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# More Dose-dependent Side Effects with Mercaptopurine over Azathioprine in IBD Treatment Due to Relatively Higher Dosing

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**Background:** There are substantial global differences in the preference for mercaptopurine (MP) or its prodrug azathioprine (AZA) as first-choice thiopurine to treat inflammatory bowel diseases. Studies comparing both agents are scarce. Our aim was to compare AZA and MP in thiopurine-naive patients with inflammatory bowel disease for the frequency of side effects and efficacy.

**Methods:** Post hoc analysis of the "Thiopurine response Optimization by Pharmacogenetic testing in Inflammatory bowel disease Clinics" (TOPIC) trial, in which thiopurine-naive patients with inflammatory bowel disease with an indication for a thiopurine were randomized for a genotype-based dose versus standard of care. For this study, Cox proportional hazard ratios (HRs) were calculated to compare AZA and MP for discontinuation rates within 5 months, incidence of hepatotoxicity, leukopenia, and gastrointestinal side effects. Treatment efficacy was compared by logistic regression.

**Results:** Patient characteristics were similar for patients treated with AZA (n = 494, 64.4%) and MP (n = 273, 35.6%), yet patients with MP were relatively higher dosed compared with those on AZA. Discontinuation rates within 5 months were not different, 39.3% (AZA) and 38.1% (MP), HR 0.92 (95% confidence interval, 0.72–1.17; $P = 0.50$); however, patients on MP were more often subjected to dose reductions (30% versus 14%, $P < 0.01$). Higher rates of hepatotoxicity, HR 1.93 (95% confidence interval, 1.35–2.76; $P < 0.01$) and leukopenia, HR 2.55 (95% confidence interval, 1.51–4.30; $P < 0.01$) were observed with MP, which annulled in a secondary analysis with adjustment for the higher dose and metabolite levels.

**Conclusions:** Patients treated with MP were relatively higher dosed, which resulted in more dose-dependent side effects and a higher rate of dose reductions.

*(Inflamm Bowel Dis 2017;23:1873–1881)*

**Key Words:** azathioprine, mercaptopurine, inflammatory bowel disease, side effects

Thiopurines have a central role in the treatment of inflammatory bowel diseases (IBDs) and are also used in the treatment of numerous immune-mediated diseases and malignancies as well as for the prevention of posttransplantation organ rejection.[1–3] Azathioprine (AZA) and mercaptopurine (MP) are classified as the conventional thiopurines. Both agents have proven

efficacy in the treatment of Crohn's disease (CD) and ulcerative colitis (UC).[4–7] Unfortunately, the use of thiopurines is frequently accompanied by side effects such as hepatotoxicity, gastrointestinal complaints, and flu-like symptoms or leukopenia, often leading to premature treatment discontinuation.[8,9]

After absorption, AZA is metabolized to MP, and further metabolism leads to the formation of several metabolites of which 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMPR) are considered to be the most important.[10] Current guidelines for IBD express no preference for AZA or MP because an equal efficacy is assumed and head-to-head comparisons are lacking.[5,11,12] Accompanied with marketing-related issues, this has led to major global differences in the preference for AZA or MP as first-choice thiopurine to treat IBD.[13] Although many studies have reported on thiopurine-induced side effects, none of these focused on a direct comparison of AZA and MP, or these investigations were limited by the fact that one drug was overrepresented in the study population.[14–17]

Nevertheless, some reports showed differences between AZA and MP in terms of side effects.[18] The methylnitroimidazole group, which is released in the conversion of AZA to MP, has been associated with gastrointestinal side effects, but also with enhanced immunosuppressive effects.[19,20] Furthermore, genetic variants in glutathione transferase, an enzyme involved in the conversion of AZA to MP, might affect thiopurine metabolite levels in patients on AZA but not in those on MP.[21,22] Recent data suggest that MP might be considered as an alternative in patients intolerant to AZA.[23,24] Notably, if AZA and MP would have been developed in the current era, randomized blinded trials would have been mandatory to compare their safety and efficacy profiles before routine use. Such data are currently lacking and given the cost of such studies and relatively low financial implications, it is highly unlikely that such trials will ever be performed. In the present post hoc analysis of the "Thiopurine response Optimization by Pharmacogenetic testing in Inflammatory bowel disease Clinics" (TOPIC) trial, we evaluated the hypothesis that AZA and MP are equally effective and safe in the treatment of thiopurine-naive patients with IBD.

## PATIENTS AND METHODS

### Patients

The study design, patient data, and results of the TOPIC trial have been published previously.[25] Briefly, thiopurine-naive patients with IBD with an indication for thiopurine treatment were randomized 1:1 for thiopurine dosing based on their thiopurine S-methyltransferase (*TPMT*) genotype versus the standard AZA (2.0–2.5 mg/kg) or MP dose (1.0–1.5 mg/kg). Patients assigned to the *TPMT* genotyping arm received a 50% dose reduction when they carried a heterozygote variant in *TPMT\*2*, *TPMT\*3A*, or *TPMT\*3C,* whereas carriers of a homozygote variant received 10% of the original dose or were advised to start an alternative

treatment. Patients assigned to the standard-of-care arm were genotyped for variants in *TPMT* afterward. TPMT activity was assessed in all patients after the execution of the clinical trial.

