed two or three modifications, respectively. Therefore, the results should be interpreted with caution, since an alternative conclusion might be that patients with more aggressive disease course were able to maintain similar clinical benefit with a combination therapy and/or modifications in the dose or interval of the biological therapy.

More recently, however, anti-TNF agents have been used earlier in the disease course, including in patients naïve to AZA. The first piece of evidence arises from the pediatric literature^[40]. In 112 children with moderate-to-severe disease, IFX induction and scheduled maintenance therapy, every 8 wk, in the REACH study was associated with 63.5% and 55.8% clinical response and clinical remission rates, respectively. All patients were required to have started concomitant immunomodulators (AZA, 6-MP or MTX) at least 8 wk prior to study entry and approximately one third of the patients were also simultaneously receiving steroids. The average disease duration was as low as two years. Although the defini-

tion of remission was different (instead of CDAI, the pediatric index, PCDAI, was used) and disease duration was short, these were among the highest remission rates reported for anti-TNF agents.

In adult CD patients with early disease (< 2 years) naïve to purine analogues and MTX, the outcome was similar to that found in the pediatric population. The large, blinded, double-dummy, controlled SONIC trial compared AZA monotherapy (2.5 mg/kg per day), IFX monotherapy, and combined IFX and AZA therapy^[41]. The average disease duration was 2.3 years (range 0-43 years). At 26 wk, the steroid-free remission rates in patients receiving combined immunosuppressive therapy with IFX and AZA were higher than with IFX monotherapy (56.8% *vs* 44.4%, $P < 0.05$). In turn, these were also higher than remission rates in patients receiving AZA monotherapy (30.0%, $P < 0.01$). A course of steroids was allowed in all patients until week 12, to compensate for the slow onset of the therapeutic effect of AZA. The proportion of patients receiving systemic steroids at baseline in combination with AZA, IFX or in the combination group was similar ($n = 40, 52$ and 47 patients, respectively); however, the dose used was below that recommended for induction therapy (mean dose of 24 mg/d). It is even more difficult to explain the large difference in clinical remission off steroids at week 26, since the number of patients receiving steroids at this time point ($n = 60, 60$ and 58 , respectively) and the mean dose were virtually identical (range: 9.4-11.6 mg/d) in all three groups. Therefore, the lower clinical efficacy is not reflected by differences in steroid use. Moreover, steroids should have been tapered off by week 12, where possible. As a consequence, the end result in at least one-third of the patients reflects an insufficient steroid induction therapy in combination with either AZA, IFX or the combination of the two drugs. Nevertheless, the total disappearance of mucosal ulcers was also higher in the combined IFX-AZA group (43.9% IFX and AZA *vs* 16.5% AZA, $P < 0.001$). Nonetheless, a significant bias cannot be excluded, since patients with lesions at baseline who did not undergo endoscopy at week 26, or who had results that could not be evaluated were assumed to have a lesion. These patients numbered 50 of 109 (45.9%) in the AZA group, 29 of 93 (31.2%) in the IFX group, and 31 of 107 (29.0%) in the combination-therapy group. In addition, it is difficult to interpret the data since a significant proportion of the patients had negative ileocolonoscopy at inclusion.

At week 50, assuming that patients not entering the study extension would not be in a steroid-free remission, the overall proportion of patients in steroid-free remission was 46.2% with the IFX-AZA combination, 34.9% under IFX monotherapy, and 24.1% with AZA monotherapy ($P < 0.03$). To select patients with objective signs of inflammation (an elevated C-reactive protein and/or active disease at endoscopy) seems to be important, since in a subgroup analysis, benefit from more aggressive combination therapy was restricted to patients with

objective signs of active inflammation.

Whether these results would affect the management of non-immunosuppressive-naïve patients remains debated. Of note, in a very recent cohort study by Sokol *et al*^[42] IBD flare-ups, perianal complications, and a switch to adalimumab were less frequently observed in patient-semester with combined immunosuppressant and biological use than in those without immunosuppressives (19.3% *vs* 32.0%, $P = 0.003$; 4.1% *vs* 11.8%, $P = 0.03$; 1.1% *vs* 5.3%, $P = 0.006$). Maximal C-reactive protein (CRP) level and IFX dose/kg observed during the semesters were lower in semesters with immunosuppressives. In a multivariate analysis, immunosuppressive co-treatment was associated with a decreased risk of disease flare-up (OR: 0.52; 95% CI: 0.35-0.79). Moreover, the effectiveness of co-treatment with immunosuppressants was time-independent.

