




Sustained effectiveness, safety and therapeutic drug monitoring of tioguanine in a cohort of 274 IBD patients intolerant for conventional therapies

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Summary

Background: Tioguanine (or thioguanine) is an alternative drug for IBD patients who fail prior conventional immunomodulating therapy.

Aim: To report effectiveness, safety and therapeutic drug monitoring in a cohort of patients with prolonged tioguanine maintenance therapy.

Methods: In this nationwide, multicentre study, medical records of tioguanine-using IBD patients were retrospectively reviewed. Response to therapy was defined as clinical effectiveness without (re)initiation of corticosteroids, concurrent biological therapy or surgical intervention. All adverse events that occurred during the follow-up were listed and graded according to the common terminology criteria (CTC).

Results: Two hundred and seventy-four patients (female 63%, Crohn's disease in 68%) were included with median treatment duration of 51 months, 1567 patient-years of follow-up and median 20 mg/d tioguanine dosage. Tioguanine was tolerated in 79%, clinical effectiveness at 6 months was documented in 66% and sustained clinical effectiveness during 12 months in 51% of patients. Forty-one per cent of patients developed adverse events: 5% were graded as severe. Adverse events comprised infection requiring hospitalisation in three and skin cancer in eight patients (two melanomas). Asymptomatic nodular regenerative hyperplasia of the liver occurred in two out of 52 patients with liver biopsies (3.8%) and portal hypertension in three whereof one potentially associated with tioguanine (0.4%). Clinical effectiveness was correlated with 6-thioguanine nucleotide threshold concentrations $>682 \text{ pmol}/8 \times 10^8 \text{ RBC}$ ($P < 0.05$).

Conclusions: Long-term tioguanine therapy for at least 12 months was effective in 51% and well tolerated as a maintenance treatment for IBD in about 70% of patients. Adverse events were common, but mainly mild or moderate. 6-Thioguanine nucleotide threshold concentration $\geq 700 \text{ pmol}/8 \times 10^8 \text{ RBC}$ is proposed as target level with higher odds for clinical effectiveness.

Melek Simsek and Debbie S. Deben designate shared first co-authorship.

The Handling Editor for this article was Professor Jonathan Rhodes, and it was accepted for publication after full peer-review.

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1 | INTRODUCTION

Over the last decades, several drugs have been introduced for the management of Crohn's disease and ulcerative colitis, being the two main phenotypes of IBD.¹ Novel costly pharmaceutical agents (primarily biologicals) have been developed, although with limited data on long-term usage and specific safety issues.^{2,3} Nevertheless, there has been a growing interest in optimisation of conventional drugs for the treatment of IBD patients.^{4,5} One of these drugs was tioguanine, an alternative thiopurine-derivative, which has shown promising therapeutic results in the treatment of both Crohn's disease and ulcerative colitis.⁶⁻⁸ Especially in IBD patients who failed prior therapy with conventional thiopurines, that is azathioprine or mercaptopurine, tioguanine has been reported to be both effective as well as tolerated in up to 80%.^{7,9,10} Potential benefits of tioguanine over conventional thiopurines have been ascribed to pharmacokinetic differences between the thiopurine-derivatives (Figure 1).^{11,12} Unlike azathioprine and its metabolite mercaptopurine, tioguanine is metabolized in fewer metabolic steps and bypasses several intermediate metabolites, possibly evading associated adverse events.¹³

Despite the slowly growing evidence of the therapeutic advantages of tioguanine in IBD, there appears to be a reluctance to its use in clinical practice.¹⁴ Association between high dosed tioguanine and hepatotoxicity, in particular nodular regenerative hyperplasia, has been reported.¹⁵ Presuming publication bias on the balance between effectiveness and safety profile, limited and precarious research on tioguanine in IBD patients has been continued and additional data on toxicity have become available.⁶ Prevalence of histological liver alterations, such as nodular regenerative hyperplasia, has been low on uncharted, but adequate dosing (0.2-0.3 mg/kg, though not exceeding 25 mg/d) and has appeared to have an asymptomatic course in general.¹⁶⁻¹⁸ Following these results, tioguanine has been conditionally licensed as a certified IBD treatment in the Netherlands since 2015 for patients who previously failed

azathioprine or mercaptopurine due to intolerable adverse events or nonresponse.⁸

Azathioprine and mercaptopurine, both as monotherapy or in combination with anti-tumour necrosis factor (TNF) drugs, are well established drugs in the management of both Crohn's disease and ulcerative colitis. These drugs are effective in the maintenance of remission of active IBD and exert steroid-sparing effects.^{19,20} Also, early start of thiopurines reduces risk of first and recurrent abdominal surgery.²¹ Long-term use of thiopurines, however, has been associated with an increased risk for opportunistic infections, non-melanoma skin cancer and lymphoproliferative disorders.²² These risks increase in patients with combination therapy with corticosteroids and biologicals.²³ Similar data on the long-term safety profile of tioguanine are limited, presumably due to the short duration of the follow-up which has been reported in literature until now.^{6,10} Moreover, utility of therapeutic drug monitoring during tioguanine treatment has not been verified yet.

In view of the increasing use of tioguanine as an alternative drug for IBD, in particular in the Netherlands, more studies evaluating the long-term effects of tioguanine during longer follow-up periods are warranted. The primary aim of this study was to assess long-term safety and effectiveness profile in relation to therapeutic drug monitoring of tioguanine in IBD patients among three real-life cohorts from tioguanine expert centres in the Netherlands. Furthermore, we evaluated if outcomes have been related to disease and treatment characteristics and performed a meta-analysis of the sustained clinical effectiveness of tioguanine of at least 1 year in IBD patients.