Indication for thiopurine treatment was determined by the treating physician. Indication was not reported on the case report form. Physicians were free in the choice whether to start AZA or MP, and advised to start full regular dose immediately. The main exclusion criteria of the TOPIC trial were previous use of thiopurines, cotreatment with allopurinol, a baseline white blood cell count $<3.0 \times 10^9$/L, and baseline liver test abnormalities (alanine transaminase [ALT], aspartate transaminase [AST], or alkaline phosphatase) $\geq 2$ times the normal upper limit (ULN), a known TPMT enzyme activity or *TPMT* genotype. Biochemical and hematological safety parameters were assessed at baseline and weeks 1, 2, 4, 6, 8, and 20. The patients were followed for 20 weeks. The TOPIC trial was approved by the institutional ethic committees, and all patients provided written informed consent (clinicaltrials.gov, NCT00521950).

### 6-MMPR and 6-TGN Metabolite Levels

Week 8 6-MMPR and 6-TGN metabolite levels were assessed in red blood cells by high-performance liquid chromatography, according to the Lennard method and reported as median pmol/$8 \times 10^8$ red blood cells with the interquartile range (IQR).[25] Week 8 levels were assessed in the first 301 patients included in the TOPIC trial. Next to week 8 levels, 6-MMPR, and 6-TGN levels were also assessed at week 1 in 267 patients according to the same method.[26,27]

### Study Design

In this study, we compared AZA and MP for their safety and efficacy. All patients who complied with the study protocol and started with AZA or MP were included in an intention-to-treat analysis. The AZA dosage in mg/kg bodyweight was divided by 2.08 for comparison with the MP dose. The molecular weight of MP (Mw = 152.18 g/mol) is 55% of the molecular weight of AZA (Mw = 277.27 g/mol), resulting in a conversion factor of 2.08 (1/0.55–1/0.88), when converting MP into an equivalent pharmaceutical dose of AZA, assuming 100% bioavailability.[18,28] In 30 patients, the dose was escalated within 1 or 2 weeks. In these cases, we used the final dose for analysis.

The primary outcome was the proportion of the patients who were still using the initial thiopurine after 5 months. Dose reductions and temporarily interruption were accepted; however, when the thiopurine was discontinued for more than 20% of the study time (1 month or longer), the primary endpoint was not reached. If the indication for discontinuation after temporarily interruption was identical to the final reason to stop, the first date of discontinuation was used for analysis. When a different indication led to definite treatment discontinuation after temporarily discontinuation, the last date was used for analysis.

Secondary outcomes were signs of hepatotoxicity (defined as increase more than 2 times the ULN of ALT or conjugated bilirubin, or a combined increase in AST and alkaline phosphatase

provided that one of them is above 2 times the ULN).[29] When baseline levels were between 1 and 2 times the ULN, a 2-time increase of the baseline value was required. Furthermore, the frequency of gastrointestinal side effects (defined as occurrence of nausea, vomiting, or decreased appetite during the study as reported by the patient), leukopenia (defined as a white blood cell count ≤3.0 × 10^9/L), and the incidence of thiopurine-induced pancreatitis (TIAP) were compared for AZA and MP. TIAP was defined as the presence of amylase or lipase 3 times the ULN according to the reference value of the local laboratory in combination with radiological or clinical signs suggestive for pancreatitis in the absence of another likely cause.[30] Treatment response was evaluated using the Harvey–Bradshaw index in CD and partial Mayo score in UC. Treatment response was defined as a reduction of 3 points or more at week 20 compared with week 0.

## Statistical Analysis

All the statistical analyses were performed using SPSS version 20.0.0.1 (SPSS Inc., Chicago, IL). The patient characteristics between patients on AZA and MP were compared by the chi-square test for dichotomous variables, and student's *t* tests or Mann–Whitney U tests were used for continuous variables. We performed Cox proportional hazards survival analysis to calculate the hazard ratio for AZA and MP with 95% confidence intervals (CIs) for the time to treatment discontinuation, gastrointestinal side effects, signs of hepatotoxicity, and leukopenia. Kaplan–Meier curves with separate lines for AZA and MP were plotted to illustrate differences in treatment discontinuation, gastrointestinal side effects, signs of hepatotoxicity, and leukopenia. Log-rank tests were used to compare the Kaplan–Meier curves.

Secondary multivariate Cox proportional hazard models were computed to calculate the hazard ratio for AZA and MP adjusted for confounders. Differences in the patient characteristics between AZA and MP users with a *P*-value <0.1 were included in a Cox proportional hazard model to calculate the adjusted hazard ratio with 95% CIs for AZA compared with MP for the primary outcome treatment discontinuation as well as the secondary outcome signs of hepatotoxicity, leukopenia, and gastrointestinal side effects. As follows from the comparison of baseline characteristics between AZA and MP, metabolite levels were included as covariate in this analysis. Week 1 metabolite data were chosen instead of week 8 levels because most adverse events, among others, hepatotoxicity and leukopenia, occurred in the first weeks of treatment initiation. As a consequence, week 8 metabolite levels were either biased in these patients because of already applied dose reductions or were not available because of treatment discontinuation before week 8 because of the adverse event. Moreover, patients with severe hepatotoxicity or leukopenia are more likely to be subjected to dose reductions or treatment discontinuation, which also might lead to biased results. With the use of week 1 metabolite levels, this was obviated because patients did not develop the adverse event yet. Week 1 metabolite levels were available for 267 patients, and Cox proportional hazard analyses were performed in these patients.

