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Experts Opinion on the Practical Use of Azathioprine and 6-Mercaptopurine in Inflammatory Bowel Disease

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Abstract: The relevance of azathioprine and 6-mercaptopurine therapy in inflammatory bowel disease, Crohn's disease, and ulcerative colitis, has been challenged in recent publications. In this article, a panel of experts gives advice, based on the relevant literature, on indications and practical use of azathioprine/6-mercaptopurine, prevention, and management of drug adverse reactions and special situations such as vaccination, pregnancy, and lactation.

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Key Words: azathioprine, 6-mercaptopurine, thiopurines, inflammatory bowel disease, therapy

Thiopurines are purine antimetabolites that inhibit cell proliferation, especially of lymphatic cells. Their use in clinical medicine dates back more than 60 years, when they were originally investigated as chemotherapeutics, in particular for the treatment of acute and chronic (lymphatic) leukemia, as well as non-Hodgkin's lymphoma. In the 1980s, thiopurines were found to be effective in Crohn's disease (CD) and later also in ulcerative colitis (UC). For more than 20 years, thiopurines prodrugs and derivatives, i.e., azathioprine (AZA), 6-mercaptopurine (6-MP), or 6-thioguanine nucleotides (6-TGN), have been used for the long-term and maintenance treatment of glucocorticoid-dependent or glucocorticoid-refractory inflammatory bowel disease (IBD) (Fig. 1). Recent publications, however, have challenged the place of AZA and 6-MP in the armamentarium for the treatment of CD and UC[1–3] (Table 1). In this article, a panel of experts review the data and facts concerning AZA/6-MP to answer the following questions: (1) what are AZA and 6-MP and how do they work?, (2) which patient with IBD could benefit from AZA/6-MP?, (3) choice of monotherapy or combo therapy with AZA/6-MP, (4) how to start, maintain, and when considering to stop AZA/6-MP?, (5) AZA/6-MP adverse drug reactions: patient information, prevention, and management, (6) special situations such as vaccination, surgery, pregnancy, and lactation while on AZA/6-MP.

METHODS

A literature review on the use of AZA/6-MP was performed focusing on the questions outlined above. The available literature was assessed according to the GRADE approach (Grades of Recommendation, Assessment, Development, and Evaluation). The GRADE working group has developed a system for grading the quality of evidence.[4] The GRADE approach specifies four levels of quality (high, moderate, low, and very low). In a first step, the literature was ranked according to the evidence level, in a second step factors were taken into account that can either downgrade the evidence (such as risk of bias, inconsistency, indirectness, imprecision, and publication bias), or upgrade the evidence (such as a large consistent effect, dose response, confounder only reducing the size of a particular effect). In a third step, the final grade (high, moderate, low, and very low) was assigned. In a fourth step, factors were analyzed that can affect a recommendation on the use of a particular measure (such as balance of desirable and undesirable effects, cost-effectiveness, and preference of patients). In the fifth step, the recommendation for the use of a particular measure was provided (strong for using, weak for using, strong against using, and weak against using). The grading of evidence and recommendation provided here reflects the consensus of the panel, defined as a ≥70% majority vote.

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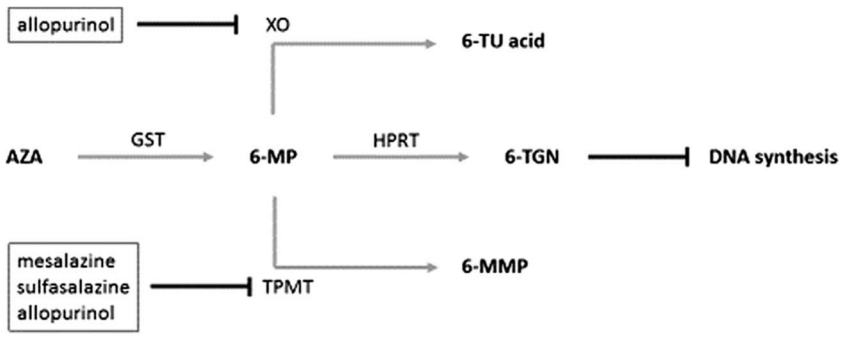

FIGURE 1. Simplified metabolic pathway of thiopurines. 6-TU, 6-thiouric acid (inactive metabolite); GST, glutathione transferase (but nonenzymatic process also active); HRPT, hypoxanthine phosphoribosyl transferase. Black blunt arrows indicate inhibitory pathway.

Question 1: What Are AZA/6-MP, How Do They Work?

AZA/6-MP are purine analogs and act as immunosuppressive/immunomodulating drugs. AZA/6-MP are metabolized through a complex metabolic pathway to 6-TGN antimetabolites that are incorporated into nucleic acid, thereby inhibiting DNA replication and RNA, as well as protein synthesis.

AZA is a prodrug cleaved by glutathione-S-transferase to 6-MP, which is then metabolized to its active form through a complex metabolic pathway. In a simplified manner, it involves the following three main enzymes: thiopurine methyltransferase (TPMT), xanthine oxidase, and hypoxanthine phosphoribosyl transferase that convert 6-MP to 6-thiouric acid (inactive), 6-methylmercaptopurine (6-MMP), and precursors of the active 6-TGN. 6-TGN are antimetabolites, purine antagonists due to their structural similarity that are incorporated into nucleic acids and inhibit DNA replication. This ultimately prevents T-lymphocyte proliferation leading to an immunosuppressive activity. Other proposed mechanisms of action include inhibition of several immune- and inflammation-related genes involved in intestinal inflammation and trafficking of leukocytes to the gut such as tumor necrosis factor (TNF)–related apoptosis-inducing ligand, TNF receptor superfamily member 7, and alpha-4-integrin among activated but not resting T lymphocytes or through T-cell apoptosis induction by blocking the CD28-dependent Rac1 protein activation.[5–8]

TPMT has several genetic variants that may result in reduced or absent TPMT activity. Among whites, approximately 0.3% are homozygous for low enzyme activity (complete deficiency), 11% are heterogeneous (partial deficiency), and 89% are homozygous for high enzyme activity (high activity). An assay of TPMT activity in red blood cells (RBCs) or a TPMT genetic test can identify patients with reduced TPMT activity, allowing for the adjustment of AZA/6-MP dose (see below).[5,9–17]

Question 2: Which Patient with IBD Can Benefit from AZA/6-MP?

In CD, AZA/6-MP seem to offer no advantage over placebo for the induction of remission or for clinical improvement but permit the reduction of steroid consumption. However, AZA/6-MP are less effective than anti–TNF-alpha drugs for the induction of steroid-free remission.

GRADE: high, recommendation: weak.