2 | MATERIALS AND METHODS

2.1 | Study design and patient population

This was a nationwide, multicentre, retrospective cohort study using data from three tioguanine expert centres located in the western (Amsterdam UMC, location VU medical centre at Amsterdam),

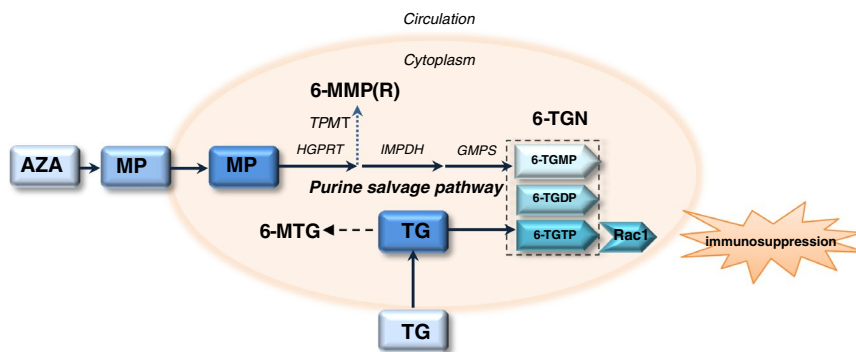


FIGURE 1 Simplified metabolism of thiopurines in a target cell. Bold lines represent the purine salvage pathway in which the pharmacologically active end-metabolites (6-thioguanine nucleotides (6-TGN) are formed and dotted lines represent the competing pathways. The 6-TGNs consist of 6-thioguanine monophosphate (6-TGMP), 6-thioguanine diphosphate (6-TGDP) and 6-thioguanine triphosphate (6-TGTP). The 6-TGTP nucleotides target Rac1 and induce T-cell apoptosis. AZA: azathioprine; MP: mercaptopurine; TPMT: thiopurine S-methyltransferase; 6-MMP: 6-methylmercaptopurine; 6-MMPR: 6-methylmercaptopurine ribonucleotides; HGPRT: hypoxanthine-guanine phosphoribosyltransferase; IMPDH: inosine monophosphate dehydrogenase; GMPS: guanosine monophosphate synthetase; TG: tioguanine; 6-MTG: 6-methylthioguanine

eastern (Rijnstate Hospital at Arnhem) and southern (Zuyderland medical centre at Sittard-Heerlen) part of the Netherlands. Patients were identified by retrieving hospital pharmacy dispensing records of the first 6 months of 2015. During this period, tioguanine was temporarily designated as a hospital dispensed medicinal product obligating patients to exclusively collect their (three months) prescription from the hospital pharmacies.

Using this study design, we collected data from a real-life cohort consisting of all patients who collected their first or repeat prescription in this specific period and studied their follow-up until treatment discontinuation or June 2018. Adult patients (≥ 18 years) treated with tioguanine for IBD and complete documentations of follow-up, were included in the analysis. Patients were categorised into regular users or recent users, depending on the start date of tioguanine. Patients who started collecting tioguanine in the first 6 months of 2015 (recent users) had a shorter follow-up than patients who collected repeat prescription (regular users). Therefore regular users and recent users were analysed separately.

The diagnosis of Crohn's disease and ulcerative colitis was based on the standard combination of clinical, radiologic, endoscopic and histological findings.²⁶ Indications for tioguanine were categorised into 'induction of remission of active disease', 'maintenance of remission' and 'concomitant immunomodulation in patients with insufficient response to biological monotherapy'. In all centres, tioguanine was prescribed by experienced gastroenterologists, according to the expert-based guidelines provided by the European tioguanine working party.¹⁴

2.2 | Data collection

Demographic, clinical, biochemical and endoscopic data were retrieved from the medical patient files using a pre-determined structured data-sheet incorporated into Castor Electronic Data Capture (EDC) (version 1.4, Amsterdam, the Netherlands).²⁷ Data included patient and disease characteristics, such as age at time of tioguanine initiation, gender, IBD disease duration and phenotype according to Montreal classification,²⁸ bowel resection history and previous and current (co)treatment. Furthermore, laboratory parameters including haemoglobin, erythrocyte sedimentation rate (ESR), white blood cell count (WBC), platelet count (PC), C-reactive protein (CRP), faecal calprotectin (FCP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma glutamyl-transferase (GGT), bilirubin and albumin within 3 months prior to tioguanine initiation, at 6 months of therapy and collected at the last patient visit, were recorded. When available, concentrations of the active 6-thioguanine nucleotides (6-TGN) during steady-state (between 4 and 6 months of therapy) were recorded.

2.3 | Clinical effectiveness

Clinical effectiveness was defined by a physician's global assessment (graded as (a) diminished, ie improved condition, (b) unchanged or (c) worsened disease activity), combined with normalised parameters of inflammation (ie CRP < 10 mg/L or FCP < 200 μ g/g) and, when available, improvement of endoscopic and pathophysiological findings.

Primary nonresponse was assigned when criteria for clinical effectiveness were not met within the first 6 months of tioguanine therapy. Secondary nonresponse was defined as loss of response after 6 months of tioguanine therapy after an initial response. For steroid-induced remission, clinical effectiveness was only met if steroids were withdrawn within 6 months and not restarted for at least 12 months after starting tioguanine. Reinitiating corticosteroids, initiation of concurrent biologicals or IBD-related surgical intervention during tioguanine treatment were assigned as nonresponse to therapy. Furthermore, sustained clinical effectiveness, defined as an ongoing use of tioguanine treatment after at least 12 months of follow-up without either (re)initiation of corticosteroids, biologicals or IBD-related surgical intervention, was also evaluated.

2.4 | Drug withdrawal and adverse events

Occurrence of adverse events and reasons for withdrawal during the entire follow-up were recorded. Adverse events were defined as symptoms, signs or laboratory abnormalities that occurred after initiation of tioguanine and were listed according to the common terminology criteria for adverse events (CTCAE) (Version 5.0, released November 27, 2017).²⁹ The CTCAE are a set of criteria for the standardised classification of adverse events of chemotherapeutic drugs using a range of grades from 1 to 5 (1: mild, 2: moderate, 3: severe, 4: life-threatening and 5: adverse event-related death). Additionally, we collected all available histopathological evaluations of liver biopsies after initiation of tioguanine until end of follow-up.