The treatment response in AZA and MP was compared by the chi-squared test. For further analysis, the response rates in CD and UC were merged to have sufficient numbers. Logistic regression was used to calculate the odds ratio of treatment response adjusted for confounders. A *P*-value of <0.05 was considered statistically significant.

## RESULTS

### Patients

In the TOPIC trial, 796 patients were randomized, of whom 29 did not start with a thiopurine or were excluded because of protocol violations.[25] The remaining 767 patients treated with either AZA (n = 494, 64.4%) or MP (n = 273, 35.6%) were included in this analysis. The baseline characteristics of both groups are depicted in Table 1 and showed no differences between AZA and MP users except for the median dose in mg/kg, which was higher in patients taking MP (1.21 mg/kg, IQR 1.10–1.30) compared with the converted rate of AZA (1.05 mg/kg, IQR 0.99–1.11), *P* < 0.001. Furthermore, both week 1 and week 8 6-MMPR and 6-TGN metabolite levels were significantly higher in patients treated with MP (Table 1). Otherwise, no differences in characteristics were observed between the patients assigned to AZA and MP.

### Treatment Discontinuation

Overall, 298 patients (38.9%) discontinued the initial thiopurine within 5 months after starting treatment. In patients taking AZA, n = 194 (39.3%) discontinued treatment and in patients taking MP, n = 104 (38.1%), *P* = 0.75. The Kaplan–Meier curves showed no difference in treatment discontinuation rates between AZA and MP, *P* = 0.57 (Fig. 1). The hazard ratio for AZA versus MP with 95% CIs for discontinuation in the first 5 months was 0.92 (0.72–1.17; *P* = 0.50).

Of the patients still taking the initial prescribed thiopurine at week 20 (n = 469), those treated with MP were more often subjected to dose reductions compared with the patients treated with AZA (30.1% versus 13.7%, *P* < 0.001). In addition, at week 20, the median dose of the patients still taking the initial prescribed thiopurine was significantly more decreased in patients taking MP (n = 168), 1.14 mg/kg (IQR 0.82–1.25), compared with those treated with AZA (n = 300), 1.04 mg/kg (IQR 0.96–1.11), *P* < 0.001.

### Signs of Hepatotoxicity

Signs of hepatotoxicity (defined as more than 2 times the ULN increase of ALT or conjugated bilirubin, or a combined increase in AST and alkaline phosphatase provided that one of them is above 2 times the ULN) were reported in 59 patients treated with AZA (11.9%) and in 62 patients taking MP (22.7%), hazard ratio 1.93 (95% CI, 1.35–2.76; *P* < 0.001). In 88% of the patients (106 of 121) with hepatotoxicity, this developed in the first 8 weeks of treatment. Time to hepatotoxicity was not significantly

**TABLE 1.** Baseline Characteristics of the Patients on AZA (AZA Users) and MP (MP Users)

| | Total | AZA Users | MP Users | P |
|---|---|---|---|---|
| Total, n (%) | 767 (100) | 494 (64) | 273 (36) | |
| Sex, male, n (%) | 346 (45) | 220 (46) | 126 (47) | 0.66 |
| Age, yr, median (IQR) | 40.5 (26.6–53.0) | 40.1 (26.5–52.7) | 41.3 (26.6–54.3) | 0.43 |
| Weight, kg, mean (SD) | 74.4 (16.1) | 74.2 (14.9) | 74.6 (18.2) | 0.72 |
| Disease, n (%) | | | | |
|   CD | 463 (60) | 297 (60) | 166 (61) | |
|   UC | 297 (39) | 194 (39) | 103 (38) | |
|   Indeterminate colitis | 7 (1) | 3 (1) | 4 (2) | 0.74 |
| Study arm, n (%) | | | | |
|   Genotyping | 398 (52) | 253 (51) | 145 (53) | |
|   Standard | 369 (48) | 241 (49) | 128 (47) | 0.61 |
|   Heterozygous *TPMT* genotype, n (%) | 72 (9) | 46 (9) | 26 (10) | 0.92 |
| TPMT activity, mg/mmol Hb.h, mean (SD) | 90.3 (22.4) | 90.5 (22.9) | 90.0 (21.5) | 0.78 |
| Dosage, mg/kg, median (IQR)[a] | | 2.18 (2.07–2.31) | 1.21 (1.10–1.30) | — |
| Dosage after conversion by 2.08, median (IQR)[b] | | 1.05 (0.99–1.11) | 1.21 (1.10–1.30) | <0.001 |
| Baseline disease activity | | | | |
|   CD (HBI), mean (SD)[c] | 3.5 (3.0) | 3.4 (2.9) | 3.6 (3.3) | 0.57 |
|   UC (partial Mayo), mean (SD)[d] | 3.8 (1.7) | 3.8 (1.7) | 3.9 (1.8) | 0.58 |
| Comedication during the trial, n (%) | | | | |
|   5-aminosalicylic acid | 385 (50) | 253 (51.2) | 132 (48.4) | 0.45 |
|   Corticosteroids[e] | 440 (58) | 275 (55.9) | 165 (60.4) | 0.18 |
|   Biologics | 82 (11) | 49 (10) | 33 (12) | 0.35 |
| Metabolite levels, pmol/$8 \times 10^8$ RBCs | | | | |
|   Week 1 6-TGN levels, median (IQR) | 152 (110–206) | 134 (100–186)[f] | 189 (129–241)[g] | <0.001 |
|   Week 1 6-MMPR levels, median (IQR) | 2013 (946–3595) | 1802 (897–3119)[f] | 3051 (1069–5716)[g] | <0.001 |
|   Week 8 6-TGN levels, median (IQR) | 243 (179–366) | 221 (169–337)[h] | 269 (196–391)[i] | 0.04 |
|   Week 8 6-MMPR levels, median (IQR) | 2908 (771–6592) | 2274 (678–5270)[h] | 4147 (1027–10,651)[i] | 0.003 |

