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Original Article

Favourable Tolerability and Drug Survival of Tioguanine Versus Methotrexate After Failure of Conventional Thiopurines in Crohn's Disease

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Abstract

Background and Aims: Both methotrexate and tioguanine can be considered as treatment options in patients with Crohn's disease after failure of conventional thiopurines. This study aimed to compare tolerability and drug survival of methotrexate and tioguanine therapy after failure of conventional thiopurines in patients with Crohn's disease.

Methods: We conducted a retrospective, multicentre study, including patients with Crohn's disease initiating monotherapy methotrexate or tioguanine after failure [all causes] of conventional thiopurines. Follow-up duration was 104 weeks or until treatment discontinuation. The primary outcome was cumulative therapy discontinuation incidence due to adverse events. Secondary outcomes included total number of [serious] adverse events, and ongoing monotherapy.

Results: In total, 219 patients starting either methotrexate [$n = 105$] or tioguanine [$n = 114$] were included. In all 65 [29.7%] patients (methotrexate 43.8% [46/105 people], tioguanine 16.7% [19/114 people], $p < 0.001$) discontinued their treatment due to adverse events during follow-up. Median time until discontinuation due to adverse events was 16 weeks (interquartile range [IQR] 7-38, $p = 0.812$). Serious adverse events were not significantly different. Patients treated with methotrexate experienced adverse events more often [methotrexate 83%, tioguanine 46%, $p < 0.001$]. Total monotherapy drug survival after 104 weeks was 22% for methotrexate and 46% for tioguanine [$p < 0.001$].

Conclusions: We observed a higher cumulative discontinuation incidence due to adverse events for methotrexate [44%] compared with tioguanine [17%] in Crohn's disease patients after failure of conventional thiopurines. The total adverse events incidence during methotrexate use was higher, whereas serious adverse events incidence was similar. These favourable results for tioguanine treatment may guide the selection of immunosuppressive therapy after failure of conventional thiopurines.

Key Words: Inflammatory bowel diseases; immunosuppressives; side effects

1. Introduction

Thiopurines and methotrexate [MTX] are immunosuppressants that are widely used to maintain remission in patients with Crohn's disease [CD], with over three decades of experience for each therapy.[1,2] Up to 71% of CD patients are exposed to an immunomodulator in the first 5 years of disease.[3] Current international guidelines recommend a conventional thiopurine, such as azathioprine [AZA] or mercaptopurine [MP], as the first-choice drug in regular step-up therapy for CD.[4,5] However, subsequent incidence of failure of conventional thiopurine monotherapy due to adverse events [AE] is as high as 43%, resulting in the use of alternative immunosuppressive therapies including MTX and tioguanine [TG].[6]

The relatively high number of AE due to conventional thiopurines can partially be explained by thiopurine metabolism. Briefly, AZA is converted to MP and eventually converted into the pharmacologically active metabolites 6-tioguanine nucleotides [6-TGN]. Simultaneously, MP is methylated by thiopurine methyltransferase [TPMT] to 6-methylmercaptopurine [6-MMP]. High levels of 6-TGN correlate with efficacy of thiopurines but also with myelotoxicity, and high levels of 6-MMP are associated with hepatoxicity and other AE.[7,8] TG, a non-conventional thiopurine, requires fewer metabolisation steps towards the active 6-TGN. This results in minimal to undetectable 6-MMP levels and is therefore expected to cause less AE. TG

is administered orally with doses of 0.2-0.3 mg/kg once daily and is an alternative treatment option for CD after failure of conventional thiopurines.[9] Most frequently reported AE include gastrointestinal complaints, hypersensitivity reactions, and elevated liver enzymes.[10]

MTX is a folate antagonist interfering with the thymidylate biosynthesis. The precise mechanism explaining the immunosuppressive effect is unclear.[11] MTX can be administered orally, subcutaneously, or intramuscularly and is most commonly prescribed at a dose of 15 mg per week with an optional 25 mg per week induction dosing for CD patients.[5,12] Frequently reported AE due to MTX therapy include liver toxicity, gastrointestinal complaints, and fatigue.[13]

After failure of conventional thiopurines, guidelines and international consensus-based recommendations advise to consider MTX or TG as subsequent therapy.[5,14] Both therapies have shown clinical benefit as maintenance therapy in CD.[15,16] Cumulative AE discontinuation incidences of 26% for MTX and 20% in TG have been reported in separate studies, but a head-to-head comparison to assess safety and effectiveness has never been performed.[9,17]

This study aimed to compare safety, tolerability, and effectiveness of TG and MTX in CD patients after failure of conventional thiopurines.