2.5 | Thiopurine metabolites

In all centres, tioguanine metabolites were determined in red blood cells (RBC) using a method by Dervieux and Boulieu.³⁰ Due to a lack of standardised operating procedures for measuring thiopurine metabolites, we considered that analysed 6-TGN concentrations might differ between the three hospital laboratories.^{31,32} To evaluate possible differences in 6-TGN concentrations, blood samples were cross-checked between the participating hospitals. 6-TGN concentrations were comparable between the laboratories of Zuyderland medical centre and Rijnstate hospital. However, the laboratory of Amsterdam UMC assessed 6-TGN concentrations that were 1.2 times higher than the other two hospitals. Therefore, collected 6-TGN data of Amsterdam UMC were corrected with a factor 0.83 before data analysis.

2.6 | Statistics

Data were presented as numbers with percentages, medians with interquartile range (IQR) or means with standard deviations, when appropriate. Depending on the distribution, parametric or nonparametric tests including the Mann-Whitney *U*, Wilcoxon signed-rank test, Kruskal-Wallis and the student *t* test or for categorical variables the chi-square test, were used to test for differences within and between groups. To analyse the association between clinical response and 6-TGN concentrations, a quartile analysis was performed. To

this end, 6-TGN concentrations were subdivided in quartiles and its association with clinical response was tested with a chi-square test. In addition, a receiver operator characteristic (ROC) curve was made. Cut-off for 6-TGN threshold concentrations was chosen based on a test specificity of $\pm 90\%$. A Kaplan-Meier survival curve was used for the comparative analysis of drug survival.

Furthermore, a meta-analysis of the sustained clinical effectiveness of tioguanine after a continued use of at least 12 months was conducted to identify a long-term treatment effect. Data from our study were combined with results of previous studies in which long-term clinical effectiveness of ≥ 12 months was described. To avoid overlap of included patients, previous studies from our research group were excluded from the meta-analysis.^{33,34} Sustained clinical effectiveness to therapy was reported as the proportion of patients with ongoing clinical effectiveness of tioguanine for at least 12 months relative to all compared patients in the study population. Pooled proportion with associated 95% CI was calculated using the random effects model to account for possible heterogeneity between the studies. Heterogeneity was assessed by the χ^2 and I^2 tests: the χ^2 test was used to assess the presence of heterogeneity and the I^2 test was used to determine the percentage of heterogeneity due to variation between studies. The GRADE guidelines were used to assess the quality of evidence of the included articles.³⁵ Statistical analyses were performed using SPSS Statistics (version 22.0, IBM, New York, NY, USA) and the meta package for R Version 3.2.5 (R foundation for Statistical Computing, Vienna, Austria). Two-tailed probability (P) values < 0.05 were considered statistically significant.

2.7 | Ethical considerations

The research medical ethics committee (REC) of the Amsterdam UMC, Zuyderland medical centre and Rijnstate hospital approved this study with file-number 2017-274. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki (2013) as reflected in a prior approval by the institution's human research committee. As data were anonymously provided, written informed consent for data analysis and reporting was waived by the REC.

3 | RESULTS

3.1 | Patient and therapy characteristics

A total of 305 patients treated with tioguanine for IBD were identified, of whom 31 cases were excluded (Figure 2). In this remaining cohort of 274 patients, 85 patients (31%) were recent users and 189 patients (69%) regular users. The patient characteristics, which were in general similar between the three hospitals, are summarised in Table 1. A total of 173 patients (63%) were female, 186 patients (68%) had Crohn's disease (36% had a stricturing and 12% a penetrating disease behaviour) and median disease duration at time of tioguanine initiation was 10 years (IQR 2-17).

Indications for tioguanine were to induce remission in 106 (39%), to maintain steroid-free remission in 147 (54%) and use as

concomitant immunomodulation in 21 patients (7%). Tioguanine dosages ranged between 8 and 40 mg/d, with a median daily dosage of 20 mg. In 18 patients (8%), the daily dosage was between 30 and 40 mg. All patients were previously unsuccessfully treated with immunomodulating drugs before tioguanine was initiated. A total of 96% failed conventional thiopurines (azathioprine in 37%, mercaptopurine in 52% and both in 11%) due to intolerance (92%) or resistance (8%). Furthermore, prior to tioguanine, 20% and 6% of patients failed biologicals and methotrexate respectively. Ninety out of 186 IBD patients with Crohn's disease (48%) had a history of bowel resection prior to tioguanine therapy.

3.2 | Clinical effectiveness

Clinical effectiveness of tioguanine therapy was documented in 182 of 274 patients (66%) within 6 months. In 16 patients (6%), primary clinical response was insufficiently documented. Therapeutic response after these 6 months was reported in another 13 patients (5%). Response rates did not depend on gender (male: 60% and female: 70%, $P = 0.17$), type of IBD (ulcerative colitis: 72%, Crohn's disease: 65% and IBD unclassified: 50%, $P = 0.33$) or the indication for tioguanine (induce remission: 66%, maintain remission: 68% or as a concomitant drug in biological therapy: 57%, $P = 0.82$). Among the entire cohort, a biochemical response at 6 months was observed based on the decrease in ESR, CRP, WBC and FCP and increase in haemoglobin and albumin compared to baseline (Table 2).

3.3 | Sustained clinical effectiveness

Sustained clinical effectiveness of tioguanine during at least 12 months, in the absence of (re)initiation of corticosteroids, biologicals or IBD-related surgical intervention, was reported in 71% of responding patients (139 of 195) and 51% of the total cohort (139 of 274). Secondary loss of response was reported in 29% of the responding patients (56 of 195). Bowel resection was performed in nine patients (all with Crohn's disease), corticosteroids were reinitiated in 41 and concurrent biologicals were started in 19 patients. Loss of response did not depend on the prior indication for tioguanine therapy, disease characteristics, gender or age.

Sustained clinical effectiveness of at least 12 months of tioguanine has been reported in five previous studies outside of our own research group. The main results of these studies are depicted in Table 3 and were included in a meta-analysis together with the results of this current study. In total, 483 patients with tioguanine therapy for IBD were described. Of these, 231 patients experienced an ongoing clinical effect of tioguanine therapy of more than 12 months (pooled proportion: 44%, 95% CI: 34%-55%) (Figure 3). The heterogeneity between the studies was 74%.