[a]Dose levels include 38 patients (AZA n = 24 and MP n = 14, P = 0.98) with a heterozygous *TPMT* variant randomized to the genotype arm and subsequently received a 50% dose reduction from the start according to the study protocol.
[b]AZA dose as divided by 2.08.
[c]Available for 349 patients.
[d]Available for 251 patients.
[e]Available for 763 patients, steroids with limited systemic bioavailability, such as budesonide, were not included.
[f]Available for 182 patients.
[g]Available for 85 patients.
[h]Available for 150 patients.
[i]Available for 84 patients.
HBI, Harvey–Bradshaw index; RBCs, red blood cells.

different between AZA and MP (P = 0.64) (Fig. 2). The median (IQR) rise of ALT was not different between AZA users, 90 U/L (65–148), and MP users, 96 U/L (68–133), P = 0.83 (Fig. 3).

Because patients with MP were relatively higher dosed and, therefore, probably showed higher metabolite levels, we also calculated the hazard ratio adjusted for these variables. This secondary analysis in 267 patients, with adjustment for dosage in mg/kg and week 1 6-MMPR and 6-TGN metabolite levels, showed a hazard ratio of 1.79 (95% CI, 0.82–3.92; P = 0.15).

## Leukopenia

Leukopenia (defined as a white blood cell count $\leq 3.0 \times 10^9$/L) was observed in 58 patients (7.6%). Eight of these patients (4 patients on AZA and 4 on MP) had a variant in *TPMT* and were randomized to the standard-of-care group and subsequently received the standard thiopurine dose. Leukopenia was observed in 24 patients (4.9%) treated with AZA and in 34 patients (12.5%) treated with MP, hazard ratio 2.55 (95% CI, 1.51–4.30; P < 0.001). Secondary analysis with adjustment for dosage in mg/kg and week 1 6-MMPR and 6-TGN
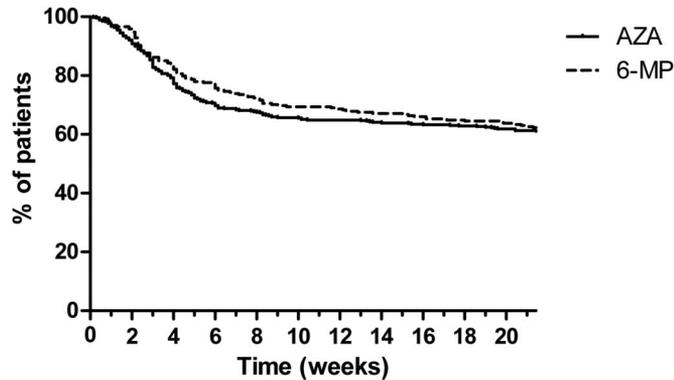
FIGURE 1. Time to treatment discontinuation for patients assigned to AZA and MP. No difference in treatment discontinuation was observed between AZA and MP, $P = 0.57$.

metabolite levels showed a hazard ratio of 0.92 (95% CI, 0.28–3.05; $P = 0.89$). The time to development of leukopenia was not significantly different between AZA and MP ($P = 0.62$) (Fig. 4).

## Gastrointestinal Side Effects

Gastrointestinal side effects were reported by 345 patients (45.0%), of whom 216 patients (43.7%) were treated with AZA and 129 patients (47.3%) with MP, hazard ratio 1.09 (95% CI, 0.87–1.3; $P = 0.46$). Median time to the occurrence of gastrointestinal side effects was 14 (range, 7–34) days. No difference was observed in the Kaplan–Meier curve between AZA and MP, $P = 0.52$ (Fig. 5). In the secondary analysis, the hazard ratio for AZA or MP on gastrointestinal side effects was hazard ratio 1.38 (95% CI, 0.90–2.10; $P = 0.17$).
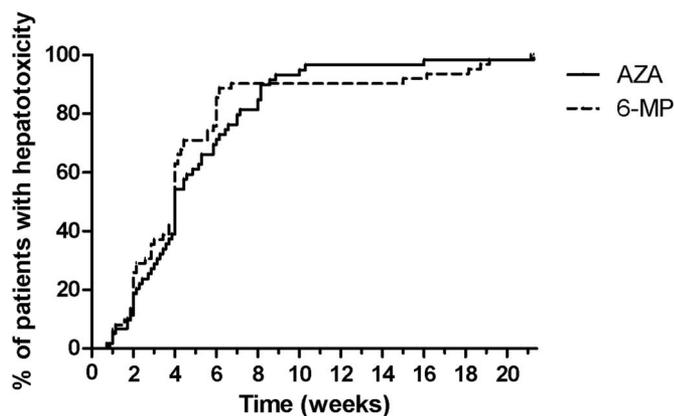


FIGURE 2. Time to development of signs of hepatotoxicity (defined as more than 2 times the ULN increase of ALT or conjugated bilirubin, or a combined increase in AST and alkaline phosphatase provided that one of them is above 2 times the ULN) in patients taking AZA or MP. No difference was observed in the time to the development of signs of hepatotoxicity between AZA and MP ($P = 0.64$).
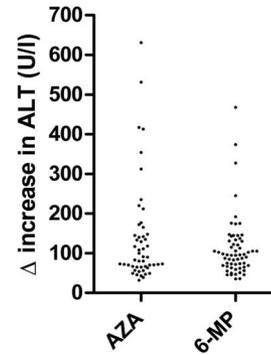


FIGURE 3. Maximum increase in ALT in units/litre compared with baseline in patients on AZA (n = 59) and patients on MP (n = 62) who developed signs of hepatotoxicity. No difference was observed between AZA and MP ($P = 0.83$).