For induction of remission or clinical improvement in active CD, AZA/6-MP offered no advantage over placebo in a Cochrane review published in 2013, as 48% (107/225) of patients achieved clinical improvement or remission with AZA/6-MP compared with 36% (75/209) with placebo (8 studies, 434

TABLE 1. Type, Frequency, Prevention and Management of AZA Adverse Reactions*

Name of Adverse Event	Type	Frequency and When	Monitoring/Detection
Leukopenia	Dose dependent	10%, anytime	CBC, (6-TGN)
Agranulocytosis	Dose dependent	0.3%, within 4–8 wk	CBC, (TPMT activity)
Gastrointestinal intolerance	+/− Dose dependent	5%–20% within 6–8 wk	None, (patient information)
Hepatitis	Dose dependent	15%–28%, anytime but increases gradually overtime	LFTs, (6-MMP)
Pancreatitis	Dose independent	3%–15%, within 3 mo	Lipase
Flu-like illness	Dose independent	5%, within 2 wk	(Patient information), body temperature
Rash	Dose independent	4%, within 3 wk	Patient information

*CBC, complete blood count; GM-CSF, granulocyte–monocyte colony-stimulating factor; LFT, liver function test.

patients).[18] However, a statistically significant difference was seen regarding steroid sparing (defined as prednisone dose <10 mg/d) between AZA versus placebo, as 64% (47/163) of AZA patients were able to reduce their prednisone dose to <10 mg/d compared with 46% (32/70) of placebo patients (relative risk [RR] 1.34, 95% CI: 1.02–1.77). Hazlewood came to the same conclusion that AZA was not different from placebo for inducing remission (odds ratio (OR) 1.2 95% credible interval of 0.76–2.1) in his comparative effectiveness network meta-analysis.[19] However, the technical review article by the American Gastroenterological Association Institute comments that compared with placebo, AZA/6-MP therapy showed a trend toward fewer failures to achieve remission at 12 to 17 weeks (RR: 0.87; 95% CI: 0.71–1.06).[20]

The open-label RAPID treatment strategy trial by Cosnes et al. compared the efficacy of randomly assigned early treatment (within 6 months after diagnosis) with AZA (2.5 $mg \cdot kg^{-1} \cdot d^{-1}$, n = 65) versus conventional management (AZA only in cases of corticosteroid dependency, chronic active disease with frequent flares, poor response to corticosteroids, or development of severe perianal disease, n = 67).[1] The study, conducted in 24 GETAID French IBD centers, enrolled adults with a diagnosis of CD for less than 6 months who were at risk of disabling disease (age <40 years, active perianal lesions, and corticosteroid use within 3 months of diagnosis). Over an observation period of 3 years, a median of 67% of trimesters were spent in steroid-free and anti-TNF-alpha–free remission (interquartile range, 11%–85%) in the AZA group, compared with 56% in the conventional management group (interquartile range, 29%–73%; $P = 0.69$). There was a statistically not significant trend for a decreased proportion of trimesters with any corticosteroid use. The median duration of a significant (i.e., >10 mg/d prednisone or >3 mg/d budesonide) corticosteroid dose exposure per trimester was 9 days[5,9,10,21–32] in patients in the early AZA group, and 11 days[10,11,18,24–34] in patients in the conventional management group ($P = 0.052$). Regarding the secondary endpoints, there were no differences between the 2 groups in the proportions of trimesters with disease flare, hospitalization, intestinal surgery, or the use of TNF antagonists. However, a higher cumulative proportion of patients in the AZA group were free of perianal surgery than in the conventional management group (96% ± 3% and 82% ± 6% at month 36, respectively; $P = 0.036$). The authors concluded that early AZA was not more effective than conventional management in adult patients with newly diagnosed CD in terms of lengthening the period on clinical remission over 3 years after diagnosis.

In the double-blind, placebo-controlled AZathioprine for Treatment of Early Crohn's disease (AZTEC) trial (n = 131), AZA 2.5 mg/kg started within the first 8 weeks after diagnosis of CD was not associated with an increased rate of sustained corticosteroid-free remission at week 76 compared with placebo (44% versus 36%, $P = 0.48$).[2] The rates of relapse (defined as CDAI >175) and corticosteroid requirements were also similar between the 2 groups. However, a post hoc analysis of relapse, defined as a Crohn's Disease Activity Index (CDAI) score >220, showed lower relapse rates in the AZA group than in the placebo group (8/68 = 12% versus 19/63 = 30%; $P = 0.01$). Panés et al. concluded that early AZA was not more effective than placebo in adult patients with newly diagnosed CD for achieving sustained steroid-free remission over 76 weeks.

The fact that only 81 (60%) of the 136 randomized patients in RAPID and 69 (53%) of the 131 in AZTEC trial completed the studies and the relatively small sample sizes of the two studies raise some concerns about the authors' conclusions. In his comment, Lakatos emphasizes that the interpretation should rather be that patients with CD with a mild phenotype at diagnosis do not necessarily benefit from systematic early introduction of AZA therapy.[35] Nevertheless, infliximab monotherapy outperformed AZA monotherapy at week 26 for corticosteroid-free clinical remission (44.4% versus. 30.0%; NNT 7) in the 2010 Study Of biologic and immunomodulator Naive patients In Crohn's disease (SONIC) trial that randomized 508 treatment-naive, moderate-to-severe, diagnosed (median disease duration of 2.3 years) patients with CD to AZA, infliximab, or a combination of both.[19,36,37] A post hoc analysis of the SONIC trial (n = 188)[38] evaluated different composite remission measures at week 26 in a subgroup of patients with CD with CDAI scores, C reactive protein (CRP), and endoscopic data available at baseline and week 26 (n = 188). Assessed composite remission measures were: clinical remission (CDAI < 150) and mucosal healing (MH, absence of any mucosal ulcerations), also called as "deep remission," and alternative composite endpoints: clinical remission + normalized CRP (CRP < 0.8 mg/dL); normalized CRP + MH; and clinical remission + normalized CRP + MH. Seventy-two percent of the patients achieved clinical remission, 48% achieved MH at week 26. All composite outcomes were significantly greater (Bonferroni significance level, $P \leq 0.016$) with combination therapy (i.e., infliximab and AZA; 52%–64%) versus AZA monotherapy (13%–29%; $P \leq 0.005$ for all comparisons). Composite remission rates including MH were significantly greater with combination therapy (52%–57%) versus infliximab (26%–32%; $P \leq 0.015$ for all comparisons except normalized CRP + MH, $P = 0.017$) and versus AZA monotherapy (13%–20%; $P \leq 0.002$ for all comparisons). In the SONIC trial, patients in the AZA group had only AZA to induce remission, which may explain the lack of efficacy (steroid-free remission at week 26). This was in contrast with the Combination Of Maintenance Methotrexate-Infliximab Trial study where patients received conventional steroids and in addition also infliximab and methotrexate or placebo.[39]

In CD, AZA (2–2.5 $mg \cdot kg^{-1} \cdot d^{-1}$) or 6-MP (1–1.5 $mg \cdot kg^{-1} \cdot d^{-1}$) are effective as maintenance therapy for patients:

1. Whose disease is chronically active or flares frequently
2. Who are dependent on steroids, have experienced steroid-related side effects, or for whom steroids no longer work, as it reduces the need for steroid treatment.

GRADE: high, recommendation: strong.

In CD and in UC, corticosteroid-induced remission is a common clinical scenario, and the discontinuation of

corticosteroids is associated with high relapse rates.[40,41] In addition, many patients whose disease is steroid dependent or refractory experience serious steroid-related side effects. Steroid-sparing strategies are thus needed for a large proportion of patients exposed to this class of medication.

In a Cochrane review by Prefontaine, AZA/6-MP have been shown to have a positive effect on maintaining remission in CD.[42] Indeed, the OR for maintenance of remission with AZA was 2.32 (95% CI: 1.55–3.49) with a NNT of 6, whereaas the one with 6-MP was 3.32 (95% CI: 1.40–7.87, NNT of 4). Furthermore, a steroid-sparing effect was noted with AZA (OR of 5.22, 95% CI: 1.06–25.68, NNT of 3). When these maintenance therapy data were analyzed for the effect of the AZA dose (range 1.0–2.5 $mg \cdot kg^{-1} \cdot d^{-1}$), the OR for response increased from 1.20 (95% CI: 0.60–2.41) at 1.0 $mg \cdot kg^{-1} \cdot d^{-1}$ to 3.01 (95% CI: 1.66–5.45) at 2.0 $mg \cdot kg^{-1} \cdot d^{-1}$ and to 4.13 (95% CI: 1.59–10.71) at 2.5 $mg \cdot kg^{-1} \cdot d^{-1}$. Furthermore, more patients seemed to respond to AZA (71% maintained remission in pooled analysis) than to 6-MP (51% maintained remission). However, 1 study by Hanauer et al,[43] published in 2004, used a low dose of 6-MP (50 mg/d), which may account for this difference.