3.4 | Drug survival

The median duration of tioguanine use of the entire cohort was 51 months (IQR 36-89), 1567 patient-years of follow-up. A total

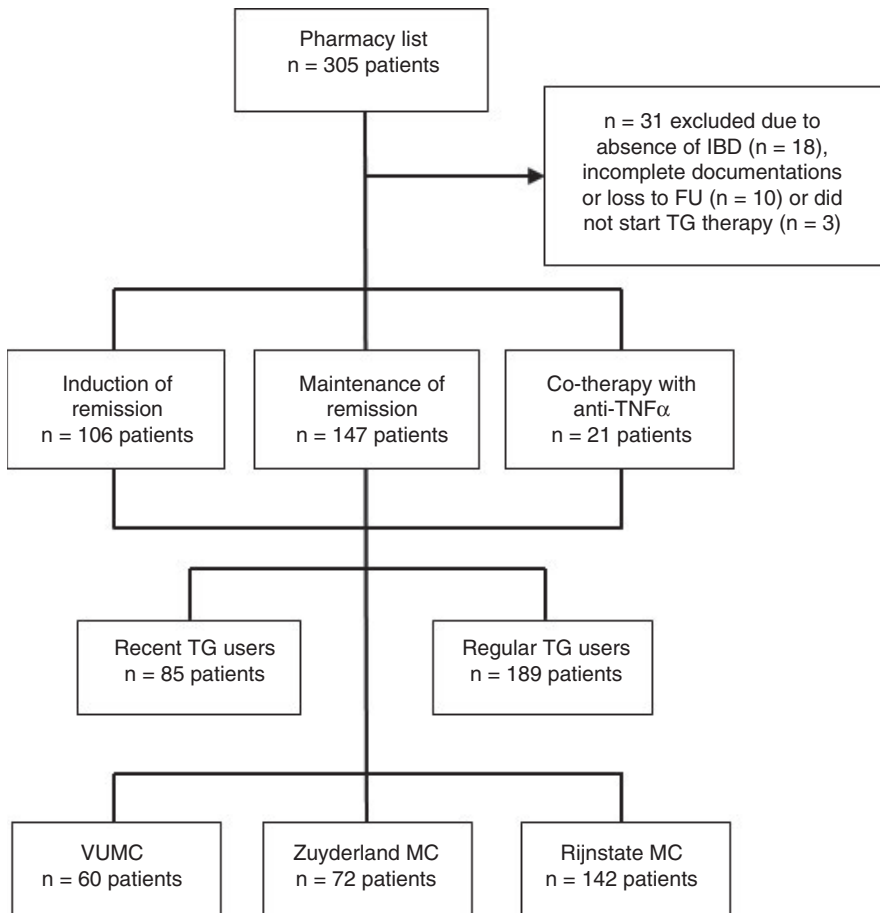


FIGURE 2 Flowchart of patient inclusion. IBD: inflammatory bowel disease; FU: follow-up, TG: tioguanine

of 216 out of 274 patients (79%) tolerated prolonged tioguanine therapy: 19 patients (7%) discontinued therapy due to sustained clinical remission and 197 patients (72%) used tioguanine until end of follow-up. The proportion of patients who continued tioguanine in the three different hospitals is depicted in a Kaplan-Meier survival curve in Figure 4. In total, 77 patients (28%) discontinued therapy with tioguanine (median duration 41 months, IQR 23-72) due to sustained clinical remission ($n = 19$), intolerance or severe adverse events ($n = 29$), at patient's own request ($n = 17$), insufficient therapeutic response ($n = 5$), pregnancy ($n = 5$) and death ($n = 2$). The death of these two patients was attributed to underlying comorbidity: a 74-year-old man suffered from prostate cancer and an 86-year-old woman had cardiovascular disease and died from cardiac arrest.

To compare regular users with recent users, we separately analysed the subgroup of recent users ($n = 85$) and the subgroup of regular users ($n = 189$). The subgroup of recent users had a duration of therapy ranging from 2 to 42 months with a median duration of 32 months. Forty-five of 85 recent users (64%) continued therapy until the end of follow-up. In the subgroup of regular users, 144 patients (76%) continued tioguanine therapy until the end of follow-up. The difference in drug survival between recent and regular users was statistically significant ($P = 0.031$). Patient and drug characteristics of the subgroups were similar, as well as the adverse events and 6-TGN concentrations.

3.5 | Adverse events

In 112 patients (41%), a total of 186 adverse events were reported during 1567 patient-years of follow-up of tioguanine therapy. Several patients reported more than one adverse event. Adverse events are listed and graded according to the CTCAE in Table 4. Out of 186 adverse events, 121 were graded as mild (65%), 55 as moderate (30%) and 10 as severe (5%). None of the patients developed grade 4 or 5 adverse events (ie life-threatening or adverse event-related death) during tioguanine treatment.

Adverse events which were the reason to discontinue therapy were reported in 29 patients (11%) and included myelotoxicity ($n = 2$, grade 2), drug-induced liver injury (grade 2: $n = 3$, grade 3: $n = 2$), portal hypertension ($n = 3$, grade 3), general malaise ($n = 4$, grade 2), depression ($n = 1$, grade 1), headache ($n = 1$, grade 1), arthralgia or myalgia ($n = 4$, grade 1), alopecia ($n = 3$, grade 1), rash ($n = 2$, grade 2), fever ($n = 2$, grade 2), enterocolitis ($n = 2$, grade 1), opportunistic infection ($n = 1$, grade 3) and malignant neoplasm ($n = 2$, grade 3) (Table 4). Overall, 6-TGN concentrations were not associated with haematological or hepatobiliary-related adverse events (area under curve (AUC): 0.53, 95% CI: 0.43-0.64).