2. Materials and Methods

2.1. Study design and population

This multicentre retrospective cohort study was conducted in three academic and two general teaching hospitals in The Netherlands and patients were recruited from 2012 until 2020. Patients with an established diagnosis of CD and initiating monotherapy MTX or TG for CD after failure [for any reason] of conventional thiopurines [AZA or MP] were included. Patients with prior MTX or TG experience, MTX or TG use prescribed for a different indication [e.g., for rheumatic disease], or patients receiving concomitant biologic treatment at baseline were excluded. The decision to prescribe MTX or TG was made by the treating physician combined with patient preference, independently of study protocols. Follow-up duration from starting treatment was 104 weeks or until treatment discontinuation.

2.2. Data collection

Data were collected from prospectively maintained local databases in the five participating hospitals. To assess safety, tolerability, and effectiveness, the following variables were collected during treatment: demographics and disease status [sex, age, smoking status, Montreal classification, concomitant CD-related medication], safety parameters [AE, defined as possibly/probably/definitely drug-related, and SAE, defined as mortality, hospitalisation, intravenously administered antibiotics/antiviral medication]. All [S]AE were categorised by the Common Terminology Criteria for Adverse Events [CTCAE] [version 5.0]. Furthermore, we assessed disease activity (physician global assessment [PGA], C-reactive protein [CRP] and faecal calprotectin [FCP] levels, and CD-related surgery), and drug survival [change and duration of therapy, reason for discontinuation].

2.3. Study outcomes and definitions

The primary study outcome was the cumulative treatment discontinuation incidence due to AE within 104 weeks following treatment initiation. Secondary outcomes included ongoing TG or MTX monotherapy, biologic- and corticosteroid-free survival, number of [S]AE, infection, and clinical and biochemical remission proportions.

Monotherapy was defined as TG or MTX use without the use of any concomitant biologic therapy. Clinical remission was defined as PGA <1. Biochemical remission was defined as CRP ≤5 ml/L and/or FCP level ≤250 µg/g when available.

Reasons for discontinuation were classified as AE, primary non-response [insufficient clinical and/or biochemical response during the full duration of treatment], secondary non-response [insufficient clinical and/or biochemical response, after a period of response], stable remission, [intention to] pregnancy, patient's own request, or comorbidity.

2.4. Statistical analysis

Continuous variables were presented as means ± standard deviations [SD], and analysed with independent sample t tests for normally distributed variables, or medians with interquartile ranges [IQR] with Mann-Whitney U tests for non-normally distributed variables. Categorical and binary variables were presented as percentages [%] and compared by chi square tests. Logistic regression analysis was used to assess the association between treatment [MTX or TG] and the outcomes of interest. The following variables were assessed: sex, body mass index [BMI], disease duration, age at baseline, smoking, Montreal location and behaviour classification, [history of] perianal disease, prior intestinal surgery, prior biological use, baseline PGA, baseline CRP, baseline FCP, steroid use at baseline, and conventional thiopurine failure reason. Time-to-event analysis of treatment discontinuation was conducted using the Kaplan–Meier method. Competing risk analysis based on Fine and Gray's proportional sub hazards model was performed to account for competing reasons for therapy discontinuation [AE, lack/loss of response, or remission]. Patients who discontinued treatment due to pregnancy or comorbidity and patients lost to follow-up were considered censored cases in competing risk analysis.

To correct for confounding by indication, inverse probability treatment weighting [IPTW] was performed. Propensity scores were calculated to compare groups, based on the probability of receiving MTX or TG. To calculate propensity scores, the following variables, based on literature research and agreed upon before analysis, were used: disease duration at baseline, current smoking, complicated disease [defined as Montreal B2/B3], proximal disease location [Montreal L4], history of perianal fistula, prior biological use, history of intestinal surgery, baseline steroid use, and objective disease activity at baseline. Next, a stabilised weight was calculated for every patient, accounting for the degree of influence in the total population. Analyses for baseline differences, and primary and secondary outcomes, were repeated with addition of the weights, and standardised mean differences [SMD] between treatment groups were calculated. An SMD < .2 was considered a small, and an SMD <0.5 was considered a medium, between-group imbalance after weighting.