In the setting of steroid resistant CD, however, the use of an anti–TNF-alpha drug might be more appropriate than AZA/6-MP, although no other trial than SONIC (follow-up until week 50) directly compared the two drug classes. At week 50, the corticosteroid-free clinical remission rate in SONIC was 24.1% for AZA alone (n = 170), 34.9% for infliximab alone (n = 169) ($P = 0.03$), and 46.2% for the combo therapy (n = 169), P value for combo therapy versus infliximab alone = 0.04 and P value for combo therapy versus AZA alone <0.001.[36]

In perianal fistulizing, CD AZA/6-MP have limited impact, thus anti-TNF treatment should be favored.

GRADE: moderate, recommendation: weak for using AZA/6-MP.

No controlled clinical study evaluated AZA/6-MP as therapy for CD with fistula closure as a primary endpoint. A meta-analysis[44] of 5 randomized placebo-controlled clinical trials in which fistula closure was a secondary endpoint showed beneficial effects of AZA/6-MP in 54% of cases, whereas only 21% responded to placebo (OR 4.4, 95% CI: 1.5–13.2).[45] A limitation of this analysis was that 66% of the pooled patients came from a single study. In another meta-analysis, an odds ratio of 4.44 (95% CI: 1.5–13.2) favoring healing or decreased discharge with antimetabolites was reported.[44] In a third meta-analysis, looking at response rates to placebo in patients with fistulizing CD, Ford et al[46] found a pooled rate of 15.6% (95% CI: 10.9%–20.9%) among all RCTs, for complete fistula closure. AZA/6-MP might therefore be used in fistulizing CD but would rarely be sufficient alone. The panel thus recommends that anti-TNF treatment with infliximab, adalimumab, or certolizumab pegol[47,48] should be used as an integral part of the combined medical and surgical therapeutic approach to fistulizing CD, in line with the ECCO recommendations.[49]

In CD, AZA/6-MP seem to be effective for maintenance of surgically induced remission, although efficacy outcomes between AZA/6-MP and 5-aminosalicylic acid (5-ASA) agents are uncertain.

AZA/6-MP prescription postoperatively is recommended in the presence of:

1. Intolerance to 5-ASA
2. Poor prognostic factors (high relapse rate, penetrating disease, repeat surgery, multiple admissions for flares, involvement of the upper gastrointestinal tract, early age at diagnosis, smoking, and extensive ulceration of the mucosa)
3. Ulcerations at the endoscopic control 6 months postsurgery (Rutgeerts' score ≥i2).

GRADE: moderate, recommendation: weak.

The panel recommends furthermore to step up to an anti-TNF agent in case of Rutgeerts' score ≥i2 at the endoscopic control in patients already under AZA/6-MP.

Based on 2 small studies, AZA/6-MP (n = 168) are superior to placebo for maintenance of surgically induced remission in patients with CD. In the AZA/6-MP group, 48% of patients experienced a clinical relapse compared with 63% of placebo patients (RR 0.74, 95% CI: 0.58–0.94).[43,50] A pooled analysis of five studies (n = 425 patients) showed no difference in clinical relapse rates at 1 or 2 years between AZA/6-MP and 5-ASA agents. In the AZA/6-MP group, 66% of patients had a clinical relapse compared with 54% of 5-ASA patients (RR 1.15, 95% CI: 0.99–1.34).[18]

In a Cochrane systematic review, one small study, which used intravenous or oral methotrexate (25 mg/wk), showed no statistically significant difference between methotrexate and AZA; 44% of patients under methotrexate (12/27) failed to enter remission, compared with 37% of patients under AZA (RR 1.20, 95% CI: 0.63–2.29).[41]

De Cruz (n = 174) conducted a strategy comparison trial aimed at the identification of the optimal strategy to prevent postoperative disease recurrence—The Post-Operative Crohn's Endoscopic Recurrence (POCER) treat-to-target study.[51] The authors compared standard treatment comprising 3 months of metronidazole tailored to clinical risk stratification of recurrence (low risk: no treatment, high risk (smoker, perforating disease, ≥second operation): thiopurine, high risk and thiopurine intolerant: adalimumab) to colonoscopy at 6 months with treatment step-up in case of recurrence (i.e., Rutgeerts' score ≥i2) (thiopurine, fortnightly adalimumab with thiopurines, or weekly adalimumab). The second strategy was the best therapeutic strategy for prevention of postoperative CD recurrence. At 18 months, endoscopic recurrence occurred indeed in 60 (49%) patients in the active care group (colonoscopy at 6 months) compared with 35 (67%) patients in the standard care group (no colonoscopy), $P = 0.03$. Complete mucosal normality was maintained in 27 (22%) of 122 patients in the active care group versus 4 (8%) in the standard care group ($P = 0.03$), with no differences in incidence and type of adverse and severe adverse events. Furthermore, Savarino showed in a randomized trial (n = 51) that the rate of endoscopic

recurrence at 2 years after ileocolonic resection was significantly lower on adalimumab (6.3%) compared with AZA (64.7%) or mesalamine (83.3%).[52] Clinical recurrence (12.5%) was also significantly lower and quality of life significantly higher IBD Questionnaire score (IBDQ = 220) in the ADA group compared with the AZA 2 $mg \cdot kg^{-1} \cdot d^{-1}$ (64.7%, IBDQ = 90) and mesalamine groups 3 g/d (50%; IBDQ = 98).

In UC, AZA/6-MP is recommended for patients who:

1. Have failed or cannot tolerate mesalamine or sulfasalazine
2. Require repeated courses of steroids.

GRADE: moderate, recommendation: strong.

Like in CD, therapeutic strategies in UC aim to treat flares and prevent recurrence of the disease. For a mild to moderate disease, 5-ASA is the treatment of choice for flares and the maintenance of remission. In case of severe flares or 5-ASA inefficiency/intolerance, corticosteroids remain the first choice to induce remission, but their tapering/discontinuation is associated with high rates of relapse. In addition, many patients whose disease is steroid dependent or refractory experience steroid-related side effects. Steroid-sparing strategies are thus needed for a large proportion of patients exposed to this class of medication.[40,53,54] Furthermore, the recently approved introduction of MMX-budesonide for the treatment of UC is likely to renew the problem of steroid dependence.[54]

In a prospective single-center study in the UK where the use of biological therapies for chronic relapsing and remitting UC is not routinely allowed in accordance with National Institute for Health and Care Excellence guidance, all patients with UC treated with AZA were identified from an electronic database. There were 164 (64.3%) of 255 patients who were still receiving AZA at the last follow-up, of whom 154 (60.4%) were considered to have achieved sustained clinical benefit.[55]

Fraser reviewed the charts of patients attending the Oxford IBD clinic from 1968 to 1999. The overall remission rate under AZA—remission was defined as no need for oral steroids for at least 3 months and relapse as active disease requiring steroids—was 58% (n = 346). For patients in receipt of more than 6 months of treatment, remission rates were 87%. Interestingly, there was no difference in relapse rates between CD and UC.[56]

