The pharmacokinetic effect of adalimumab on thiopurine metabolism in Crohn's disease patients

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KEYWORDS
Azathioprine; Mercaptopurine; Adalimumab; Drug interaction; Therapeutic drug monitoring; Crohn's disease

Abstract

Background and aims: A drug interaction between infliximab and azathioprine has previously been reported in Crohn's disease patients: the concentration of the main active thiopurine metabolites, the 6-thioguanine nucleotides (6-TGN), increased 1–3 weeks after the first infliximab infusion by 50% compared to baseline. The aim of this prospective study was to determine the effect of adalimumab on thiopurine metabolism in Crohn's disease patients, evaluated by 6-TGN and 6-methylmercaptopurine ribonucleotides (6-MMPR) concentration measurement.

Abbreviations: AZA, azathioprine; CDAI, Crohn's disease activity index; 95% CI, 95% confidence interval; CRP, C-reactive protein; CD, Crohn's disease; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; HPLC, high performance liquid chromatography; IBD, inflammatory bowel disease; IMP, inosine monophosphate; ITPase, inosine triphosphate pyrophosphatase; 6-MMPR, 6-methylmercaptopurine ribonucleotides; 6-MP, mercaptopurine; MCV, mean corpuscular volume; RBC, red blood cells; TDM, therapeutic drug monitoring; 6-TGN, 6-thioguanine nucleotides; 6-TGTP, 6-thioguanine triphosphate; TPMT, thiopurine S-methyltransferase; UC, ulcerative colitis.

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1. Introduction

The immunomodulating thiopurines, azathioprine (AZA) and mercaptopurine (6-MP), are effective for induction and particularly maintaining remission in the treatment of moderate to severe inflammatory bowel disease (IBD). Additionally, these drugs act as steroid-sparing agents.1–3

Neither AZA nor 6-MP has intrinsic pharmacological activity. AZA is a pro-drug that is converted to 6-MP by glutathione-S-transferase in the liver. 6-MP needs to undergo extensive metabolic transformations, involving pivotal enzymes like thiopurine S-methyltransferase (TPMT), xanthine oxidase (XO), hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and inosine triphosphate pyrophosphatase (ITPase), yielding to a variety of pharmacologically active metabolites. The thiopurine metabolites 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMPR) are considered to be clinically the most important. The proposed thiopurine metabolism in humans is given in Fig. 1.4

Patients with 6-TGN concentrations above the proposed therapeutic threshold of 235 pmol/8 × 10^8 red blood cells (RBCs) are more likely to be in clinical remission than patients with a 6-TGN concentration below this threshold.5,6 Further, the risk for myelotoxicity increases with 6-TGN above 490 pmol/8 × 10^8 RBC.7,8 High concentrations of 6-MMPR (above 5700 pmol/8 × 10^8 RBC) are associated with an increased risk of hepatotoxicity and treatment failure.8

In clinical practice therapeutic drug monitoring (TDM) of the thiopurine metabolites 6-TGN and 6-MMPR is a practical tool to optimise drug therapy in order to improve efficacy or avoid thiopurine toxicity.9,10

The biological drugs infliximab and adalimumab are monoclonal antibodies directed against tumour necrosis factor-α (TNF-α), a pro-inflammatory cytokine produced by macrophages and activated T-cells, which plays a key role in the pathophysiology of inflammatory bowel diseases. Infliximab and adalimumab are effective for induction and maintenance of remission of Crohn’s disease (CD) and ulcerative colitis (UC).11

According to national and international guidelines, anti-TNF-α treatment is indicated for steroid-refractory, steroid-dependent, or complex fistulizing CD.11–13

In the SONIC-trial it was shown that patients with moderate-to-severe CD, who were treated with infliximab in combination with AZA, were more likely to have a corticosteroid-free clinical remission than those receiving AZA or infliximab monotherapy.14

In 2003, Roblin et al. described a pharmacokinetic interaction between infliximab and AZA in CD patients: 1–3 weeks after the first infliximab infusion the mean 6-TGN concentration increased in infliximab responders with 1.5 times the baseline level. Interestingly, the increase of 6-TGN concentration correlated with the clinical response to infliximab and AZA combination therapy. Three months after the first infliximab infusion, 6-TGN concentrations were comparable with the baseline levels, which suggests a reversible effect of infliximab on AZA metabolism.15 Elevation of the active 6-TGN may result to an increased clinical efficacy, but may also increase the risk of severe myelotoxic side effects.9,16 Stringent safety monitoring is thus warranted.

The aim of this prospective study was to evaluate the effect of adalimumab on thiopurine pharmacokinetics in CD patients being treated with stable thiopurine maintenance therapy.