A p-value of <0.05 was considered statistically significant. Baseline and regression analyses were performed using SPSS version 25.0 for Windows [SPSS Inc., IBM Corp., Chicago, IL, USA]. R version 3.6.2 [Vienna, Austria] was used for competing risk analysis [*survminer* version 0.4.2 package] and IPTW analyses [*tableone* version 0.13.0 package].

2.5. Ethical statement

The study was reviewed and approved by the Committee on Research Involving Human Subjects at the Radboudumc, reference number 2020-6788. All patients provided signed informed consent for the use of data.

3. Results

3.1. Baseline characteristics

A total of 219 CD patients with previous conventional thiopurine failure and subsequent switch to monotherapy MTX [*n* = 105] or TG [*n* = 114] were included. The median follow-up was 91.0 weeks [IQR 27.0-104.0]. Baseline characteristics are presented in Table 1. The majority of patients (MTX: *n* = 80 [76.2%], TG: *n* = 91 [79.8%], *p* = 0.774) previously discontinued a conventional thiopurine because of AE. Of these, 34 [32.4%] MTX- and 27 [23.7%] TG-treated patients had subsequently failed a second conventional thiopurine prior to baseline [*p* = 0.884], with AE failure in 70.6% and 66.7% of these patients, respectively. MTX patients had a significantly longer disease duration at baseline [6.8 vs 2.8 years, *p* = 0.001] and more often upper gastrointestinal tract involvement [15.2% vs 7.0%, *p* = 0.052]. MTX patients had more history of previous surgery [36.2% vs 19.3%, *p* = 0.005] and a higher rate of prior biologic therapy use [26.7% vs 14.9%, *p* = 0.031]. Median maintenance doses were 15.0 mg weekly [IQR 15.0-15.0 mg] for MTX, and 20.0 mg daily [IQR 20.0-20.0 mg] for TG. MTX was used subcutaneously in 96 [91.4%] patients.

3.2. Safety

In total, 65 [29.7%] patients discontinued treatment due to AE. MTX-treated patients experienced significantly more AE-related discontinuations: 46 [43.8%] patients vs 19 [16.7%] TG-treated patients (unadjusted odds ratio [OR] 3.898, 95% CI 2.086-7.287, *p* <0.001) [Figure 1]. Competing risk analysis confirmed a significantly higher cumulative AE discontinuation incidence in MTX-treated patients [Figure 2].

Table 1. Baseline characteristics of methotrexate and tioguanine-treated IBD patients with corresponding *p*-values before and after IPTW, and SMD after IPTW.

	Before IPTW			After IPTW	
	MTX [*n* = 105]	TG [*n* = 114]	*p*	*p*	SMD
Age at start treatment, median [IQR]	48.0 [33.5–58.5]	41.0 [28.0–55.0]	0.101	0.238	-0.165
Disease duration [years], median [IQR]	6.8 [2.2–20.6]	2.8 [0.6–14.3]	0.001	0.914	00.15
Sex [female] *N* [%]	64 [61.0]	71 [62.3]	0.840	0.812	0.033
BMI, mean [SD]	25.6 [5.7]	25.0 [4.5]	0.441	0.307	-0.155
Current smoker *N* [%]	29 [27.6]	31 [27.2]	0.944	0.919	0.014
Crohn's disease location, *N* [%]			0.884	0.949	
Ileum	43 [40.0]	49 [43.0]			0.058
Colon	20 [19.0]	19 [16.7]			-0.151
Ileum and colon	41 [39.0]	46 [40.4]			0.075
Upper GI involvement, *N* [%]	16 [15.2]	8 [7.0]	0.052	0.915	0.016
Disease behaviour, N [%]			0.359	0.952	
Non stricturing, non-penetrating	69 [65.7]	85 [74.6]			0.011
Stenosing	25 [23.8]	20 [17.5]			-0.011
Penetrating	11 [10.5]	9 [7.9]			0.001
Perianal disease	15 [14.3]	9 [7.9]	0.130	0.864	-0.024
Prior intestinal surgery, *N* [%]	38 [36.2]	22 [19.3]	0.005	0.980	-0.004
Perianal surgery, *N* [%]	4 [3.8]	7 [6.1]	0.430	0.164	0.220
Prior biologic therapy, *N* [%]	28 [26.7]	17 [14.9]	0.031	0.972	-0.005
Clinical disease activity [PGA]			0.836	0.759	
Remission	15 [14.3]	14 [12.3]			-0.019
Mild disease	57 [54.3]	68 [59.6]			0.090
Moderate disease	21 [20.0]	20 [17.5]			-0.067
Severe disease	4 [3.8]	3 [2.6]			-0.019
Unknown	8 [7.6]	9 [7.9]			-0.032
Corticosteroid use at baseline	44 [41.9]	55 [48.2]	0.346	0.947	0.009
Baseline CRP, median [IQR]	5 [1.8–10.0]	5 [3–14]	0.508	0.915	-0.015
Baseline faecal calprotectin [*N* = 29/45]	180 [111–500]	229 [100–601]	0.646	0.355	0.194
Previous failure of one conventional thiopurine	71 [67.6]	87 [76.3]	0.151	0.344	0.137
Previous failure of two conventional thiopurines	34 [32.4]	27 [23.7]			