A Cochrane review pooled six studies (n = 286) of mostly poor quality, comparing AZA, 6-MP, placebo, mesalamine, or sulfasalazine for a duration of at least 12 months. AZA was better than placebo for maintenance treatment, as 56% of patients treated with AZA were disease free after 1 year of treatment compared with 35% of patients who received placebo. Therefore, given the established effectiveness and safety of aminosalicylates (i.e., mesalamine or sulfasalazine) for remission maintenance in UC, AZA/6-MP cannot be recommended as first-line treatment in this setting. However, AZA/6-MP may be an effective maintenance treatment of patients who have failed or cannot tolerate mesalamine or sulfasalazine and for patients who require repeated courses of steroids.[57]

The randomized double-blind UC-SUCCESS trial evaluated the efficacy and safety of 16 weeks of treatment with infliximab monotherapy, AZA monotherapy, or the combo therapy of both in anti–TNF-naive moderate-to-severe UC. Patients treated with the combo therapy were statistically significantly more likely to achieve corticosteroid-free remission at week 16 than those receiving infliximab alone (40% [31 of 78] versus 22% [17 of 77; $P = 0.017$]) or AZA alone, 24% (18 of 76; $P = 0.032$). Furthermore, the combination therapy led to significantly better MH than AZA monotherapy.[58]

Armuzzi et al investigated the long-term outcome of consecutive patients with steroid-dependent UC treated with infliximab. At the median duration of follow-up of 41.5 months (interquartile range 26–45), 64% (46/96) of patients showed a sustained clinical response, and colectomy-free survival was 77%. Combo therapy was an independent predictor of sustained clinical response ($P < 0.0001$). AZA/6-MP–naive status was protective from colectomy ($P = 0.025$). In this study, Mayo endoscopic subscore of 3 at baseline ($P = 0.04$) and high CRP after induction ($P = 0.001$) were independent predictors of colectomy.[25]

In view of efficacy, cost, safety, and tolerability issues, the panel recommends thiopurine maintenance therapy for patients with a low risk of disease progression (i.e., moderate activity, and not defined as at high risk of disease progression, see below) who responded to corticosteroids. For corticosteroid-dependent patients or patients at high risk of disease progression (extensive severe colitis, flares requiring hospitalization, high erythrocyte sedimentation rate, or high concentration of C-reactive protein), the panel recommends an anti-TNF agent, either as monotherapy or preferably in combination with a thiopurine, in accordance with the Toronto consensus,[30] it should indeed be noted that, in the UC-SUCCESS trial, compared with the combo therapy, those receiving infliximab alone performed slightly worse (22% [17 of 77; $P = 0.017$]) than patients receiving AZA alone, (24% [18 of 76; $P = 0.032$]), or vedolizumab (with the exception of fulminant colitis due to lack of data).

AZA/6-MP can induce MH in CD and in UC.

GRADE: moderate.

MH has emerged as an important treatment goal for patients with IBD as it is associated with sustained clinical remission, as well as reduced rates of hospitalization and surgical resection. In UC, MH may represent the ultimate therapeutic goal because inflammation is limited to the mucosa. In CD, which is a transmural disease, MH can be considered as an initial event in the suppression of inflammation of deeper layers of the bowel wall rather than as a sign of complete healing of gut inflammation; for that reason, it is viewed by some as a minimum therapeutic goal.[59,60] Few original data have been published on that topic but the data indicate that AZA is able to induce and maintain MH.

D'Haens[61] showed in 2 studies that AZA can lead to MH. In severe recurrent Crohn's ileitis after ileocecal resection, 15 of 19 patients treated with AZA have been reevaluated by endoscopy or radiology. AZA therapy resulted in induction and maintenance of clinical remission in all 15 patients for at least 6 months after

complete weaning off corticosteroids. Complete macroscopic healing of the neoterminal ileum was observed in 6 of 15 patients, near-complete healing with only superficial erosions remaining in 5 of 15 patients, partial healing in 3 of 15 patients, and unchanged inflammatory lesions in 1 patient. D'Haens[62] also studied MH in 20 patients with CD on AZA with long standing steroid-free remission i.e., taking AZA for at least 9 months (mean duration 24.4 ± 13.7 months) and no corticosteroids for at least 3 months. He showed that full MH could be achieved in 54% (7/13) of patients with ileitis, and 70% (14/20) with colitis with most of the remaining patients having at least partial healing, and only a small minority experienced no healing at all (1/20 for colitis and 1/13 for ileitis). Mantzaris et al[63] also showed the healing potential of AZA in a controlled study. This study showed the superiority of AZA (n = 38) over budesonide (n = 39) in patients with steroid-dependent Crohn's ileocolitis or proximal colitis who had achieved clinical remission on conventional steroids, as complete or near-complete healing was achieved in 83% of AZA-treated patients compared with only 24% of budesonide-treated patients ($P < 0.0001$). In the SONIC trial, MH occurred in 16% of patients receiving AZA monotherapy, 30% of patients receiving infliximab monotherapy, and 44% of patients receiving combo therapy at week 26.[36] In the above-mentioned post hoc analysis of the SONIC trial, 48% of patients with CD achieved MH at week 26, and combination therapy (i.e., infliximab and AZA) was significantly more effective than AZA or infliximab monotherapy.[38] A 2014 Effective Health Care Program comparative effectiveness review found moderate strength of evidence for the benefit of infliximab over AZA for MH, as well as the benefit of combo therapy over AZA for disease activity and MH. However, the data used for analysis were almost entirely drawn from the SONIC trial.[22,36]

Limited data are available to assess thiopurines-mediated MH in UC. An open-label study with AZA[64] showed MH in 22/32 (69%) patients after at least 6 months therapy, indicating that MH may be maintained with AZA. Another study found that AZA therapy for 6 months resulted in MH and complete remission in 19/36 (53%) patients with UC compared with 7/36 (19%) patients on 5-ASA.[24] As already mentioned above, MH at week 16 in the UC-SUCCESS trial was achieved in 63% (49 of 78) of the combo therapy (infliximab and AZA) group versus 55% (42 of 77, $P = 0.295$) of the infliximab alone group and 37% (28 of 76, $P = 0.001$) of the AZA alone group.[58]

Question 3: Choice of Monotherapy or Combo Therapy?

In CD, the combination of AZA/6-MP and infliximab (combo therapy) is superior to infliximab alone for the induction of steroid-free remission.

1. Severe CD, characterized by early disease onset, extensive, and deep intestinal ulcerations or complex fistulizing perianal disease, may benefit from combo therapy with AZA/6-MP and an anti–TNF-alpha drug (especially infliximab, as the value of combo therapy in adalimumab or certolizumab studies has not been shown convincingly) to maximize rapid control
2. Six months to 1 year of combination therapy is recommended, with no data available for longer duration.

GRADE: high, recommendation: strong.

In UC, combo therapy for 16 weeks with AZA/6-MP and infliximab seems to be the best option for moderate to severe UC in anti-TNF-alpha-naive patients. There are, however, no data for longer treatment durations.

GRADE: high, recommendation: strong.