2. Materials and methods

2.1. Patient selection

A prospective study was performed in a group of CD patients under surveillance between July 2009 and December 2011 in five hospitals in the Netherlands (two university hospitals (Maastricht University Medical Centre and the VU University Medical Centre) and three general district hospitals (Orbis Medical Centre Sittard, Laurentius Hospital Roermond and Catharina Hospital Eindhoven)).

Steroid-dependent or steroid-refractory patients with ileocolonic, colonic or peri-anal (fistulizing) CD during maintenance AZA/6-MP therapy, who were scheduled for concomitant treatment with adalimumab, were prospectively included when meeting the following inclusion criteria: age between 18 and 70 years old, diagnosis of CD for at least 6 months...
ascertained by usual clinical, endoscopic and histological criteria, AZA/6-MP maintenance therapy for at least 3 months in a stable dose for at least 6 weeks, normal renal function and no liver test abnormalities. Concomitant mesalazine therapy was registered and had to be stable for at least 8 consecutive weeks because of the known interference with thiopurine metabolism in vivo.17

Patients were excluded from the study when one or more of the following criteria were met: signs of myelosuppression (leukocyte count below $2.5 \times 10^9/l$ and/or platelet count below $100 \times 10^9/l$); severe anaemia (haemoglobin (Hb) concentration below 6 mmol/l); known extensive proximal small bowel CD possibly interfering with resorptive area; small bowel surgery leading to intestinal failure or short bowel

AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-MMP, 6-methylmercaptopurine; 8-OHMP, 8-hydroxy-6-mercaptopurine; 6-TUA, 6-thiouric acid; 6-MTIMP, 6-methylthioinosine monophosphate; 6-MTIDP, 6-methylthioinosine diphosphate; 6-MTTTP, 6-methylthioinosine triphosphate; 6-TIMP, 6-thioinosine monophosphate; 6-TIDP, 6-thioinosine diphosphate; 6-TITP, 6-thioinosine triphosphate; 6-TXMP, 6-thioxanthosine monophosphate; 6-TGMP, 6-thioguanine monophosphate; 6-TGDP, 6-thioguanine diphosphate; 6-TGTP, 6-thioguanine triphosphate; 6-MTGP, 6-methylthioguanine monophosphate; ‘GST, glutathione-S-transferase’ XO, xanthine oxidase; TPMT, thiorurine S-methyl transferase; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; GMPS, guanosine monophosphate synthetase; MPK, monophosphate kinase; DPK, diphosphate kinase; ITPase, inosine triphosphate pyrophosphatase; PDNS, purine synthesis de novo; DNA, deoxyribonucleic acid. 6-MTIMP, 6-MTIDP and 6-MTITP together form the 6-methyl-mercaptopurine ribonucleotides (6-MMPR). 6-TGMP, 6-TGDP and 6-TGTP form together the 6-thioguanine nucleotides (6-TGN).

(Adapted from Derijks et al.4)

Figure 1  Proposed thiopurine metabolism.
syndrome; symptomatic ileal strictures or stenosis; concomitant use of allopurinol, angiotensin I-converting enzyme inhibitors, mycophenolate mofetil, infliximab or furosemide within 6 weeks before inclusion; concomitant use of methotrexate; and current pregnancy or intention to become pregnant within 6 months before treatment or lactation.

2.2. Study design

Adalimumab therapy started with a subcutaneous induction dose of 160 mg at week 0 and 80 mg at week 2, subsequently followed by a maintenance dose-regimen of 40 mg subcutaneous every other week. Demographic and clinical data, concomitant medication and disease activity (Crohn's disease activity index (CDAI)) were collected at baseline.

The study follow-up lasted for 12 weeks starting from the first adalimumab induction dose. At baseline and at weeks 2, 4, 6 and 12, blood samples were collected for 6-TGN and 6-MMPR metabolite assessment and evaluation of haematological and biochemical safety parameters. Adverse events were also recorded at these time points.

Clinical outcome was assessed at weeks 4 and 12 of concomitant adalimumab therapy, evaluated by CDAI calculation and C-reactive protein (CRP) serum concentration measurement.

Written informed consent was obtained from all participating patients.

2.3. Outcome parameters

The primary objectives were changes in 6-TGN and 6-MMPR metabolite concentrations compared to baseline, resulting from concurrent adalimumab therapy. The secondary objectives were enzyme activity changes of three essential thiopurine metabolizing enzymes, TPMT, HGPRT and ITPase, assessed before and 4 weeks after initiation of adalimumab therapy. Clinical response and safety were also evaluated during combination therapy.

Clinical remission was defined by a CDAI <150. In addition to CDAI evaluation, CRP serum concentrations were compared to baseline.