MTX, methotrexate; TG, tioguanine; IPTW, inverse probability treatment weighting; SMD, standardised mean difference; IBD, inflammatory bowel disease; IQR, interquartile range; BMI, body mass index; SD, standard deviation; GI, gastrointestinal; PGA, Physician's Global Assessment; CRP, C-reactive protein.

Nausea was the most common AE leading to discontinuation in MTX [n = 11]. For TG therapy, abdominal pain [n = 4] and elevation of liver enzymes [n = 4] were the most common AE. All AE leading to discontinuation are displayed in Table 2. Median treatment duration until discontinuation because of AE was 17 weeks [IQR 8-40] for MTX and 15 weeks [IQR 1-36] for TG [p = 0.812]. None of the variables in the unadjusted analysis were predictive for discontinuation due to AE. There were no cases detected with a diagnosis of histological nodular regenerative hyperplasia [NRH], liver fibrosis, or cirrhosis. NRH was excluded based on clinical, biochemical, radiological, and histological data if available. Of note, only one patient in this study [MTX group] underwent a liver biopsy during follow-up. In patients who discontinued a conventional thiopurine due to AE prior to baseline, 38 of 80 [47.5%] MTX- and 16 of 91 [17.6%] TG-treated patients discontinued therapy due to AE [p < 0.001] [Supplementary Figure 1]. The number of previously failed conventional thiopurines did not predict AE discontinuation [p = 0.340]. Both patients receiving an MTX dose of >15 mg [n = 8, p = 0.063], and patients receiving oral rather than subcutaneous MTX [n = 9, p = 0.508] did not have a significantly higher risk of discontinuation due to AE. Only 4/114 TG patients were prescribed doses exceeding 20 mg daily. A TG dose of >20 mg [highest dose: 30 mg] was not predictive of discontinuation due to AE [p = 0.999]. Of all 219 patients, 20 patients [MTX: n = 7, TG: n = 13] were previously treated with a conventional thiopurine combined with allopurinol. Of these, four and no patients discontinued MTX or TG because of AE, respectively. When we further analysed these subgroups, previous allopurinol use was not predictive of discontinuation due to AE [p = 0.326].

In total, 15 [6.8%] patients experienced a serious adverse event [SAE] (MTX: n = 8 [7.6%], TG: n = 7 [6.1%], p = 0.915) [Table 3]. All cases of SAE required hospitalisation. Four infections required intravenous antibiotic/antiviral therapy [MTX: n = 1, TG: n = 3]. No patients died.

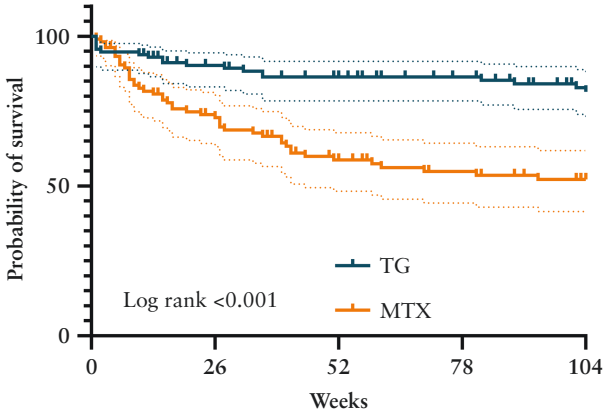

Figure 1. Kaplan–Meier survival distributions for therapy discontinuation due to adverse events. Log rank <0.001. MTX, methotrexate; TG, tioguanine.