In the Cochrane review published in 2013, the combination of AZA and infliximab was significantly superior to infliximab alone for the induction of steroid-free clinical remission in CD, as 66% (116/194) of patients under combo therapy achieved steroid-free remission compared with 48% (91/189) of patients on infliximab alone (2 studies, 383 patients; RR 1.23, 95% CI 1.02–1.47).[18] In a technical review by the American Gastroenterological Association Institute, the authors performed an analysis, combining data from the SONIC study and a study by Lemann that compared therapy with infliximab (3 doses) plus AZA (n = 58) with AZA/6-MP therapy alone (n = 57). Fewer patients failed to achieve remission in the combo group than in the AZA group (RR, 0.61; 95% CI: 0.52–0.73). The use of combo therapy reduced the number of remission failures as compared with AZA therapy alone by 274 per 1000 patients, without significant difference in the rates of serious infections.[20,65]

Based on a network meta-analysis, infliximab plus AZA or adalimumab seem to be the most effective therapies for the induction and maintenance of remission in CD.[19] In the SONIC trial that included only patients naive to immunomodulators, combo therapy at week 26 was associated with higher rate of corticosteroid-free clinical remission than infliximab monotherapy (56.8% versus 44.4%; NNT 8) and AZA monotherapy (56.8% versus 30.0%; NNT 4). At week 50, 24% of patients on AZA monotherapy achieved steroid-free remission compared with 35% of patients receiving infliximab monotherapy and 46% of patients receiving combo therapy.[36] Combo therapy was better than monotherapy in terms of MH rates, clinical remission, disease severity rating, and quality of life rating.[22,36]

In the SONIC trial, patients in the AZA group had only AZA to induce remission which may explain the lack of efficacy (steroid-free remission at week 26). This was in contrast with the Combination Of Maintenance Methotrexate-Infliximab Trial in which patients received conventional steroids and additionally infliximab and methotrexate or placebo (Feagan BG Gastroenterology. 2014;146:681–688). In Combination Of Maintenance Methotrexate-Infliximab Trial, a 50 weeks double-blind placebo-controlled trial with 126 patients, the combination of infliximab and methotrexate, although safe, was actually no more effective than infliximab alone (30.6 versus 29.8% of actuarial rate of treatment failure, respectively) in patients with CD

receiving treatment with prednisone as induction therapy within the preceding 6 weeks.[39]

A meta-analysis by Jones[66] questioned whether patients with CD who start anti-TNF therapy after failed immunomodulator therapy (subgroup of patients from 11 randomized controlled trials) should continue to receive concomitant immunomodulators. Overall, combination therapy was no more effective than monotherapy in inducing 6 months remission, inducing a response, maintaining a response, or inducing partial or complete fistula closure. Similarly, in subgroup analyses of individual anti–TNF-alpha agents, combination therapy was not more effective than anti-TNF monotherapy in inducing 6 months remission. Combination therapy was not associated with an increase in adverse events, in contrast to other published data.[38] However, the benefit of combo therapy may extend to preventing the long-term loss of response to infliximab as it is associated with lower CRP levels[67] and fewer trimesters with CD flares.[68]

In the UC SUCCESS,[58] which included only anti–TNF-naive adults, 10% of whom had previous AZA or ciclosporine, combo therapy was shown to be the best option for moderate to severe UC to achieve steroid-free remission at week 16. However, there are no data for longer durations, and thus the therapeutic strategy beyond 16 weeks remains unclear. The combo therapy group also had the best percentage of MH at week 16 with 63% (49 of 78) versus 55% (42 of 77; $P = 0.295$) for the infliximab alone group and 37% (28 of 76; $P = 0.001$) for the AZA alone group.

The combination of methotrexate and infliximab does not seem to provide any additional benefit over infliximab monotherapy. However, the available studies were, comparatively, small; further research is thus needed to determine the role of methotrexate when used in conjunction with infliximab or other biological therapies.[41]

In a retrospective study by Ben-Horin, addition of AZA/6-MP (n = 3) or methotrexate (n = 2) to infliximab was shown[28] to gradually decrease levels of antibodies to infliximab, whereas trough levels of infliximab increased and clinical response was restored.

There are several hypotheses why combo therapy may lead to greater efficacy. One reason may be the additive immunosuppressive effect, for example through a synergistic effect on apoptosis induction.[69] Another relevant mechanism might be the prevention of immunogenicity. Indeed, combo therapy has been shown to be associated with higher infliximab trough levels[36] and to reduce infliximab clearance, as well as the formation of antibodies to infliximab (ATI). ATI can cause infusion reactions and loss of response.[26,70] Combo therapy lowers the incidence of antidrug–antibodies for all anti–TNF-alpha agents, regardless of the origin of the protein sequence (chimeric, humanized, and human) and the disease population (rheumatoid arthritis and IBD), as they can block the expansion of activated immune cells. This pharmacological effect of AZA and 6-MP is considered the underlying mechanism to explain the inhibition of the anti–TNF-alpha drugs immunogenicity.[36,71–74]

Question 4: How to Start, Maintain, Monitor, and Stop AZA Treatment?

There are 2 dosing strategies for AZA and 6-MP: the first one is empiric weight-based dosing, the second one is based on TPMT phenotyping (activity measurement).

1. There is a lack of studies evaluating how quickly the AZA dose can be increased. Some clinicians start directly with the target dose of 2.0 to 2.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for AZA and 1.0 to 1.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for 6-MP. Others start at low dose (50 mg or 1 $mg \cdot kg^{-1} \cdot d^{-1}$ for AZA and 25 mg or 0.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for 6-MP) and then increase to the full dose or increase the dose every second week to reach the target dose
2. Determining TPMT activity may reduce the time to therapeutic drug levels and reduce side effects.

Complete blood counts and liver tests should be performed every week during the first month, then every second week for the next 2 months (the European Summary of Product Characteristics recommends a weekly blood count during the 8 first weeks of treatment), and then every 3 to 4 months as myelotoxicity and hepatotoxicity may also develop as late complications.

Measuring 6-TGN and 6-MMP metabolites in erythrocytes may be used to monitor therapeutic drug levels and patient's compliance and to lower the risk of toxicity.

GRADE: low, recommendation: weak.

AZA absorption in healthy subjects ranges between 16% and 50%. The molecular weight of 6-MP is 55% of AZA's, and 88% of AZA is converted to 6-MP. A conversion factor of 0.5 is therefore used to convert AZA dosing into 6-MP dose.[75]

It is a question whether to start with AZA or with 6-MP. A Cochrane review concluded that AZA and 6-MP are both more effective than placebo for maintenance of remission in CD. However, higher response rates were obtained with AZA than 6-MP. However, the one study evaluating 6-MP used a relatively low dose of the drug.[76]

There are 2 dosing strategies for AZA and 6-MP. The first one is empiric weight-based dosing and the second one is based on TPMT phenotype testing (enzyme activity measurement; this should not be measured in patients who have received recent blood transfusions). TPMT genotyping (usually performed by single-nucleotide polymorphism analysis and mutation detection) has indeed less sensitivity and is less cost-effective than phenotyping in predicting the risk of leukopenia.[77] However, outside of the USA, TPMT enzyme activity measurement is not universally and readily available, and not universally covered by health insurances.

Some clinicians start immediately with the target dose of 2.0 to 2.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for AZA and 1.0 to 1.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for 6-MP, whereas others start at low dose (50 mg or 1 $mg \cdot kg^{-1} \cdot d^{-1}$ for AZA vs. 25 mg or 0.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for 6-MP) and then increase to the full dose or increase the dose by 50 mg every 2

TABLE 3. Interpretation and Required Intervention According to Thiopurines Metabolite (6-TGN/6-MMP) Results

Overdose → reduce the dose with active disease—refractoriness to thiopurines → add or switch to an induction drug (e.g., steroids or anti-TNF agents)	High TPMT activity ("shunt" of the metabolism → leads to inefficacy of treatment)
Low or deficient TPMT activity → reduce the dose (to 30%–50%) or stop (depending of the myelotoxicity) and consider measuring TPMT activity (for assessment of risk)	poor patient compliance → patient education or underdosed → increase the dose

Tested in the framework of a stable dose (at least 2–3 weeks).