Gastrointestinal, general, musculoskeletal and skin-related adverse events contributed to almost half of all the adverse events reported in this cohort (46%), but occurred only graded as mild or moderate according to the CTCAE (Table 4). Severe adverse events

TABLE 1 Patient and disease characteristics

Baseline characteristics	N (%) or medians (IQR)
Total patients	274 (100%)
Female	173 (63%)
Age at starting tioguanine, y	43 (18-52)
IBD disease duration, y	10 (2-17)
Crohn's disease (Montreal classification)	186 (68%)
Age at diagnosis, y	
<17 (A1)	15 (8%)
17-40 (A2)	113 (62%)
>40 (A3)	55 (30%)
Behaviour	
Nonstricturing, nonpenetrating (B1)	96 (52%)
Stricturing (B2)	66 (35%)
Penetrating (B3)	21 (11%)
Perianal disease (P)	41 (22%)
Location	
Ileal (L1)	69 (37%)
Colonic (L2)	43 (23%)
Ileocolonic (L3)	71 (38%)
Upper gastrointestinal disease (+L4)	14 (8%)
Ulcerative colitis (Montreal classification)	83 (30%)
Extent	
Proctitis (E1)	4 (5%)
Left-sided (E2)	37 (45%)
Pancolitis (E3)	42 (50%)
IBD unclassified	5 (2%)
Bowel surgery in Crohn's disease	90 (48%)
Prior drug failure	100%
Azathioprine	132 (48%)
Mercaptopurine	173 (63%)
Anti-tumour necrosis factor	55 (20%)
Methotrexate	17 (6%)

Abbreviations: IBD, inflammatory bowel disease.

(5%) were hepatic-, infection- or malignancy-related. In this cohort, none of the patients developed pancreatitis during the follow-up including 43 patients (16%) with prior azathioprine or mercaptopurine-induced pancreatitis.

3.6 | Hepatobiliary disorders

Drug-induced liver injury occurred relatively frequently ($n = 51$, 19%) in this cohort primarily consisting of patients intolerant for conventional thiopurines, but was mainly mild. Severe cases were limited to two cases (1%). Among the entire cohort, liver enzymes prior to tioguanine did not differ from concentrations at 6 months tioguanine therapy (AST 20 vs 22 U/l, ALT 22 vs 21 U/l, GGT 23 vs 18 U/l, AP 73 vs 77 U/l, bilirubin 6 vs 7 U/l, all $P > 0.1$) (Table 2).

Three patients (1%) in this cohort suffered from complications of portal hypertension (splenomegaly and variceal bleeding). One of them, a 48-year-old male with ulcerative colitis, was diagnosed with hepatic sarcoidosis and a 53-year-old female with ulcerative colitis was suspected of having primary sclerosing cholangitis. In both patients, histological abnormalities indicative for nodular regenerative hyperplasia were absent in the liver biopsy specimens. The underlying cause of the portal hypertension in the third patient (a 53-year-old female with Crohn's disease) is not definitively histologically proven but may be due to vascular abnormalities of the liver.

Furthermore, all available liver biopsy reports were collected and assessed for histological abnormalities. A total of 84 liver biopsies were performed in 52 patients (19%) after a median tioguanine treatment of 96 months (IQR 66-137), mainly following routine toxicity screening. Nodular regenerative hyperplasia was observed in two liver biopsy specimens (2%) of two individual patients who were both asymptomatic and developed no clinical signs of portal hypertension during follow-up. In one of the patients (a 34-year-old male with ulcerative colitis), histological features of nodular regenerative hyperplasia were absent in a follow-up liver biopsy 2 years after tioguanine discontinuation. A part of these biopsies has been reported before.¹⁷

3.7 | Infections and malignancies

Infections necessitating hospitalisation occurred in three patients (1%). Two patients (54 and 62 years old) developed a common lower respiratory tract infection and a 47-year-old patient suffered from a reactivated cytomegalovirus (CMV) hepatitis during tioguanine treatment. Treatment with tioguanine was only permanently discontinued in the patient with the CMV infection.

Nine patients (3.3%) developed moderate (ie requiring minimal, local or non-invasive intervention) and two patients (0.7%) severe graded malignancies (ie medically significant but not immediately life-threatening) during 1567 patient-years of follow-up of tioguanine treatment (Table 4). Nonmelanoma skin cancer occurred in six (2.2%) and melanoma in two patients (0.7%). Solid-organ malignancies occurred in two other patients (0.7%) (prostate cancer in a 74-year-old male and breast cancer in a 57-year-old female patient). Only the two patients with severe graded malignancy discontinued tioguanine, including a 60-year-old female who was diagnosed with Non-Hodgkin lymphoma half a year after initiation of tioguanine therapy 20 mg/d. She was previously treated with anti-TNF and methotrexate therapy. Furthermore, Merkel cell carcinoma, a rare and aggressive type of nonmelanoma skin cancer, was found in a 73-year-old male after being co-treated with infliximab and tioguanine 20 mg/d for 2 years.

3.8 | Therapeutic drug monitoring

Median steady-state 6-TGN concentrations (between 4 and 6 months of therapy) were available in 134 (49%) patients. The median 6-TGN concentration was 737 pmol/ 8×10^8 RBC (range IQR 542-884). 6-TGN metabolite levels were higher in the clinical responders (801 (IQR

TABLE 2 Laboratory parameters prior to tioguanine and at 6 mo of continued therapy

Laboratory test (reference range)	Prior to tioguanine (median, IQR)	Available N (%)	At 6 mo (median, IQR)	Available N (%)	P-value
Haemoglobin (7.5-11 mmol/L)	8.3 (7.6-8.9)	260 (95%)	8.5 (7.9-9.0)	223 (81%)	<0.001
Erythrocyte sedimentation rate (<15 mm/h)	16 (6-34)	62 (23%)	11 (4-26)	39 (14%)	0.012
C-reactive protein (<10 mg/L)	5 (2-10)	239 (87%)	3 (1-6)	204 (74%)	<0.001
White blood cell count; (3.5-10 x 10 ⁹ /L)	7.5 (5.9-10)	258 (94%)	7.2 (5.8-9.3)	222 (81%)	0.035
Platelet count (150-400 x 10 ⁹ /L)	304 (248-371)	255 (93%)	284 (235-348)	223 (81%)	<0.001
Faecal calprotectin (200 µg/g)	248 (108-606)	127 (46%)	41 (16-94)	79 (29%)	<0.001
Albumin (35-50 g/L)	38 (35-41)	167 (61%)	39 (37-42)	121 (44%)	0.002
Alanine aminotransferase (<45 U/L)	22 (14-31)	253 (92%)	21 (16-29)	213 (78%)	0.45
Aspartate aminotransferase (<40 U/L)	20 (16-26)	198 (73%)	22 (18-26)	154 (56%)	0.11
Alkaline phosphatase (<120 U/L)	73 (60-89)	205 (75%)	77 (62-92)	155 (57%)	0.37
Gamma glutamyl-transferase (<55 U/L)	23 (15-35)	247 (90%)	18 (15-30)	207 (76%)	0.91
Bilirubin (<20 µmol/L)	6 (4-10)	126 (46%)	7 (5-10)	90 (33%)	0.89