3.3. Tolerability

During follow-up, a total of 139 [63.5%] patients experienced one or more AE (MTX: n = 87 [82.9%], TG: n = 52 [45.6%], unadjusted OR 5.763, 95% CI 3.078-10.790, p <0.001). A total of 251 AE occurred: 173 for MTX- [152 per 100 patient-years] and 78 for TG- [45 per 100 patient-years] treated patients. The most frequently occurring AE during treatment were nausea [n = 42] and infectious enterocolitis [n = 10] for MTX and nausea [n = 9] for TG [Supplementary Table 1]. Neither patients receiving an MTX dose of >15 mg [p = 0.540] nor patients receiving oral MTX [p = 0.672] had a significantly higher risk of AE.

3.4. Effectiveness

During follow-up, six [5.7%] MTX- and eight [7.0%] TG-treated patients discontinued therapy because of primary non-response. Loss of response was reported for five [4.7%] and seven [6.1%] patients, respectively [Table 4].

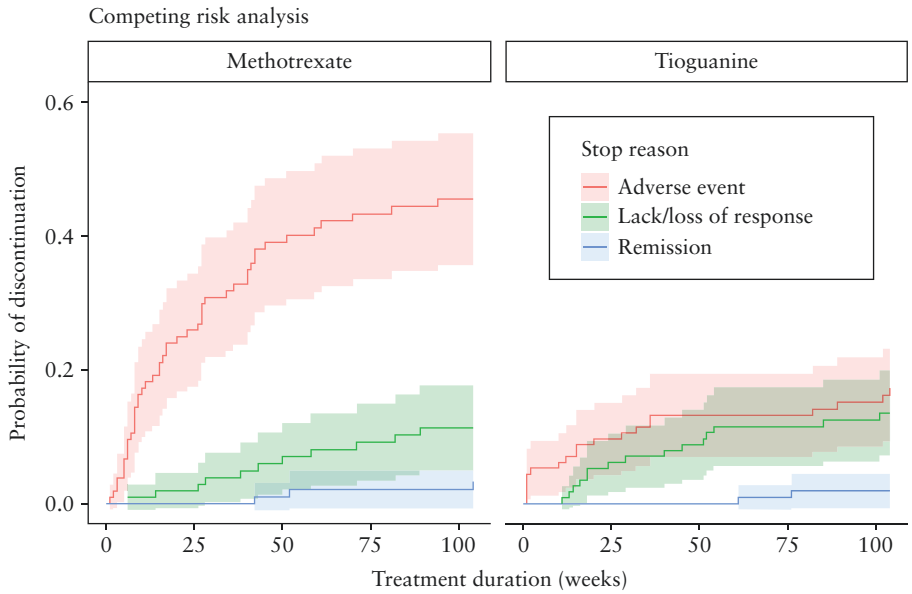

Figure 2. Cumulative incidence of discontinuation reasons in competing risk analysis.

Table 2. Adverse events leading to treatment discontinuation. Some patients experienced multiple adverse events leading to discontinuation.

Adverse event	MTX [*n* = 44]	TG [*n* = 19]
Nausea	11 [22%]	2 [8%]
Elevated liver enzymes[a]	5 [10%]	4 [16%]
Malaise	4 [8%]	1 [4%]
Abdominal pain	0	4 [16%]
Arthralgia	1 [2%]	2 [8%]
Diarrhoea	2 [4%]	1 [4%]
Fatigue	2 [4%]	1 [4%]
Headache	2 [4%]	1 [4%]
Lung infection	2 [4%]	1 [4%]
White blood cell decreased	2 [4%]	1 [4%]
Alopecia	1 [2%]	1 [4%]
Depression	2 [4%]	0
Oedema limbs	2 [4%]	0
Infections and infestations—other	2 [4%]	0
Vomiting	1 [2%]	1 [4%]
Dysgeusia	1 [2%]	0
Eczema	1 [2%]	0
Facial oedema	1 [2%]	0
Fever	1 [2%]	0
Gastroesophageal reflux disease	1 [2%]	0
Hair texture abnormal	1 [2%]	0
Insomnia	1 [2%]	0
Muscle cramps	0	1 [4%]
Pancreatitis	0	1 [4%]
Platelet count decreased	0	1 [4%]
Pruritus	1 [2%]	0
Psychiatric disorders—other	1 [2%]	0
Serum amylase increased	0	1 [4%]
Skin hyperpigmentation	0	1 [4%]
Total	49	25

MTX, methotrexate; TG, tioguanine.
[a]Composite term.