or 4 weeks to reach the target dose. Some advocate measurement of TPMT activity[5,18] to start directly with the full dose or to increase the dose toward the target dose more rapidly, and to reduce potential adverse reactions. TPMT activity measurement may indeed reduce time to therapeutic response and minimize toxicity, in particular leukopenic events.[78] High TPMT activity is associated with lower 6-TGN and higher 6-MMP levels, potentially explaining reduced response rates and increased hepatotoxicity.[16] In case of intermediate to high TPMT activity, the AZA/6-MP dose might be rapidly escalated toward therapeutic drug levels. By contrast, low TPMT activity requires initial dose reduction and slower dose escalation to avoid toxicity.[79]

Measuring 6-TGN and 6-MMP metabolites in erythrocytes can be used to monitor therapeutic drug levels, patient compliance, and risk of toxicity (Table 3). AZA/6-MP therapeutic efficacy has been associated with 6-TGN levels >235 pmol/8 × 10E8 erythrocytes, whereas risk of leukopenia correlates with 6-TGN level >450 pmol/8 × 10E8 erythrocytes, and risk of hepatotoxicity with 6-MMP level >5700 pmol/8 × 10E8 erythrocytes.[13]

Osterman et al[80] pooled the available data of previous studies (they identified 55 articles, 12 of which contained data suitable for inclusion) to provide a more precise estimate of the association between 6-TGN levels and IBD activity. The mean/median 6-TGN levels were higher among patients in remission than in those with active IBD (pooled difference, 66 pmol/8 × 10^8 RBCs; 95% CI, 18–113; $P = 0.006$), but with significant heterogeneity. Excluding the 1 outlier study eliminated this heterogeneity. Patients with 6-TGN levels above the threshold value of 230 to 260 pmol/8 × 10^8 RBCs were more likely to be in remission (62%) than those below the threshold value (36%) (pooled odds ratio, 3.3; 95% CI: 1.7–6.3; $P < 0.001$), but with significant heterogeneity. Again, excluding the 1 outlier study eliminated this heterogeneity.

Recently, Moreau[81] performed a meta-analysis on 17 studies enrolling 2049 patients with IBD and clearly established an association between 6-TGN levels and clinical remission rates in patients with IBD and explained the heterogeneity of results among selected studies by the lack of standardization in 6-TGN assays. Including only studies using the reference method by Lennard et al (n = 10), the pooled odds ratio for clinical remission among patients with 6-TGN levels over a cutoff value between 230 and 260 pmol/8 × 10^8 RBC was 3.15 (95% CI: 2.41–4.11).

In the absence of studies evaluating how quickly an AZA/6-MP dose can be increased, we recommend a dose increase every second week, provided the medication is well tolerated and in the absence of hematologic toxicity (in particular white blood cell count >4 × 10E3/L). Complete blood counts and liver function tests should be regularly monitored, irrespective of the chose initial dose. TPMT genotyping or phenotyping cannot be a substitute, but could help choosing the appropriate dose,[82] We propose monitoring complete blood counts and liver function tests every week for the first month, then every second week for the 2 following months, and then every 3 months,[5] as leukopenia and hepatotoxicity can also occur as a late adverse drug reaction (Fig. 2).

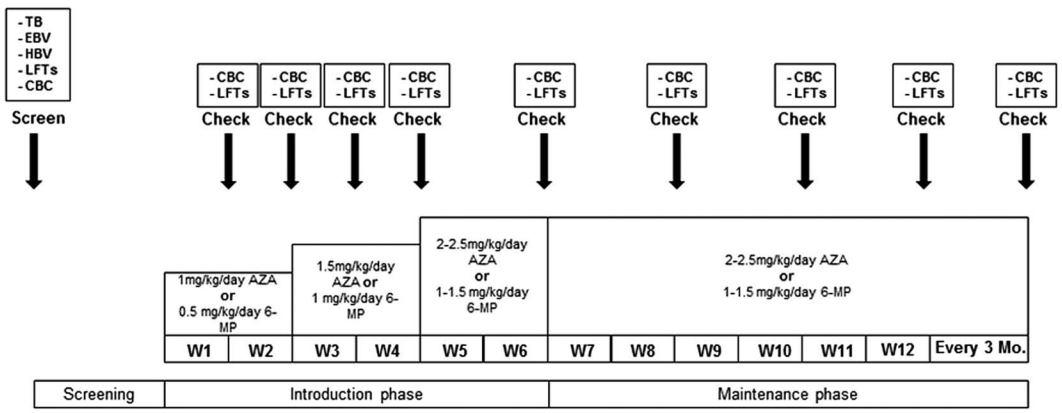

FIGURE 2. AZA therapy introduction and monitoring scheme. Proposed screening, dosing, and surveillance scheme for the introduction of AZA/6-MP introduction. CBC, complete blood counts; EBV, Epstein–Barr virus; HBV, Hepatitis B virus serology; LFTs, liver function tests; Mo, months; TB, tuberculosis screening; W, week.

Mesalamine and sulfasalazine have been shown in some studies to increase 6-TGN levels in 82% of study patients in 1 study and 100% in another study, possibly as the consequence of TPMT inhibition. This effect should therefore increase therapeutic efficacy and decrease side effects such as hepatotoxicity. However, this combination therapy has been associated with increased rates of myelotoxicity, although the available data are to some extent contradictory.[83]

For that reason, in accordance with Amin et al, we propose to consider the preventive reduction of the AZA/6-MP dose by 25% combined with continuous laboratory monitoring.[5,84,85]

In patients with CD and UC with long-term clinical and endoscopic remission, an interruption of AZA/6-MP may be considered after 4 years of therapy.

GRADE: moderate, recommendation: weak.

When patients with CD achieve sustained steroid-free remission under AZA, it is usually stable and long standing. A controlled withdrawal study from the GETAID indicated sustained remission over more than 5 years in 80% of patients when treatment was continued, compared with 40% of patients when it was stopped.[86] In another study on patients with CD in remission for >3.5 years under AZA, the remission rate was higher after 18 months if AZA therapy was not interrupted, as 3 patients suffered a relapse in the AZA group (n = 40) and 9 in the placebo group (n = 43). Kaplan–Meier estimates of the relapse rate at 18 months were 8% ± 4% and 21% ± 6%, respectively.[87] In a single-center retrospective study, Oussalah et al. assessed the cumulative probability of infliximab failure in patients with CD in remission who stopped AZA after receiving infliximab in combination with AZA for at least 6 months. Survival without infliximab failure was 85% (±5%) at 12 months, and 41% (±18%) at both 24 and 32 months in 48 patients with CD.[88]

Patients with UC who discontinue AZA or 6-MP treatment have a high relapse rate, as 87% of complete responders who discontinued 6-MP subsequently relapsed[89]; after AZA withdrawal, a third of the patients relapsed within 12 months, half within 2 years, particularly in case of history of extensive colitis, lack of sustained remission during AZA maintenance or in case of short treatments (3–6 months).[33] Few data help us to decide when considering a discontinuation of AZA/6-MP. In his retrospective Oxford IBD clinics study, Fraser showed that after discontinuing AZA while still in remission, the percentage of patients with UC and patients with CD (n = 222) remaining in remission at 1, 3, and 5 years was 63%, 44%, and 35%, respectively; it is interesting to note that the duration of AZA treatment did not affect the relapse rate after treatment discontinuation.[56] In a multicenter retrospective cohort study from 11 centers across the UK, Kennedy et al investigated the success of planned thiopurines withdrawal (with continuous thiopurines use ≥3 years) in 237 patients (129 CD, 108 UC) in sustained clinical remission. The median duration of thiopurines use before withdrawal was 6.0 years (interquartile range 4.4–8.4). At 1 year postwithdrawal, moderate/severe relapses were observed in 23% patients with CD and 12% patients with UC ($P = 0.035$), and at 2 years in 39% patients with CD versus 26% patients with UC.[17]