TABLE 3 Summary of studies on the sustained clinical effectiveness of 12 mo of tioguanine therapy in IBD patients

Study	Design	Number of patients	Dosage (mg/d)	Follow-up (months)	Sustained effect of 12 mo	No sustained effect	Quality of studies
Qasim et al, 2007 ⁴¹	Prospective	40	40	8 (1-23)	10/40 (25%)	30/40 (75%)	Very Low
Ansari et al, 2008 ⁴²	Retrospective	30	20-60	22 (1-62)	17/30 (57%)	13/30 (43%)	Low
Almer et al, 2009 ⁴³	Prospective	23	20-60	7 (1-81)	5/23 (22%)	18/23 (78%)	Low
Pavlidis et al, 2014 ⁴⁴	Retrospective	62	40-60	8 (1-45)	37/62 (60%)	25/62 (40%)	Moderate
Ward et al, 2016 ¹⁰	Retrospective	54	20-40	16 (IQR 5-37)	23/54 (43%)	31/54 (57%)	Moderate
Simsek et al, 2019	Retrospective	274	20-40	51 (IQR 36-89)	139/274 (51%)	135/274 (49%)	Moderate

667-1003) vs 492 (IQR 388-569) pmol/8×10⁸ RBC, $P < 0.05$). With quartile analysis, an increased proportion of patients with clinical effectiveness with 6-TGN concentrations of ≥ 542 pmol/8×10⁸ RBC of the second quartile was found ($P < 0.001$) (Figure 5A). An additional analysis on 6-TGN concentrations and prediction of clinical response was performed with a ROC curve to calculate a threshold concentration. The AUC for the 6-TGN concentrations to predict clinical response was 0.89 (95% CI: 0.82-0.95) (Figure 5B). A cut-off level for effectiveness of therapy of 682 pmol/8×10⁸ RBC yielded a test specificity of 89% and a sensitivity of 74%. Patients with a 6-TGN concentration above 682 pmol/8×10⁸ RBC had higher odds for clinical response than patients with concentrations below this (OR: 22.2, 95% CI: 7.1-68.7, $P < 0.001$). Rounding this value to 700 pmol/8×10⁸ RBC yielded an equivalent test specificity of 89% and a sensitivity of 70%.

Median 6-TGN concentrations were not statistically significantly different between patients with anti-TNF combination therapy ($n = 14$, 785 pmol/8×10⁸ RBC) and patients without ($n = 120$, 723 pmol/8×10⁸ RBC, $P = 0.89$). Furthermore, there was no association found between tioguanine dose and clinical response or between dose and serum 6-TGN concentrations.

4 | DISCUSSION

In this study, we reported on long-term effectiveness, safety and therapeutic drug monitoring data of tioguanine in three cohorts of IBD patients from tertiary referral centres spread over the Netherlands with a median follow-up over 4 years and 1567

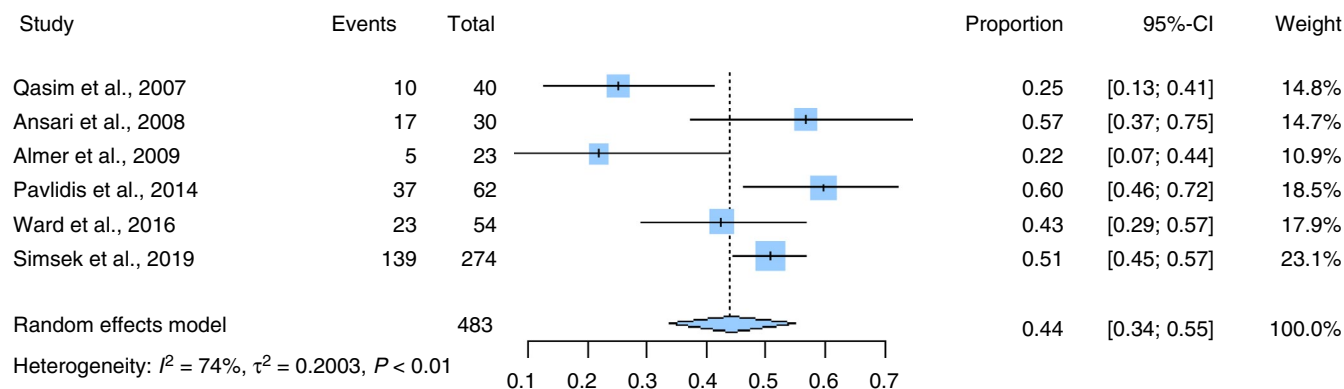


FIGURE 3 Forest plot of sustained clinical effectiveness of tioguanine therapy in IBD

patient-years. Clinical effectiveness of tioguanine (in median dosage of 20 mg/d) within 6 months was observed in two-thirds of patients who all failed prior immunomodulating therapies. Sustained clinical effectiveness during 12 months was reported in about 70% of the primary responders and 50% of the total cohort. A pooled proportion of our current results and the literature for sustained clinical effectiveness of tioguanine for IBD was 44%, 95% CI: 34%-55%. Furthermore, adverse events were documented in 40% of the patients but were mainly clinically tolerable. Intolerable or severe adverse events leading to tioguanine withdrawal occurred in 11%, mainly in patients who recently started therapy. Tioguanine was a beneficial therapy over the long-term with approximately 70% of patients continuing therapy until the end of follow-up.