## Thiopurine-induced Acute Pancreatitis

In total 14 patients (1.8%), of whom 11 on AZA (2.2%) and 3 on MP (1.1%) developed a TIAP for which the initial thiopurine was stopped. Characteristics of patients with a TIAP are depicted in Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B529. Mean time to TIAP was 20 ± 5 days, and no cases of severe (necrotizing) pancreatitis were reported. One patient with a TIAP on AZA was later switched to MP; however, this was stopped after 1 day because of fever (no laboratory measurements were performed).

## Treatment Efficacy

Information about treatment response was available for 351 patients (45.7%), AZA (n = 242) and MP (n = 109). Baseline disease activity scores (mean ± SD) were not different for patients of whom treatment response was known or unknown (Harvey–Bradshaw index, 3.43 ± 2.88 versus 3.62 ± 3.36, $P = 0.63$) and (partial Mayo score, 3.74 ± 1.73 versus 3.90 ± 1.70, $P = 0.50$), respectively. In CD, 38 patients on AZA (26%) and 21 patients on MP (26%) achieved a reduction in the
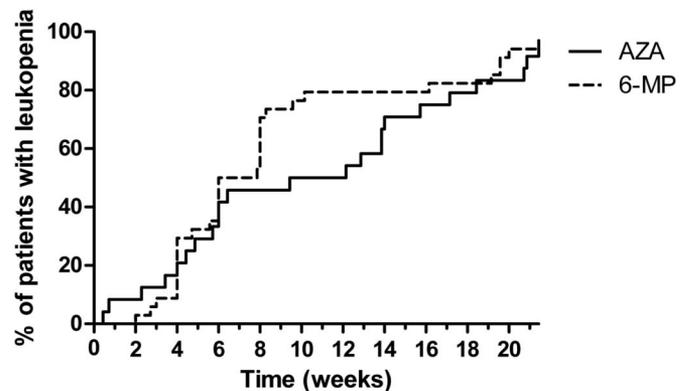


FIGURE 4. Time to development of leukopenia (defined as a white blood cell count ≤3.0 × 10⁹/L) in patients taking AZA or MP. No difference was observed between patients on AZA and MP ($P = 0.62$).
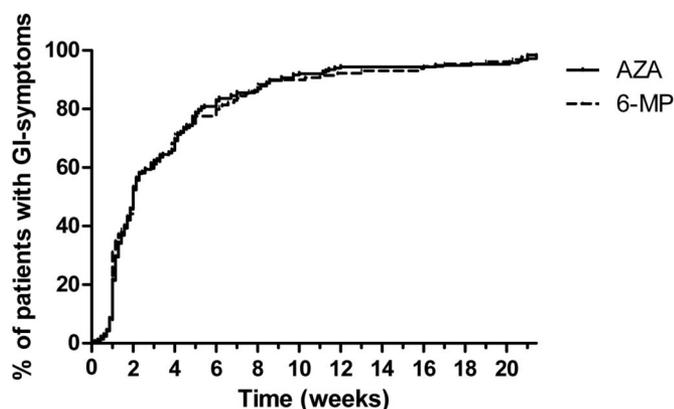
FIGURE 5. Time to development of gastrointestinal side effects in patients taking AZA or MP. No difference was observed between AZA and MP ($P = 0.52$).

Harvey–Bradshaw index of 3 points or more, $P = 0.90$. In UC, a reduction in the partial Mayo score of 3 points or more was seen in 22 patients on AZA (24%) and 9 patients on MP (32%), $P = 0.42$. For further analysis, the response rates in CD and UC were merged to have sufficient numbers. No difference in treatment response was observed between AZA (26%) and MP (27%), odds ratio 1.12 (95% CI 0.67–1.87; $P = 0.66$). In this group, week 1 metabolite levels and thiopurine dose were available for 126 patients. Secondary analysis in this group with adjustment for week 1 metabolite levels and thiopurine dose did not influence the odds ratio 1.11 (95% CI, 0.44–2.88; $P = 0.88$).

## DISCUSSION

Our data showed that in thiopurine-naive patients with IBD, the treatment discontinuation rates within the first 5 months were similar between AZA and MP users. The higher rates of signs of hepatotoxicity and leukopenia in patients taking MP are most likely explained by the relatively higher average dose and subsequent higher 6-MMPR and 6-TGN metabolite levels compared with patients with AZA, rather than by interdrug differences. Furthermore, no differences were found in treatment efficacy and gastrointestinal side effects.