In a review article on "Why, when and how to de-escalate therapy in inflammatory bowel diseases," Pariente and Laharie calculated that in patients receiving AZA/6-MP alone, relapse rate

TABLE 2. Vaccine Characteristics and Use During Immunosuppressive Therapy with AZA/6-MP (Adapted from Refs. 42,45,76)

Vaccine Type	Vaccine Target	Antigen Type	Restriction of Use	Prevaccination Washout Period	Postvaccination Waiting Period efore AZA Therapy
Inactivated vaccines	Diphtheria, tetanus, pertussis, poliomyelitis, Hepatitis A, Hepatitis B, tick-borne encephalitis	Proteins	No restriction	No wash out	1 mo
	Haemophilus influenza type b, *Pneumococcus* (PCV 13 and PPSV 23), *Meningococcus C*, *Meningococcus* ACW135Y, typhoid (injectable)	Polysaccharides (conjugated or not)			
	Human papilloma virus	Virus-like particles			
Live vaccines	Measles-mumps-rubella, varicella, zoster, yellow fever, typhoid 21a (oral), rotavirus, tuberculosis (BCG)	Living particles	Contra-indicated	>3 mo	1 mo

at 12 months after AZA/6-MP cessation was around 20% in CD and 30% in UC.[90] A multidisciplinary European expert panel on "When do we dare to stop biological or immuno-modulatory therapy for CD" considered it appropriate to stop AZA/6-MP monotherapy after 4 years of clinical remission; for combo therapy, anti–TNF-alpha withdrawal, while continuing the immunomodulator, was considered appropriate after 2 years of clinical remission.[91]

We recommend performing thorough clinical and biological monitoring before stopping AZA in UC and in CD—stool calprotectin, CRP, WBC, colonoscopy, and imaging—and to consider deescalation only in the presence of clinical and biological remission, including MH. We propose to continue the regular monitoring of stool calprotectin levels after drug withdrawal.

Question 5: AZA Adverse Drug Reactions, Patient Information, Prevention, and Management?

Around 15% to 20% of patients have to discontinue AZA because of adverse drug reactions. Myelosuppression and hepatotoxicity are generally dose dependent and related to intracellular concentration of active metabolites. Severe adverse drug reactions are encountered only rarely if the dose does not exceed 2.5 $mg \cdot kg^{-1} \cdot d^{-1}$ (AZA) or 1.5 $mg \cdot kg^{-1} \cdot d^{-1}$ (6-MP).[18] However, the development of leukopenia, anemia, or pancytopenia, due to the accumulation of the active 6-TGN metabolite, can be observed months to years after the initiation of therapy.[92] Hepatotoxicity but also in some cases nausea, myalgia, and fatigue, seem to be associated with the production of the methylated 6-MMP products. In 15% to 20% of patients ("hypermethylators"), this mechanism is exacerbated. A high TPMT activity (>14 U/mL RBC) indeed results in more 6-MMP, which leads to increased hepatotoxicity,[5,16] whereas low TPMT activity is associated with high 6-TGN levels and thus myelotoxicity.

Blocking xanthine oxidase with allopurinol and reducing the AZA dose to only a quarter—as concomitant prescription of allopurinol and AZA/6-MP can lead to severe myelotoxicity—can solve this issue and optimizes the proportion of active metabolites.[93,94] Indeed, some pharmacodynamics studies have shown that the combination therapy for allopurinol with low-dose 6-MP or AZA (25%–33% of normal weight–based dose) resulted in increased 6-TGN levels while decreasing 6-MMP, and thus decreasing 6-MMP/6-TGN ratios, thereby reducing hepatotoxicity and enhancing therapeutic efficacy. This is due to the observations that 6-MMP levels correlate with a risk of hepatotoxicity if levels exceed 5700 pmol/8×10^8 RBCs. However, the exact mechanism of action of allopurinol, a shunt of the metabolization toward the favored 6-TGN, remains not clearly understood, as the inhibitory effect of allopurinol includes xanthine oxidase and the TPMT. This approach requires extremely careful monitoring and should be restricted to experienced IBD centers.[34,95]

Adverse gastrointestinal events are frequent. In mild cases of gastrointestinal intolerance, taking the medication before sleeping or splitting the dose can help to control symptoms. For more severe cases, a switch to 6-MP is a good option.

GRADE: low, recommendation: weak.

Adverse gastrointestinal events mostly encountered in daily practice are nausea, vomiting, and/or abdominal pain. In case of mild gastrointestinal intolerance, taking the medication before sleeping or split-dose administration (2 or 3 times per day) can help to control symptoms. Another option is to switch from AZA to 6-MP, as these symptoms seem to be linked to the imidazole derivative.[96] Shih et al. found that dividing the total daily thiopurines dose (e.g., 75 mg twice a day instead of 150 mg once daily) led to a reduction in 6-MMP levels (11,785 versus 5324; $P = 0.0001$) without reducing 6-TGN levels (239 versus 216, P = NS) or the clinical disease activity. Furthermore, elevated transaminases and flu-like symptoms resolved in 18 of 20 patients studied.[97]

Dose-independent adverse reactions, such as pancreatitis, flu-like syndrome, and rashes are reported less frequently. In case of pancreatitis, further use of any kind of thiopurines is contraindicated as the mechanism is thought to be allergic or idiosyncratic. Recently, Heap et al. performed a genome-wide association study on 172 cases and 2035 controls with IBD, and identified strong evidence of an association within the class II HLA region. It seems that HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurines immunosuppressants.[98] However, this view has been recently challenged in a case report of 2 pediatric patients successfully treated with 6-MP after AZA pancreatitis[99] Owing to numerous false-positive results, there should be no routine monitoring of lipase levels, but of typical symptoms. When the drug is stopped, lipase level usually returns to reference range within a few days.

Patients should be advised that the long-term use of AZA/6-MP (mostly >2 years) alone or in combination with anti–TNF-alpha agents has been associated with a slightly increased risk of lymphoma, nonmelanoma skin cancer, and urinary tract cancer.

Skin follow-up by a dermatologist should be performed once a year in white patients. Patients should avoid excessive sun exposure and use a high-factor sun block.

The risk of cervix dysplasia and cancer is also increased and requires annual check-ups.

GRADE: moderate, recommendation: strong for the above surveillance strategy.

The long-term use of AZA/6-MP (mostly >2 years) as monotherapy or combo therapy has been associated with the development of lymphoproliferative disorders,[27,100–104] urinary tract cancer,[105] and nonmelanoma skin cancers (basal cell carcinoma and squamous cell carcinoma).[106–108] For dark skin types less vulnerable to skin cancer development induced by exposure to ultraviolet radiation from the sun, check-ups every 2 to 3 years are probably sufficient (weak evidence).

Subgroups of patients at higher risks have been identified: combo therapy in males <30 years for hepatosplenic T-cell lymphoma,[109] and mono-AZA therapy in patients >65 years for lymphoproliferative disorders.[27] Lymphoma risk is thus the major limiting factor of the prolonged use of AZA/6-MP, but the absolute cumulative risk of lymphoproliferative disorder remains extremely low, <1% after 10 years of exposure.[27]

An update of the immunization status should ideally be performed before starting AZA/6-MP, as well as serologies for Hepatitis B virus, Hepatitis C virus (HCV), varicella zoster virus (VZV), and Epstein–Barr virus, as severe varicella and severe primary infectious mononucleosis (some with hemophagocytic lymphohistiocytosis), especially in young male patients, have been recently reported.[29,110] In accordance with ECCO guidelines,[111] we propose that screening for HCV using antibody testing should be considered and if positive, it should be confirmed by detection of HCV RNA because the potential risk of worsening liver function as a result of immunosuppressive therapy, concomitant infection with other viruses such as Hepatitis B virus or HIV or by potentiating the effects of hepatotoxic medications. Immunomodulators may furthermore influence active chronic HCV infection. Immunomodulators are not contraindicated but in our view should be used with caution after discussion with a hepatologist.