Table 3. Serious adverse events for methotrexate and tioguanine during follow-up.

	CTCAE term	Frequency
Methotrexate	Enterocolitis infections	4
	Ileus	1
	Neoplasms benign	1
	Pneumonitis	1
	Gallbladder obstruction	1
	Total	8
Tioguanine	Infections and infestations—other	3
	Lung infection	2
	Abdominal infection	1
	Abdominal pain	1
	Total	7

MTX, methotrexate; TG, tioguanine; CTCAE, Common Terminology Criteria for Adverse Events.

Table 4. Discontinuation: all causes.

	Methotrexate discontinued [*n* = 67]	Tioguanine discontinued [*n* = 41]
Treatment duration—weeks, median [IQR]	27.0 [8.0-51.0]	29.0 [13.5-60.5]
Discontinuation reason, *N* [%]		
Adverse event	46 [68.7]	19 [46.3]
Remission	3 [4.5]	2 [4.9]
Primary non-response	6 [9.0]	8 [19.5]
Loss of response	5 [7.5]	7 [17.1]
Pregnancy	3 [4.5]	0 [0.0]
Patient request	2 [3.0]	3 [7.3]
Comorbidities	2 [3.0]	2 [4.9]

IQR, interquartile range.

During follow-up, 29 patients initiated concomitant biologic therapy in each group [MTX 27.6%, TG 25.4%, $p = 0.715$]. Median time to biologic initiation was 29.0 [IQR 16.5-51.5] weeks for MTX and 39.0 [IQR 20.3-47.3] weeks for TG [$p = 0.788$]. Monotherapy drug survival probability at 104 weeks was 21.6% for MTX- and 45.7% for TG-treated patients [$p < 0.001$, Supplementary Figure 2].

After censoring for discontinuation [all reasons], 22 MTX-treated patients [survival probability = 46.3%] and 43 TG-treated patients [survival probability = 56.8%] reached biologic- and corticosteroid-free drug survival after 104 weeks [$p = 0.150$, Supplementary Figure 3]. Only baseline clinical remission was predictive for biologic- and corticosteroid-free drug survival in the unadjusted logistic regression [OR 0.299, 95% CI 0.133-0.671, $p = 0.003$]. Biochemical [$p = 0.239$] and clinical [$p = 0.538$] remission proportions at 104 weeks were not significantly different between treatment groups.

3.5. Inverse probability treatment weighting

After IPTW, no significant baseline differences were present. All baseline variables except history of perianal surgery had an SMD <0.2, indicating a small between-group difference after weighting. As in the univariate analysis, discontinuation due to AE was significantly higher in MTX-treated patients [OR 3.302, 95% CI 1.783-6.115, $p < 0.001$]. Similarly, AE incidence was higher [OR 5.967, 95% CI 3.166-11.248, $p < 0.001$] and biologic- and corticosteroid-free survival was lower [OR 0.391, 95% CI 0.208-0.736, $p = 0.004$] in MTX-treated patients.

3.6. Subsequent therapy during follow-up

At the end of follow-up, 108 [49.3%] patients had discontinued treatment (MTX: $n = 67$ [63.8%], TG: $n = 41$ [36.0%], $p < 0.001$). Most patients (MTX: $n = 24$ [35.8%], TG: $n = 14$ [34.1%], $p = 0.860$) were subsequently treated with an anti-tumour necrosis factor [TNF] agent. One TG-treated patient received vedolizumab. Three patients started ustekinumab (MTX: $n = 1$ [1.5%], TG: $n = 2$ [4.9%], $p = 0.299$). Ten [14.9%] MTX-treated patients received

subsequent TG therapy, and nine [22.0%] TG-treated patients received subsequent MTX. Four [6.0%] MTX- and two [4.9%] TG-treated patients underwent surgery following therapy discontinuation [$p = 0.810$]; for 11 [16.4%] and five [12.2%] patients, respectively, no subsequent therapy was initiated [$p = 0.549$].