Safety was evaluated by measurement of the following haematological and biochemical parameters at baseline and at weeks 2, 4, 6 and 12: haemoglobin count, leukocyte count, platelet count and erythrocyte count, mean corpuscular volume (MCV), haematocrit, CRP, aspartate aminotransaminase (ASAT), alanine aminotransaminase (ALAT), alkaline phosphatase (AP), gammaglutamyl transferase (GGT), bilirubin, lactate dehydrogenase (LDH), albumin, amylase and creatinine.

2.4. Analytical procedures

2.4.1. 6-TGN and 6-MMPR concentrations

Blood samples were immediately stored in a refrigerator (2–8 °C) and subsequently sent to the laboratory of the Department of Clinical Pharmacy & Toxicology of the Orbis Medical Centre (Sittard-Geleen, The Netherlands), where the samples were processed and stored at −20 °C until required.

6-TGN and 6-MMPR metabolite concentrations were measured with a modified high performance liquid chromatography (HPLC) method of Lennard et al. as published previously. The lower-limits of quantification for 6-TGN and 6-MMPR were 40 pmol/8 × 10⁸ RBC and 300 pmol/8 × 10⁸ RBC, respectively. The inter-assay variability for both thiopurine metabolites was less than 10%.

2.4.2. TPMT, HGPRT and ITPase enzyme activity

TPMT enzyme activity was measured in erythrocyte lysates as described by Jacques-Aigrain, using reversed phase HPLC for quantification.

ITPase enzyme activity was measured in erythrocyte lysates by the production of inosine monophosphate (IMP) from inosine triphosphate. IMP was quantified using ion-pairing HPLC with an external calibrant.

HGPRT enzyme activity was measured by the production of IMP from hypoxanthine and phosphoribosyl pyrophosphate with a modified HPLC method of Jacomelli et al. using dried blood spot. IMP was quantified using ion-pairing HPLC with an external calibrant.

The enzyme activity measurements were performed at the Laboratory of Biochemical Genetics of the University Hospital Maastricht (Maastricht, The Netherlands). The inter-assay variation of each enzyme assay was less than 10%.

2.5. Statistical analysis

Data are presented descriptively as the median and range. The data were compared using the non-parametric Wilcoxon test since the outcome parameters did not show a normal distribution. Missing data were excluded from the relevant statistical analysis, when necessary. Correlation coefficients were calculated and significance of correlations was tested using Spearman's or Kendall's tau test, when appropriate. A p-value of less than 0.05 was considered to be statistically significant.

SPSS for Windows version 17.0 was used for statistical analysis.

Power calculation of the sample size was based on a clinically relevant change of 6-TGN concentrations of more than 150 pmol/8 × 10⁸ RBC and a standard deviation of 75 pmol/8 × 10⁸ RBC, according to a previous report from our group. Based on these data at least 6 patients should be included to be able to demonstrate a statistically significant difference in 6-TGN levels with a probability of 80%.

2.6. Medical ethics

The study was initially approved by the local Medical Ethical Committee of Orbis Medical Centre, Sittard (The Netherlands) and subsequently by all other participating medical centres.

3. Results

3.1. Patient demographics

Thirteen patients (6 men, 7 women) with a median age of 24 years (range 18–46) were initially included.

Patient characteristics at baseline are given in Table 1. In two patients adalimumab dose was increased to 40 mg every week because of a lack of response after 4 weeks and 10 weeks
of treatment, respectively. The median daily AZA dose was 1.78 mg/kg (range 1.29–2.24; n = 4), and the median daily 6-MP dose was 0.77 mg/kg (range 0.27–1.23; n = 9). One patient required a reduced maintenance dose of 6-MP (0.27 mg/kg/day) due to a previously revealed heterozygous TPMT genotype, assessed prior to thiopurine treatment.

One patient treated with AZA was excluded from data analysis due to non-compliance. Further analysis was performed with data from the remaining 12 patients.

### 3.2. Thiopurine metabolite concentrations

At baseline, the median 6-TGN concentration was 206 pmol/8 × 10⁸ RBC (range 64–405) and the median 6-MMPR concentration was 2123 pmol/8 × 10⁸ RBC (range 300–11,693).

In the case of two patients the thiopurine blood samples of week 4 and week 6 were lost during shipping.

**Fig. 2** shows the individual 6-TGN and 6-MMPR concentration curves in time, based on the levels assessed at baseline and at weeks 2, 4, 6 and 12. Overall, 6-TGN and 6-MMPR metabolite concentrations did not significantly change during the first twelve weeks of concomitant adalimumab and thiopurine therapy (Table 2, Fig. 3).

Before adalimumab therapy, in 8 of 13 patients (62%) 6-TGN concentrations were found to be below the therapeutic threshold of 235 pmol/8 × 10⁸ RBC. In 7 of 11 patients (64%) who completed the study follow-up period, still suboptimal 6-TGN concentrations (<235 pmol/8 × 10⁸ RBC) were found at week 12.