The baseline characteristics were similar for the patients on AZA and MP. This excluded a preference for one of both agents in particular patient groups. Our data on dosage and metabolite levels showed that patients with MP were relatively higher dosed in mg/kg bodyweight compared with AZA users. This might well explain the higher rates of dose-dependent side effects like leukopenia and signs of hepatotoxicity with MP.[31] Adjustment for the baseline differences in thiopurine dose and week 1 metabolite levels annulled these higher rates of side effects. Week 1 6-MMPR and 6-TGN levels have been shown to be promising factors to predict leukopenia and hepatotoxicity.[26,27] Most side effects were seen in the first weeks of treatment initiation and often led to dose reduction or treatment discontinuation. As a consequence, steady-state week 8 metabolite levels were not available

or biased because of dose reductions in these patients. For this reason, we used week 1 metabolite levels for our secondary analysis. Both drug dose and thiopurine metabolite levels were significantly higher in MP users. Because most studies showed no clear correlation between drug dose and thiopurine metabolite levels, we included both variables in the multivariate analysis.[32,33]

Current studies exploring the safety profile of thiopurines are mostly limited by the inclusion of patients only taking AZA or MP.[15,16,34] This problem is mainly due to global variation in the preference for either AZA or MP and is partly caused by the fact that not everywhere both drugs are registered for the treatment of IBD.[13] Although not specifically designed for this purpose, some studies did compare AZA and MP in their analysis. In a recent study, a 5-fold higher rate of leukopenia was found in patients taking MP compared with AZA.[14] Despite the large study population of almost 4000 patients, the lack of detailed information on the dosage and metabolite levels precluded detailed dose-related analysis as in our study. The incidence of hepatotoxicity in the current study was 15.8%, which is within the range of the highly variable incidence reported in previous studies.[9,35] This wide range (4%–17%) is probably the result of differences in study protocols and definitions of drug-induced liver toxicity.[35] The frequent routine laboratory controls in the TOPIC trial in combination with our criteria for hepatotoxicity and the fact that in the TOPIC trial patients started with the full regular dose probably contributed to the high rate found in our study. Present criteria for drug-induced liver toxicity include an increase of ALT or AST 5 times the ULN.[36] Only 11 patients in our study would meet these criteria, yet most of the patients with liver test abnormalities between 2 and 5 times the ULN received a dose reduction or the treatment was (temporarily) discontinued. Given the clinical relevance, we applied the more liberal criteria formulated by Benichou.[29] There are many factors involved in the development of thiopurine-induced hepatotoxicity, including thiopurine dose, bodyweight, sex, and age.[27]

In the TOPIC trial, patients were randomized for dose adjustment based on their *TPMT* genotype versus standard dosing. This resulted in a group of patients with a heterozygous variant in *TPMT* treated with the standard dose as well as a group who received 50% of the standard dose. Table 2, Supplemental Digital Content 2, http://links.lww.com/IBD/B530 provides detailed information about side-effect rates for these different groups. Absolute numbers are low, which precludes additional analyses to compare AZA with MP within these subgroups. However, taking the small groups into account, no large differences were seen between AZA and MP with respect to hepatotoxicity, leukopenia, and gastrointestinal side effects.

As mentioned above, our results show that when official clinical guidelines are applied, MP is prescribed in a relatively higher dose than AZA. This finding has been described previously in a large retrospective analysis, where patients with AZA received 1.94 mg/kg (0.93 mg/kg after conversion by 2.08) and MP was dosed at around 1.20 mg/kg.[8] A reason might be that the only available pharmaceutical dosage form

of MP is 50 mg, whereas for AZA, there are 25 and 50 mg tablets available in Europe. Introduction of 25 mg tablets of MP (and AZA, if not yet available) would allow more accurate dosing and will circumvent the need of scoring tablets to acquire the optimal tailored dose. In addition, given that the absolute dosage is lower in MP, increasing the dose with 25 or 50 mg leads to a larger effect in terms of percentages in a patient on MP compared with a patient taking AZA. Another explanation might be that in clinical practice, a more convenient conversion rate of 2.0 is used instead of 2.08 to calculate the AZA dose from the equivalent MP dose.[28] Current guidelines recommend AZA in a dose between 2.0 and 2.5 mg/kg and MP between 1.0 and 1.5 mg/kg; however, when the 2.08 rate is applied to the recommended AZA dose, MP should actually be dosed between 0.96 and 1.20 mg/kg.[6,37,38] The equal discontinuation rates, despite more dose-dependent side effects with MP, can be explained by the higher rate of dose reductions applied in patients treated with MP. This advocates for a slightly lower starting dose of MP.

In the TOPIC trial, a high rate (38.9%) of treatment discontinuation was observed within the first 5 months, primarily because of side effects. This rate is in line with previous studies which reported discontinuation rates of approximately 30% within the first months.[8,39] The high discontinuation rate underlines the potential impact of thiopurine-induced adverse events. As expected, gastrointestinal side effects were the most frequently reported adverse event and occurred in approximately 50% of the patients. Our data corroborate with a recent study in almost 4000 patients with IBD on thiopurine therapy, which also found similar rates of gastrointestinal side effects in patients treated with AZA and MP.[14] Some studies have shown the gain of switching from AZA to MP in the presence of adverse events.[24] Although some patients were rechallenged after discontinuing the initial thiopurine, no detailed information was available to report on success rates of this switch.

TIAP is considered to be a non–dose-dependent adverse reaction linked with single nucleotide polymorphisms in the class II human leukocyte antigen region but also with blood group B.[40,41] This makes a difference between AZA and MP in frequency unlikely. Our results showed a higher rate of TIAP in AZA compared with MP; however, given the low number of cases, we had insufficient power to perform statistic analysis.