4. Discussion

In this multicentre study, we compared the 2-year safety, tolerability, and effectiveness of MTX and TG in CD after failure of conventional thiopurines. MTX-treated patients showed a significantly higher cumulative AE-related discontinuation incidence [43.8%], compared with TG-treated patients [16.7%][$p < 0.001$]. This difference was sustained after performing competing risk analysis and IPTW. In addition, more MTX-treated patients experienced AE [82.9% vs 45.6%, $p < 0.001$] and the total cumulative incidence of AE was higher [152 vs 45 per 100 patient-years]. These differences resulted in a higher monotherapy survival in TG-treated patients [21.6% vs 45.7%, respectively, $p < 0.001$].

The high cumulative AE discontinuation incidence in MTX-treated CD-patients in our study is comparable to previously reported cohort studies, showing cumulative discontinuation incidences due to AE ranging from 30% to 40%.[18,19] When considering earlier studies until 2013, cumulative AE-related discontinuation incidences range from 10% to 30%.[17,20–22] These differences might be the consequence of the increase in emerging therapies for CD after MTX, causing a lower threshold for therapy switch. A recent study of Vasudevan *et al*. with a comparable study design to our study, comparing MTX with conventional thiopurines in 782 IBD patients, showed cumulative discontinuation incidences of 40% in MTX versus 19% in conventional thiopurines [$p < 0.001$]. In the analysis of patients with prior immunomodulator intolerance, these incidences were even higher, 45% in MTX and 28% in conventional thiopurine-treated patients. The most common reason for MTX discontinuation was nausea [18%]. These results are similar to our observations in MTX-treated CD patients. The cumulative TG discontinuation incidence due to AE of 16.7% was similar to our previous study where we compared a different cohort of TG-treated inflammatory bowel disease [IBD] patients with patients treated with low-dose thiopurine combined with allopurinol [$n = 94$, 20%].[9] A systematic review of 353 IBD patients treated with TG showed an overall discontinuation incidence of 20%. The majority of these patients failed treatment due to AE, a large proportion of the patients receiving a high daily dose of ≥40mg.[10]

We observed a high AE incidence in MTX and TG in this study [82.9% vs 45.6%]. Most previous MTX studies reported total AE incidences based on retrospective data, causing a significant risk of reporting bias. Previous studies reported AE incidences from 27% during a mean follow-up of 17 months to 79% in 3 years in CD patients, making comparisons with our results difficult.[23,24] The MERIT-UC study, a recent 'negative' phase 3 trial on MTX in ulcerative colitis, reported an AE incidence of 93% in 48 weeks.[25] Given the prospective design with close monitoring, this may illustrate the effect of reporting bias. Vasudevan *et al*. reported a higher cumulative MTX SAE incidence [13.2%] compared with our incidence of 7.3%. This could be explained by the longer median follow-up duration of 48 months. The total AE [45.6%] and SAE [7.0%] incidences in TG-treated patients were comparable to a recent study describing a cohort of 274 TG-treated IBD patients [41% and 5%, respectively].[16]

Even though discontinuation because of loss/lack of response was similar in both groups, monotherapy survival was higher in TG-treated patients. Corticosteroid- and biologic-free survival probability was also higher in TG-treated patients [56.8% vs 46.3%]. However, this analysis was performed with censoring of discontinued patients [$n = 108$, 49.3%] which likely resulted in insufficient power to reach statistical significance.

This study showed favourable proportions of AE-related discontinuation and overall monotherapy drug survival for TG versus MTX. Other reasons for discontinuation, such as remission or lack/loss of response, were not significantly different. These results could support clinicians in therapy selection after failure of conventional thiopurines. In addition, other factors may also affect therapeutic decisions, such as the route of administration. When switching to MTX, most patients would undergo regular injections, as oral MTX therapy is not preferred because of the lower bioavailability with high variations between patients.[26] In patients with barriers to injectables, this could lead to loss of therapy adherence, as previous research found that IBD patients prefer oral therapy over injectables.[27] Finally, MTX treatment cannot be monitored by regular metabolite levels since these tests are not available in routine daily care, whereas in thiopurines, levels of 6-TGN can be used to monitor and optimise therapy.