The therapeutic benefit of tioguanine for IBD has been reported in multiple studies, although with varying response ratios ranging from 22% to 80%.⁷ On the other hand, tioguanine has been associated with hepatotoxicity, particularly nodular regenerative

hyperplasia, a finding which has been at least partly based on publication bias.^{15,36} In this cohort, drug-induced liver injury occurred in about 20% of patients. Out of this, 96% was graded as mild or moderate according to the CTCAE. Hepatic adverse events graded as severe occurred in two cases. Furthermore, three patients developed symptoms of portal hypertension including a patient with hepatic sarcoidosis and another with primary sclerosing cholangitis. We attribute these to the underlying conditions which are associated with portal hypertension as one of their most common complications.^{37,38} Portal hypertension in the third patient might be ascribed to nodular regenerative hyperplasia (prevalence of 1/274, 0.4%), although confirming histological liver examination is lacking.

In two out of 52 patients (3.8%) in this cohort, histological features of asymptomatic nodular regenerative hyperplasia were observed during (routine) toxicity screening including liver biopsy. Both of these patients were asymptomatic at the time of diagnosis and did not develop clinical signs of portal hypertension during follow-up. Notably, the relatively small number of patients with clinical relevant

FIGURE 4 Kaplan-Meier survival curve showing the time to tioguanine withdrawal in months in all three hospitals. In 77 out of 274 (28%), tioguanine was discontinued (median duration 41 mo, IQR 23-72) due to sustained clinical remission in 19, intolerance or severe adverse event in 29, insufficient response in 5, pregnancy in 5, not adverse event-related death in 2 and at patient's own request in 17 patients

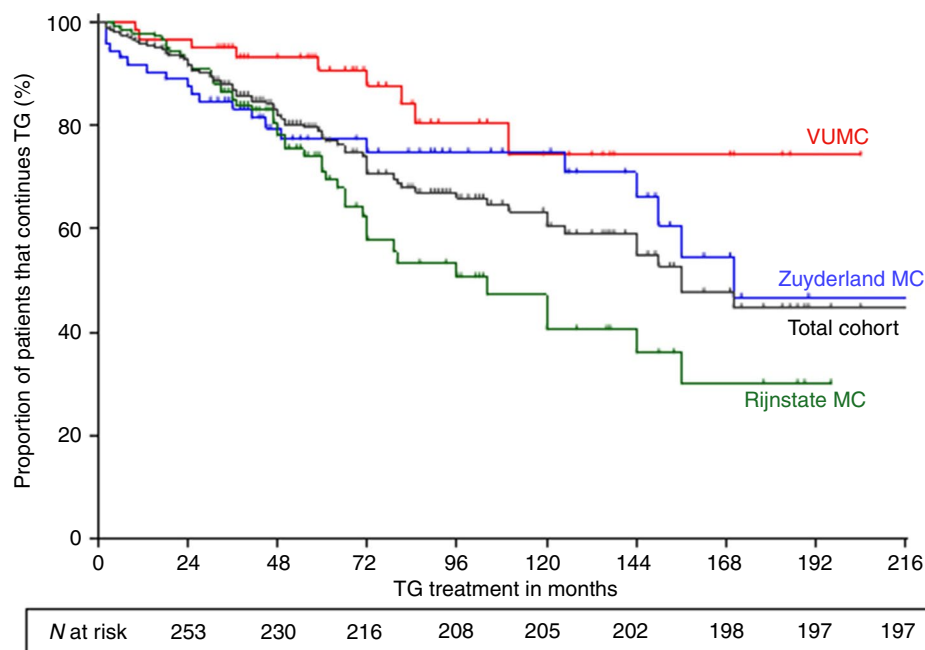


TABLE 4 Adverse events (n = 186) during 1567 patient-years of follow-up of tioguanine therapy, listed and graded according to the common terminology criteria (CTC)

	Grade 1 (mild) N = 121		Grade 2 (moderate) N = 55		Grade 3 (severe) N = 10	
General, neurological and psychiatric disorders	Flu-like symptoms	7	Malaise	4	–	
	Depression	1	Fever	5		
	Headache	2	Headache	1		
	Agitation	2	Dysesthesia	2		
Blood, lymphatic and vascular disorders	Bone marrow suppression	9	Bone marrow suppression	2	–	
	Flushing	4	Thromboembolic event	2		
Hepatobiliary disorders	DILI ^a	43	DILI ^a Cholecystitis	6	DILI ^a Portal hypertension 2 3	
Musculoskeletal and skin disorders	Pruritus	2	Arthralgia/myalgia	4	–	
	Alopecia	11	Eczema Rash	4		
	Arthralgia/myalgia	12		2		
	Rash	5				
Gastrointestinal disorders	Nausea	6	Diarrhoea	3	–	
	Abdominal pain	4	Enterocolitis	2		
	Diarrhoea	5				
	Enterocolitis	4				
Infections and infestations	Vaginal infection	1	HSV reactivation	1	CMV reactivation	1
			Bacteremia	2	Lung infection	2
			Lung infection	3		
			Urinary tract infection	2		
Neoplasm benign and malign	Skin papilloma	2	Basal cell carcinoma	3	Non-Hodgkin lymphoma	1
			Cervical dysplasia	1	Squamous cell carcinoma	2
				2		
				1		
				1		
				1		

No patient had a Grade 4 or 5 adverse event. Abbreviations: AE, adverse event; CMV, cytomegalovirus; DILI, drug-induced liver injury; HSV, Herpes Simplex Virus.