This study provides novel insights into the comparability of AZA and MP as a result of some exclusive characteristics. First, this study focused on the comparison of AZA and MP, which was possible because of the large number of thiopurine-naive patients assigned to AZA and MP. In addition, the similarity of the baseline characteristics does not suggest selection bias.

Limitations of this study are that the current analyses were not prespecified in the original study protocol. Furthermore, week 1 metabolite levels used for the secondary analysis were only available for 267 patients.[26] Importantly, there were no differences in baseline characteristics between the patients with known week 1 metabolite data compared with the patients without week 1 metabolite data, Table 3, Supplemental Digital Content 3, http://links.lww.com/IBD/B531. The use of week 1 metabolite levels is still limited to clinical trials. Secondary analyses with the use of week 8 metabolite levels instead of week 1 levels showed consistent results, however with less cases included, Table 4, Supplemental Digital Content 4, http://links.lww.com/IBD/B532. Furthermore, in the TOPIC trial, the Harvey-Bradshaw index and partial Mayo score were used to assess treatment efficacy. Ideally, colonoscopies should have been implemented for the assessment of mucosal healing; however, because the TOPIC trial primarily focused on safety outcomes rather than effectiveness, these were not included.[25] Furthermore, thiopurines were started both for active disease and maintenance of remission, which limits the assessment of the treatment effect. Also, the use of co-medication, such as corticosteroids or biologic drugs, is an important confounder in the evaluation of treatment effects. Considering these limitations, the treatment response of 26% and 27% in AZA and MP users, respectively, is in line with the data from the SONIC trial.[42,43] In the TOPIC trial, physicians were advised to start the full dose immediately, in which the recommended dose depended on the *TPMT* genotype and whether the patient was randomized to the intervention arm or not. Beginning with the full thiopurine dose might result in relative higher rates of side effects compared with a step-up approach (starting with half the dose and increase to full dose after 1 or 2 weeks if the drug is tolerated). With a step-up approach, the difference in dose-dependent side effects between AZA and MP might decrease because first signs of side effects already may result in a postponement of further increase to the intended dose.

In conclusion, in this study, we showed that when current clinical guidelines for IBD are followed, the patients treated with MP are relatively higher dosed, suffer from higher rates of dose-dependent side effects, and subsequently need more frequent dose reductions compared with those treated with AZA. This might be prevented when the initial MP dose is adjusted to 0.96 to 1.20 mg/kg bodyweight and 25 mg tablets MP are introduced for a more accurate tailored dose; however, feature studies are necessary to further evaluate this. Despite these differences, overall treatment discontinuation rates were equal for AZA and MP.

## ACKNOWLEDGMENTS

## REFERENCES

1. van Gelder T, van Schaik RH, Hesselink DA. Pharmacogenetics and immunosuppressive drugs in solid organ transplantation. *Nat Rev Nephrol.* 2014;10:725–731.
2. Lamers MM, van Oijen MG, Pronk M, et al. Treatment options for autoimmune hepatitis: a systematic review of randomized controlled trials. *J Hepatol.* 2010;53:191–198.
3. Kirchgesner J, Lemaitre M, Rudnichi A, et al. Therapeutic management of inflammatory bowel disease in real-life practice in the current era of anti-TNF agents: analysis of the French administrative health databases 2009–2014. *Aliment Pharmacol Ther.* 2017;45:37–49.

4. Rispo A, Testa A, De Palma GD, et al. Different profile of efficacy of thiopurines in ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis.* 2015;21:2570–2575.

5. Terdiman JP, Gruss CB, Heidelbaugh JJ, et al. American Gastroenterological Association Institute guideline on the use of thiopurines, methotrexate, and anti-TNF-alpha biologic drugs for the induction and maintenance of remission in inflammatory Crohn's disease. *Gastroenterology.* 2013;145:1459–1463.

6. Mottet C, Schoepfer AM, Juillerat P, et al. Experts opinion on the practical use of azathioprine and 6-mercaptopurine in inflammatory bowel disease. *Inflamm Bowel Dis.* 2016;22:2733–2747.

7. Timmer A, Patton PH, Chande N, et al. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane database Syst Rev.* 2016:CD000478.

8. Jharap B, Seinen ML, de Boer NK, et al. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis.* 2010;16:1541–1549.

9. Goldberg R, Irving PM. Toxicity and response to thiopurines in patients with inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol.* 2015;9:891–900.

10. Derijks LJ, Wong DR. Pharmacogenetics of thiopurines in inflammatory bowel disease. *Curr Pharm Des.* 2010;16:145–154.

11. Mowat C, Cole A, Windsor A, et al. Guidelines for the management of inflammatory bowel disease in adults. *Gut.* 2011;60:571–607.

12. Gomollon F, Dignass A, Annese V, et al. 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 1: diagnosis and medical management. *J Crohn's colitis.* 2017;11:3–25.

13. Coskun M, Steenholdt C, de Boer NK, et al. Pharmacology and optimization of thiopurines and methotrexate in inflammatory bowel disease. *Clin Pharmacokinet.* 2016;55:257–274.

14. Chaparro M, Ordas I, Cabre E, et al. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. *Inflamm Bowel Dis.* 2013;19:1404–1410.

15. Present DH, Meltzer SJ, Krumholz MP, et al. 6-Mercaptopurine in the management of inflammatory bowel disease: short- and long-term toxicity. *Ann Intern Med.* 1989;111:641–649.

16. Bastida G, Nos P, Aguas M, et al. Incidence, risk factors and clinical course of thiopurine-induced liver injury in patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* 2005;22:775–782.