AZA-associated hemophagocytic lymphohistiocytosis is a rare, but often fatal disease.[29,110,112] Epstein–Barr virus IgG screening should be also considered before the initiation of immunomodulator therapy. In line with the ECCO guideline, we advise anti-TNF-alpha monotherapy rather than thiopurines in Epstein–Barr virus young seronegative patients.[111]

Nodular regenerative hyperplasia is a rare hepatic disorder that may lead to severe portal hypertension. The risk of developing nodular regenerative hyperplasia during AZA treatment is low. However, it seems that male patients with previous extensive ileal resection constitute a higher risk group.[113]

A recent retrospective study of the Danish population database linked the development of cervical cancer to the cumulative use of AZA.[114] The increasing HPV prevalence also seems to impact on the risk of oral cancer and dysplasia in AZA-treated patients, based on an extensive literature review.[115] For that reason, in accordance with the ECCO guideline, we recommend regular gynecological screening for cervical cancer for women with IBD, especially if treated with immunomodulators. Although current or past infection with HPV is not a contraindication for immunomodulator therapy, discontinuation of immunomodulator therapy should be considered in patients with extensive cutaneous warts and/or condylomata, as well as routine prophylactic HPV vaccination for females and males in accordance with national guidelines.[111]

Question 6: Special Situations: Vaccination, Surgery, Pregnancy, and Lactation

Patients treated with thiopurines should be considered immunosuppressed, for that reason, the use of live vaccines is contraindicated. In practice, immunizations with live vaccines should be performed 1 month before initiation of thiopurines or at least 3 months after discontinuation of these agents. Peptidic and polysaccharidic vaccines can be administered safely during immunosuppression with thiopurines, but the response may be impaired.

GRADE: moderate, recommendation: strong for all the above recommendations.

Vaccination on AZA/6-MP.

should be considered as potentially immunosuppressed despite the relatively low dose of medication used in the treatment of IBD. In this patient population, there are 2 situations to discuss separately: the use of live vaccines and the use of inactivated vaccines, the latter further divided into the use of peptidic and polysaccharidic vaccines.

The principle of live vaccines is to induce immunity by causing a very mild form of the disease through the injection of an attenuated form of the pathogenic agent to elicit a curing immune reaction. Although severe vaccine-related illnesses after live vaccines in immunosuppressed patients remains rare in the literature, AZA has been involved in the development of severe disease after the administration live polio and tuberculosis BCG vaccines.[116,117] Based on these cases, the use of live vaccines during AZA and 6-MP therapy is strictly contraindicated.[118] Live vaccines currently in use are the combined measles-mumps-rubella, yellow fever, Ty21a oral typhoid, live attenuated influenza vaccine, rotavirus, and Bacille Calmette Guerin (BCG) vaccines. Caution should also be used when close contacts of patients immunosuppressed by thiopurines are vaccinated with live vaccines, as transmission of live vaccine particles may occur, especially from diapers of infants vaccinated against rotavirus and from skin lesions (if they occur) of subjects vaccinated against varicella.[118] However, according to US CDC guidelines, therapy with $\leq 3.0\ mg \cdot kg^{-1} \cdot d^{-1}$ AZA or $\leq 1.5\ mg \cdot kg^{-1} \cdot d^{-1}$ 6-MP are not regarded as contraindications for the administration of the zoster vaccine.[119] It is thus recommended to perform vaccination with live vaccines before the introduction of AZA or 6-MP, or after a washout period of these immunosuppressors.[118,120] In practice, immunizations with live vaccines should be performed 1 month before the initiation of thiopurines or at least 3 months after discontinuation of these agents if they were previously given to the subject to be vaccinated.

Peptidic and polysaccharidic vaccines can be administered safely during immunosuppression with thiopurines, but the response may be impaired, especially for polysaccharidic vaccines that do not elicit memory T cells and are less influenced by the immunosuppression. Therefore, immune response is best elicited if those vaccines are administered at least 1 month before the initiation of the thiopurines therapy.[111] Peptidic vaccine boosters, however, can be administered efficiently during immunosuppression, as they rely on preexisting memory T cells.[120,121] Therefore, nonlive vaccines against tetanus, diphtheria, poliomyelitis, pertussis, human papilloma virus (for both males and females), influenza, *Pneumococcus* (PCV 13 and PPSV 23) hepatitis B, *Haemophilus influenza, Meningococcus*, and tick-borne encephalitis and others can be administered under AZA/6-MP (Table 2).

Surgery

Performing surgery under thiopurines therapy should be discussed according to two different scenarios: IBD-related

abdominal surgery and other surgical procedures, mostly outside of the abdominal cavity.

Surgery is a common outcome for patients with CD and concerns a minority of patients with UC, but these surgeries are rarely performed early in the course of the disease.[122,123] As a result, a large subset of patients with IBD who become surgical candidates are under immunosuppressors or anti–TNF-alpha agents. Surgery for IBD-related indications (intestinal resections, abscesses drainage, or colectomy) has not been associated with increased risks of complications.[21,31,124,125] Accordingly, the recent ECCO consensus on surgery for UC states that preoperative thiopurines do not increase the risk of postoperative complications.[126] There is less evidence to describe the risk of complications in immunosuppressed patients undergoing surgery for IBD-unrelated reasons. Most of the literature refers to surgical complications of orthopedic surgeries, in particular joint replacements, in patients immunosuppressed for rheumatoid arthritis. In this population, the risks of this type of surgery have not been found to be increased by the concomitant administration of methotrexate or AZA.[31,127,128] However, as a high rate of infectious complications has been reported in transplant recipients undergoing joint replacement, patients under combination of immunosuppressors should be considered at high risk for surgery.[129]

Pregnancy and Lactation

AZA crosses the placental barrier, but its metabolization seems reduced in the fetal liver, as 6-TG levels are reduced (compared with the mother's level) and 6-MMP not found in infant red blood cells.[130] There are multiples clinical series and a meta-analysis to show that thiopurines can be safely administered throughout conception and pregnancy.[32,131–133] This position corresponds to the ECCO consensus on reproduction in IBD.[134] As a small amount of AZA has been found in breast milk, the ECCO consensus rates as "probably safe" AZA during lactation. The available evidence, however, shows no increased rate of infections or other complications in babies breastfed by mothers taking AZA.[23,135] With respect to the extensive beneficial effects of breastfeeding, lactation should be still be encouraged in this situation.

CONCLUSION

AZA/6-MP have been used for decades in IBD, where they represented the first second-line therapy for unresponsive or steroid-dependent patients. A role in postoperative prophylaxis in CD was also recognized. These initial indications for thiopurines have been progressively challenged by the introduction and wider use of anti–TNF-alpha agents. The advent of biosimilars of the anti–TNF-alpha is likely to even further challenge the established indications of thiopurines. Furthermore, biological therapies with novel mechanisms of actions, in particular antimigration antibodies such as vedolizumab, are likely further to decrease our need for thiopurines. Finally, retrospective and longitudinal analyses of large cohorts have shown potential severe albeit rare adverse effects of these products.

Despite all of these reasons to limit the use of thiopurines, these drugs offer some advantages, in combination with anti–TNF-alpha drugs and in special situations, and may find a renewed role as maintenance therapy after MMX-budesonide use in UC. It thus remains useful to master their application, which has become more sophisticated in line with more ambitious treatment goals for the benefit of patients with IBD.

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