POSTOPERATIVE MANAGEMENT: IMMUNOSUPPRESSANTS OR MORE?

Early postoperative use of AZA at a dose of 2-2.5 mg/kg per day) seemed to delay endoscopic postoperative recurrence in comparison to historical series or placebo groups in randomized controlled trials^[43]. Furthermore, in a recent controlled, randomized, prospective trial, AZA administered for 12 mo together with metronidazole for 3 mo was more effective in preventing endoscopic postoperative recurrence assessed at 12 mo, compared to metronidazole alone in patients previously only minimally exposed to AZA^[44]. In a meta-analysis, Peyrin-Biroulet *et al*^[45] have shown that purine analogues were more effective than control arms in preventing clinical recurrence at 1 year (mean difference: 8%, NNT = 13 and 2 years, respectively (mean difference: 13%, NNT = 8). The efficacy of purine analogues was also superior to that of placebo for the prevention of clinical and endoscopic recurrence at 1- and 2-years (mean differences: 13%, NNT = 7, and 23%, NNT = 4), respectively. At 1-year, purine analogues were more effective than control arms in preventing severe (≥2-4) endoscopic recurrence (mean difference: 15%, NNT = 7); however, the rate of adverse events leading to drug withdrawal was higher in thiopurine-treated patients.

In a more recent study from Austria,^[46] the authors evaluated the impact of thiopurine treatment on surgical recurrence in patients after the first intestinal resection for CD. In a Cox regression analysis, treatment with thiopurines for no more than 36 mo (HR: 0.41; 95% CI: 0.23-0.76, $P = 0.004$) and smoking (HR: 1.6; 95% CI: 1.14-2.4, $P = 0.008$) were identified as independent predictors for surgical recurrence. In addition, a multicenter study led by Reinisch *et al*^[47] investigated the efficacy of AZA therapy for the prevention of clinical relapse in patients with endoscopic recurrence (≥2-4, but CDAI < 200). Treatment failure-defined as a CDAI score > 200 and an increase of > 60 points from baseline, or study drug discontinuation due to lack of efficacy or intolerable adverse drug reaction-occurred in 22.0% (9/41) of

AZA-treated patients and 10.8% (4/37) of mesalazine-treated patients. The difference was mainly due to the discontinuation of AZA and the adverse drug reactions that only occurred in AZA-treated patients [9/41 (22.0%) *vs* 0%, $P = 0.002$]. In contrast, clinical recurrence was significantly less frequent in patient treated with AZA *versus* mesalazine [0/41 (0%) *vs* 4/37 (10.8%), $P = 0.031$]. Hence, the efficacy of AZA, while clearly established, must be balanced against its side-effect profile, resulting in a high rate of discontinuation. Finally, preliminary data support biological therapy as a possible therapeutic option, at least in selected patient populations^[48].

ADVERSE EVENTS

6-Mercaptopurine (predominantly used as a chemotherapeutic agent) and its pro-drug, AZA (an immune modifier agent), are purine analogues that competitively interfere with nucleic acid metabolism by acting as substrate competitive antagonists for the hypoxanthine-guanine phosphoribosyl transferase enzyme (anti-metabolite activity)^[49]. Consequently, both drugs reduce cell proliferation and have immune-modifier properties. Adverse events are frequent and lead to cessation of therapy in 9% to 25% of patients^[50]. Adverse events associated with AZA and 6-MP include nausea, allergic reactions, flu-like illness, malaise, fevers, rash, abdominal pain, pancreatitis, hepatotoxicity, myelosuppression, and an increased risk of lymphoma^[51]. Classically, AZA-related adverse events have been categorized into two types: allergic, idiosyncratic or non-dose-dependent and dose-dependent.

Advances in the understanding of AZA and 6-MP drug metabolism have led to genetic and metabolite tests that help clinicians optimize the use of these drugs. A deficiency of the thiopurine methyltransferase (TPMT) enzyme appears to account for some dose- and metabolism-dependent toxicities, such as leukopenia (and possible subsequent infection), thrombocytopenia, and malignancy. TPMT exerts these side effects by limiting the production of 6-TGNs by converting 6-MP to 6-thiouric acid and 6-MMP^[52], and major 6TGN accumulation may lead to profound, potentially life-threatening myelotoxicity. Population studies have shown that the distribution of TPMT activity is trimodal: 0.3%-0.5% of the population have low to absent activity (TPMTL/TPMTL), around 10% have intermediate activity (TPMTL/TPMTH), and approximately 90% inherit normal to high enzyme activity (TPMTH/TPMTH)^[52]. In this regard, a correlation between the TPMT genotype and enzyme activity has been proven. Approximately 5% of the white population carries one or more variant TPMT alleles, with more than ten variant alleles reported^[53]. The functional consequences of alleles *2, *2A, *3B and *3C, accounting together for more than 90% of mutant alleles, have been extensively characterized.