^aDILI grade 1 is defined as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) > 1.25 and ≤ 3 upper limit of normal (ULN) and total bilirubin > 1.25 and ≤ 2 ULN, grade 2 is defined as ALT and AST > 3 and ≤ 5 ULN and total bilirubin > 2 and ≤ 3 ULN, grade 3 is defined as ALT and AST > 5 and ≤ 10 ULN and total bilirubin > 3 and ≤ 10 ULN.

hepatotoxicity, including nodular regenerative hyperplasia, in this cohort is conflicting with the existing literature, in which incidence rates up to 62% have been reported, but consistent with a prior large series from the Netherlands.^{6,15,17} The exact pathogenesis of nodular regenerative hyperplasia in tioguanine-treated IBD patients remains poorly understood, but a dose-dependent association (being dosages above 40 mg/d) has been suggested.⁶ This study affirms that a median daily dosage of 20 mg appears to be relatively safe for the long-term treatment of IBD.³⁹ Interestingly, we observed the reversibility of vascular liver abnormalities after thiopurine cessation in one of the patients, an observation which has been reported before.^{18,36}

In contrast to conventional thiopurines, data on the utility of therapeutic drug monitoring of 6-TGN measurements during tioguanine treatment are sparse.⁴⁰ In the present study, we observed that steady-state 6-TGN concentrations correlated with clinical response, but were not indicative for drug-induced toxicity. We therefore propose a rounded cut-off value for the 6-TGN levels for effectiveness of therapy at 700 pmol/8×10⁸ RBC. In addition, we observed that

approximately 60% of the 274 patients with prior unsatisfactory therapeutic response to anti-TNF monotherapy experienced clinical benefit from tioguanine and anti-TNF combination therapy. The rates of clinical effectiveness were not statistically significantly different in patients with combination therapy as compared to tioguanine monotherapy.

The present study has several limitations which need to be acknowledged. The cohort comprised three real-life cohorts from different hospitals which precluded a definitive (prospective) calculation of the benefit-risk ratio of tioguanine. Even though therapy was applied in a uniform way according to the expert-based guidelines provided by the European tioguanine working party,¹⁴ physician and patient characteristics may differ between the hospitals. This is partly shown in the difference in drug survival of each hospital, reflecting slight differences in clinical practice of applying tioguanine therapy. All data were retrospectively retrieved from medical records by which information bias could have been introduced. The safety and effectiveness profile of tioguanine relied on a

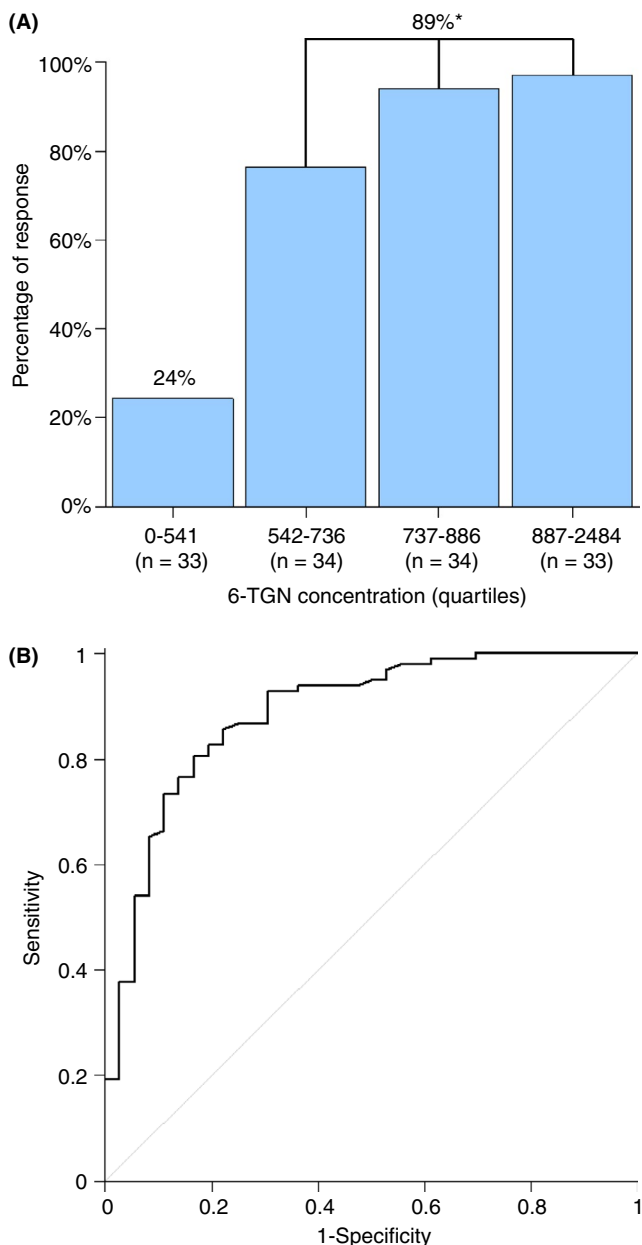


FIGURE 5 A, correlation of quartile 6-thioguanine nucleotides (6-TGN) concentrations with clinical response. Quartile analysis revealed a significantly increased clinical response starting from the second quartile with 6-TGN levels of ≥ 542 pmol/ 8×10^8 RBC ($P < 0.001$). B, Receiver operating characteristics (ROC) for 6-thioguanine nucleotides (6-TGN) concentrations between IBD patients with and without clinical response. The area under the ROC curve (AUC) for the 6-TGN levels to predict clinical response was 0.89 (95% CI: 0.82-0.95). A cut-off level for effectiveness of therapy of 682 pmol/ 8×10^8 RBC yielded a test specificity of 89% and a sensitivity of 74%

combination of various parameters including the biochemical, radiological, histological, endoscopic and pathophysiological findings and physician's global assessment, hence, was semi-standardised. Finally, we combined our long-term results with previous published data to compute a pooled proportion for the sustained clinical effectiveness

of tioguanine for IBD. This meta-analysis was limited by large heterogeneity among the studies of 74% and lack of sufficient quality of the included studies.

In conclusion, the therapeutic benefit of tioguanine needs to be balanced with associated toxicity when considering a role for this thiopurine-derivative in IBD treatment. In this large cohort, prolonged tioguanine therapy was well tolerated and effective as a maintenance treatment for IBD. Approximately 70% of patients continued tioguanine therapy during a median time of more than 4 years. Adverse events were common, but were mostly clinically tolerable. Intolerable or severe adverse events leading to withdrawal were reported in 11% patients, mainly in patients who recently started tioguanine therapy.

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AUTHORSHIP

Guarantor of the article: AvB.

Author contributions: NdB and AvB designed the study. MS, DD, CSH, MVB conceived the study and collected all data. MS and DD drafted the manuscript. MS and BILW analysed the data. CSH, HJCB, MLS, CJM, DRW, NdB and AvB critically revised the manuscript. All authors commented on the draft. All authors have approved the final draft of the article.

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