17. Shaye OA, Yadegari M, Abreu MT, et al. Hepatotoxicity of 6-mercaptopurine (6-MP) and Azathioprine (AZA) in adult IBD patients. *Am J Gastroenterol.* 2007;102:2488–2494.

18. Cuffari C, Hunt S, Bayless TM. Enhanced bioavailability of azathioprine compared to 6-mercaptopurine therapy in inflammatory bowel disease: correlation with treatment efficacy. *Aliment Pharmacol Ther.* 2000;14:1009–1014.

19. McGovern DP, Travis SP, Duley J, et al. Azathioprine intolerance in patients with IBD may be imidazole-related and is independent of TPMT activity. *Gastroenterology.* 2002;122:838–839.

20. Crawford DJ, Maddocks JL, Jones DN, et al. Rational design of novel immunosuppressive drugs: analogues of azathioprine lacking the 6-mercaptopurine substituent retain or have enhanced immunosuppressive effects. *J Med Chem.* 1996;39:2690–2695.

21. Stocco G, Cuzzoni E, De Iudicibus S, et al. Deletion of glutathione-s-transferase m1 reduces azathioprine metabolite concentrations in young patients with inflammatory bowel disease. *J Clin Gastroenterol.* 2014;48:43–51.

22. Broekman MM, Wong DR, Wanten GJ, et al. The glutathione transferase Mu null genotype leads to lower 6-MMPR levels in patients treated with azathioprine but not with mercaptopurine. *Pharmacogenomics J.* [Published online ahead of print January 3, 2017]. doi: 10.1038/tpj.2016.87.

23. Kennedy NA, Rhatigan E, Arnott ID, et al. A trial of mercaptopurine is a safe strategy in patients with inflammatory bowel disease intolerant to azathioprine: an observational study, systematic review and meta-analysis. *Aliment Pharmacol Ther.* 2013;38:1255–1266.

24. Meijer B, Seinen ML, Leijte NN, et al. Clinical value of mercaptopurine after failing azathioprine therapy in patients with inflammatory bowel disease. *Ther Drug Monit.* 2016;38:463–470.

25. Coenen MJ, de Jong DJ, van Marrewijk CJ, et al. Identification of patients with variants in TPMT and dose reduction reduces hematologic events during thiopurine treatment of inflammatory bowel disease. *Gastroenterology.* 2015;149:907–917 e907.

26. Wong DR, Coenen MJ, Vermeulen SH, et al. Early assessment of thiopurine metabolites identifies patients at risk of thiopurine-induced leukopenia in inflammatory bowel disease. *J Crohn's colitis.* 2017;11:175–184.

27. Wong DR, Coenen MJ, Derijks LJ, et al. Early prediction of thiopurine-induced hepatotoxicity in inflammatory bowel disease. *Aliment Pharmacol Ther.* 2017;45:391–402.

28. Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. *Gut.* 2001;48:591–592.

29. Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. *J Hepatol.* 1990;11:272–276.

30. Ramos LR, Sachar DB, DiMaio CJ, et al. Inflammatory bowel disease and pancreatitis: a review. *J Crohn's colitis.* 2016;10:95–104.

31. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology.* 2000;118:705–713.

32. Gilissen LP, Wong DR, Engels LG, et al. Therapeutic drug monitoring of thiopurine metabolites in adult thiopurine tolerant IBD patients on maintenance therapy. *J Crohn's colitis.* 2012;6:698–707.

33. Derijks LJ, Gilissen LP, Engels LG, et al. Pharmacokinetics of 6-mercaptopurine in patients with inflammatory bowel disease: implications for therapy. *Ther Drug Monit.* 2004;26:311–318.

34. Hindorf U, Lindqvist M, Hildebrand H, et al. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. *Aliment Pharmacol Ther.* 2006;24:331–342.

35. Gisbert JP, Gonzalez-Lama Y, Mate J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. *Am J Gastroenterol.* 2007;102:1518–1527.

36. Chalasani N, Bonkovsky HL, Fontana R, et al. Features and outcomes of 899 patients with drug-induced liver injury: the DILIN prospective study. *Gastroenterology.* 2015;148:1340–1352 e1347.

37. Amin J, Huang B, Yoon J, et al. Update 2014: advances to optimize 6-mercaptopurine and azathioprine to reduce toxicity and improve efficacy in the management of IBD. *Inflamm Bowel Dis.* 2015;21:445–452.

38. Burger D, Travis S. Conventional medical management of inflammatory bowel disease. *Gastroenterology.* 2011;140:1827–1837 e1822.

39. Newman WG, Payne K, Tricker K, et al. A pragmatic randomized controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. *Pharmacogenomics.* 2011;12:815–826.

40. Heap GA, Weedon MN, Bewshea CM, et al. HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurine immunosuppressants. *Nat Genet.* 2014;46:1131–1134.

41. Teich N, Bokemeyer B, Mohl W, et al. Blood group B is associated with azathioprine-induced acute pancreatitis in patients with IBD. *Gut.* [Published online ahead of print November 14, 2016]. doi: 10.1136/gutjnl-2016-313138.

42. Colombel JF, Reinisch W, Mantzaris GJ, et al. Randomised clinical trial: deep remission in biologic and immunomodulator naive patients with Crohn's disease—a SONIC post hoc analysis. *Aliment Pharmacol Ther.* 2015;41:734–746.

43. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med.* 2010;362:1383–1395.