Nevertheless, it is clear that there are many other causes of myelotoxicity. This was accurately demon-

strated by Colombel *et al*^[54], who found that only 27% of CD patients with myelosuppression had a documented low TPMT activity. Other confounding genetic and environmental factors include, for instance, the patient's age, renal function, AZA formulation, co-administration of mesalazine (a reversible TPMT inhibitor) and allopurinol (XO inhibitor). Thus, the determination of TPMT activity is not an exclusive test to rely on when predicting the risk of myelotoxicity. It may only be helpful in identifying a certain group of high-risk patients but as the negative predictive value is rather low, it is not beneficial in ruling out possible side effects. Also, as the prevalence of double carriage of variant TPMT alleles is as low as 1/300, continuous monitoring of red blood cell counts remains mandatory in clinical practice.

Other toxicities such as rash-fever-arthralgias (2.3%), pancreatitis (1.4%), hepatitis, nausea (1.4%), non-pancreatic abdominal pain, and diarrhea appear to be hypersensitivity reactions^[55]. Mercaptopurine may be tolerated in up to 60% of AZA-intolerant patients, and treatment should be considered, particularly if intolerance was due to hepatotoxicity, arthralgia, nausea, vomiting, flu-like illness or rash^[56,57]. A less well-known, and relatively rare, side-effect of AZA is nodular regenerative hyperplasia (NRH). In a recent French study^[58], the cumulative risk calculated was 0.5% at 5-years and 1.25% at 10-years in patients on a median AZA dose of 2 mg/kg per day.

According to a recent review by the Cochrane group, adverse events requiring withdrawal from an induction trial, principally allergy, leukopenia, pancreatitis, and nausea, were increased with active therapy with an odds ratio of 3.44 (95% CI: 1.52-7.77), and were observed in 9.3% of treated patients and in 2.3% of patients in the placebo arms^[59]. The NNT to observe one adverse event on AZA or 6-MP was 14.

In 2005, Kandiel *et al*^[59] performed a meta-analysis utilizing data from six of these cohort studies. The authors were able to pool calculated standardized incidence ratios (SIR) from all studies. When data were pooled across all studies, there were 11 observed lymphomas compared to the expected 2.63 cases, resulting in an SIR of 4.18 (95% CI: 2.07-7.51). Due to significant variability in SIR estimates amongst the studies, sensitivity analyses were performed, where each study was excluded from the group and SIR was recalculated (SIR range: 3.49-5.21). The authors concluded that IBD patients on thiopurines seemed to have a 4-fold increased risk of lymphoma, but whether this risk was due to the medications themselves or the underlying disease severity has not yet been elucidated. Nevertheless, there may be a small but real risk of lymphoma. Interestingly, treatment with AZA or 6-MP appeared to be associated with a small increased risk of Epstein-Barr virus (EBV)-positive lymphoma^[60]. Of note, EBV is a hallmark of lymphomas and lymphoproliferative disorders that arise in patients on immunosuppressive agents, which are used to limit rejection of bone marrow or solid organ transplants [post-transplant lymphoproliferative dis-

Table 1 Current status of thiopurine analogues in the treatment in Crohn's disease: Take home messages

<p>In Crohn's disease treatment paradigms have been evolving in the last decades, with biological therapy becoming available</p> <p>The efficacy of immunosuppressive therapy with purine analogues is well established in controlled trials (induction-maintenance, steroid-sparing agents, postoperative setting)</p> <p>New data indicate that earlier use of immunosuppressants alone may be more effective in maintaining remission, reducing further corticosteroid exposure, and decreasing the risk of hospitalization and surgery</p> <p>Adverse events during thiopurine therapy are frequent and lead to cessation of therapy in 9%-25% of patients</p> <p>Despite intensive research, there is still controversy in the literature regarding the clinical relevance of thiopurine S-methyltransferase (TPMT) testing. Based on recent data, the determination of TPMT activity may be helpful in identifying high-risk patients for developing major complications, especially myelosuppression. In contrast, the negative predictive value is rather low, and it is not beneficial in ruling out the possibility of a side effect. Similarly, there is no established rationale to use TPMT activity for adjusting the dose of azathioprine to enhance therapeutic efficacy. For general practice, regular, frequent monitoring of clinical symptoms and laboratory check-ups continue to be recommended</p> <p>Combination therapy with infliximab-azathioprine may have an added benefit in inducing steroid-free remission and mucosal healing compared to either infliximab or azathioprine alone, in azathioprine-naïve patients with early onset of disease</p> <p>At present, the risks and benefits of combination therapy should be assessed on a per-case basis and should be discussed with the patient in the everyday clinical practice</p>