In this age of increasing availability of IBD therapies, biologic therapies are becoming numerous and, with the introduction of biosimilars, become available at lower costs. After failure of conventional thiopurines, anti-TNF therapy is a viable therapeutic option. However, the majority of patients discontinue conventional thiopurines because of AE, despite the medication demonstrating effectiveness. Therefore, these patients may benefit from a switch within class. A Markov model to compare azathioprine, infliximab [biosimilars], and combination therapy, showed acceptable costs for azathioprine therapy with escalation to combination therapy when necessary.[28] Also, patients in newly industrialised and developing countries often do not have access to biologics, due to high costs and/or lack of insurance coverage. When choosing anti-TNF therapy, guidelines advise combination therapy with an immunomodulator to decrease the risk of anti-TNF antibodies and subsequent loss of response.[5] Although our population did not use concomitant biologic therapy, these safety results may also be applicable for TG/MTX use in combination therapy.

Strengths of this study include the carefully selected patient cohort with patients who only failed conventional thiopurines and did not have prior exposure to TG or MTX. We also created a real-world population by including patients in both academic and general teaching hospitals, in different regions of The Netherlands. In terms of methodology, we tried to approach the benefits of a randomised study using IPTW to correct for confounding by indication, as no randomised study is being performed or planned and this would not be feasible in the near future. Competing risk analysis was used to correct for discontinuation due to other reasons, in which case the primary endpoint could not be reached.

Limitations of the study include its retrospective nature, potentially resulting in reporting bias especially for minor AE, and risk of misinterpretation of documentation. However our primary outcome, AE as reason for

discontinuation, was overall well documented. Second, therapy choice might have been influenced by physician's/patient's preferences or previous medical history. Earlier research showed that clinicians may be more reluctant about initiating MTX therapy compared with thiopurines.[29] Although this is not known for TG, it could be hypothesised that clinicians approach TG treatment similarly to conventional thiopurines. In addition, clinicians may have experienced many patients discontinuing MTX due to AE, influencing personal preferences in prescribing MTX. This may have led to the present baseline population differences, showing a population of MTX patients with generally more complicated and therapy-refractory disease. To correct for these differences, we applied IPTW to account for any baseline differences. However, this cohort is not comparable with a randomised cohort and there may be residual unmeasured confounding even after applying IPTW. Third, effectiveness was compared using discontinuation reasons, monotherapy survival, biologic- and corticosteroid-free remission, and clinical/biochemical remission. Preferably, endoscopic evaluation is used for effectiveness outcomes, but this was beyond the scope of this paper that primarily focused on safety outcomes.

In conclusion, we observed higher cumulative discontinuation incidences due to AE in MTX-treated CD patients after failure of conventional thiopurines, compared with TG therapy. The total AE incidence during MTX use was higher as well, whereas SAE incidence was similar between groups. TG-treated patients had significantly higher numbers of monotherapy drug survival after 104 weeks of follow-up. These favourable results for TG treatment may aid in guiding the selection of immunosuppressive therapy after failure of conventional thiopurines in patients with CD.

Data Availability Statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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Conflict of Interest

FH has served on advisory boards, or as speaker or consultant for Abbvie, Celgene, Janssen-Cilag, MSD, Takeda, Celltrion, Teva, Sandoz, and Dr Falk, and has received unrestricted grants from Dr Falk, Janssen-Cilag, Abbvie. GD reports grants from DSM Nutritional Products and speake'rs fees from Janssen Pharmaceuticals, Abbvie, and Takeda, outside the submitted work. NKHdB has served as a speaker for AbbVie and MSD and has served as consultant and principal investigator for Takeda and TEVA Pharma BV; he has received [unrestricted] research grant from Dr Falk, TEVA Pharma BV, MLDS, and Takeda, all outside the submitted work. EHJS, MHJM, ARB, FC, VBCB, NdB, MGVMR, and TEHR have no conflict of interest to report.

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Authors Contributions

No additional writing assistance was used for this manuscript. EHJS, MHJM, ARB, FC, VBCB, NdB, MGVMR, TEHR NKHdB, GD, and FH all contributed to the design of the study; EHJS, MHJM, ARB, and FC collected data; MGVMR, TEHR, NKHdB, GD, and FH identified included patients; EHJS and MHJM analysed the data; NdB assisted in statistical analysis; EHJS drafted the manuscript. All authors revised the manuscript for important intellectual content. All authors have approved the final version of this manuscript.

Conference Presentations

A previous version of this manuscript was presented as a poster presentation at the online ECCO conference of 2021 and as a poster presentation at the Dutch Digestive Disease Days 2021.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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