order (PTLD)]. More recently, the CESAME group^[61] confirmed the above findings, through a study involving 19 486 IBD patients. The incidence rate of lymphoproliferative disorders were 0.90 per 1000 (95% CI: 0.50-1.49) patient-years in those receiving thiopurines, 0.20/1000 (95% CI: 0.02-0.72) patient-years in those who had discontinued therapy, and 0.26/1000 (95% CI: 0.10-0.57) patient-years in those who had never received thiopurines ($P = 0.0054$). The multivariate-adjusted hazard ratio of lymphoproliferative disorder between patients receiving thiopurines and those who had never received the drugs was 5.28 (95% CI: 2.01-13.9, $P = 0.0007$). Most cases associated with thiopurine exposure matched the pathological range of post-transplant disease. Importantly, there was a significant imbalance amongst the forms of disease, since 76% of patients on thiopurine therapy were CD patients *versus* only 48% of patients who never received immunosuppression. Moreover, anti-TNF therapy was also not included in the multivariate analysis, which introduced a significant bias, since there was a 7795 patient-year exposure to anti-TNF therapy and the SIR was significantly increased in patients who had received but discontinued anti-TNF therapy (SIR: 6.92) and exponentially increased in patients on combination therapy (SIR: 10.2).

Whether combined AZA/6-MP and anti-TNF therapy increases toxicity in the long-term, is still debated, but recent studies of 17 hepatosplenic T-cell lymphomas in young male patients with combined therapy have raised considerable concerns^[62]. Unfortunately, most cases were fatal. More data are needed but, in selected patients, particularly those previously exposed to purine analogues or AZA, and scheduled, long-term, maintenance monotherapy with anti-TNF antibodies is certainly a valid option. In contrast, the risk of infections was not higher during combined AZA/6-MP and anti-TNF therapy (OR: 1.6; 95% CI: 0.1-19)^[63]. In a case-control study from the Mayo Clinic, the use of steroids, AZA/6-MP was associated with a 2.2-3.4-fold elevated risk, but the risk was infinite if all three drugs were used.

CONCLUSION

In patients with Crohn's disease, treatment paradigms have been evolving in the last decades, with biological therapy becoming available. In Crohn's disease, the efficacy of immunosuppressive therapy with purine analogues is well established in controlled trials, both as induction agents and as steroid-sparing agents, showing efficacy also in the postoperative setting. In the past several years, numerous studies focusing on the optimal use of both, traditional immunosuppressants and biological therapy, investigating the conventional, accelerated step-up, and top-down approaches, have been published. Emerging new data indicate that earlier use of immunosuppressants is more effective in maintaining remission, reducing further corticosteroid exposure, and decreasing the risk of hospitalization and surgery. However, adverse events are frequent and lead to cessation of therapy in 9% to 25% of patients. Consequently, the benefit of azathioprine, while clearly established, must be balanced against its side-effect profile resulting in a high rate of discontinuation (Table 1).

Additionally, combination therapy with infliximab-azathioprine may have an added benefit in inducing steroid-free remission and mucosal healing compared to either infliximab or azathioprine alone, in azathioprine-naïve patients with early onset of disease. The added benefit of a biological-thiopurine combination is less well-established in non-azathioprine-naïve patients. Long-term combination, however, may potentially be associated with an increased risk for infection and malignancy. In recent years, several important studies on the safety of immunosuppressants, including anti-tumor necrosis factor agents, have been published and the cumulative body of evidence suggests that combined immunosuppressive therapy in patients with inflammatory bowel disease increases toxicity. At present, the risks and benefits of combination therapy should be assessed on a per-case basis and should be discussed with the patient in the everyday clinical practice. Moreover, much emphasis should be placed on defining the important predic-

tive factors in order to enable early patient stratification, thus leading to a tailored management strategy. Certainly, more research is needed in the area, since the impact of changing treatment paradigms with the increasing use of immunosuppressants and biological agents on the natural history is poorly understood. In the future, choosing among treatment paradigms, whether traditional immunosuppressants, biological or a combination in inflammatory bowel diseases may become highly dependent on the individual patient risk profile, the drugs already tried, and disease severity.

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