### 3.3. TPMT, ITPase and HGPR enzyme activity

TPMT and ITPase enzyme activity was measured in 11 of 12 analyzed patients, since the baseline sample of one patient was lost during shipping. HGPR enzyme activity was measured in 8 of 12 patients at baseline and at week 4 because three samples of the remaining patients were not appropriate for HGPR enzyme activity analysis due to logistic problems.

Compared to baseline, TPMT, ITPase and HGPR enzyme activity did not significantly change after 4 weeks of adalimumab and thiopurine co-treatment in the whole study group (Table 3).

In one patient an intermediate TPMT enzyme activity was found at baseline (0.28 μmol 6MMP/(mmol Hb × h); ref. 0.45–0.67 μmol 6MMP/(mmol Hb × h)). In another patient an intermediate ITPase enzyme activity was found (0.03 mmol IMP/(mmol Hb × h); ref. 0.00–10.00 mmol IMP/(mmol Hb × h)). In all patients a normal HGPR enzyme activity was measured (ref. 2.10–3.25 mmol IMP/(mmol Hb × h)).

### 3.4. Clinical outcome

Before combination therapy, two of 12 patients (17%) met the criteria of clinical remission (CDAI < 150), but were treated with adalimumab because of steroid-dependency. After 4 weeks of concurrent therapy, 7 of 12 patients (58%) were in clinical remission. The median CDAI score at week 4 (127, range 67–292) significantly decreased compared to baseline (257, range 97–366; p = 0.004). Likewise, after 4 weeks the median CRP concentration (5, range 1–58) decreased compared to baseline (16, range 2.9–156, p = 0.023). After 12 weeks, 64% of the patients (7 of 11) were in clinical remission. Median CDAI score at week 12 (127, range 42–370) significantly decreased compared to baseline (257, range 97–366; p = 0.008). The median CRP concentration at week 12 (3.5, range 1–97) decreased compared to baseline (16, range 2.9–156, p = 0.047), but not compared to week 4 (5, range 1–58; p = 0.796). There was no correlation between the clinical response rate, represented by the CDAI, and the 6-TGN concentrations measured at weeks 4 and 12 (p = 0.25 and p = 0.401, respectively), and there was also no correlation between CRP and 6-TGN at these time points (p = 0.354 and p = 0.272, respectively).

### 3.5. Laboratory safety parameters and adverse events

During the entire study period no relevant elevated liver tests (ALAT, ASAT, AP, GGT, bilirubin) were observed in this study population. Parameters concerning pancreas (amylase) and renal function (creatinine) did not change either (data not shown).

No alteration of MCV or erythrocyte count was observed. Compared to baseline, leukocyte count did not change at
week 2, week 4, and week 6. After 12 weeks of adalimumab therapy a decrease of the median leukocyte count was observed compared to baseline (6.1 × 10^9/l (4.0–10.6) versus 8.5 × 10^9/l (4.3–10.6), p = 0.011).

After 4 weeks one patient developed a temporary leukocytopenia with a leukocyte count of 3.5 × 10^9/l (reference value: 4–10 × 10^9/l). Two weeks later the leukocyte count normalized without intervention. Another patient developed a thrombocytopenia after 2 weeks, with a platelet count of 82 × 10^9/l (reference value: 150–400 × 10^9/l). Four weeks later platelet count dropped to 17 × 10^9/l, whereupon 6-MP and adalimumab therapy were discontinued. Autoimmune thrombocytopenia was diagnosed, allegedly induced by adalimumab treatment. The patient was successfully treated with high-dose prednisolone. Overall, in the whole study group platelet count did not statistically significantly change during the follow-up period.

4. Discussion

In the present study in CD patients on stable maintenance thiopurine therapy, no significant effect of adalimumab was observed on 6-TGN and 6-MMPR metabolite concentrations during the first 12 weeks of adalimumab and thiopurine combination therapy. Although after initiation of adalimumab therapy small individual changes in thiopurine metabolite concentrations were seen in several patients, we found no unequivocal overall effect in our study population, as opposed to the effect of infliximab on the metabolism of AZA.

TPMT, ITPase and HGPRT enzyme activities did not change after 4 weeks of concomitant adalimumab therapy. Consequently, we believe that adalimumab neither induces nor inhibits these pivotal thiopurine metabolizing enzymes in vivo.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Thiopurine metabolite levels at baseline (week 0) and 2, 4, 6 and 12 weeks after initiation of adalimumab therapy.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
</tr>
<tr>
<td>6-TGN ^a</td>
<td>206 (64–405)</td>
</tr>
<tr>
<td>Difference compared to week 0 (p-value)</td>
<td>–</td>
</tr>
<tr>
<td>6-MMPR ^a</td>
<td>2123 (300–11,693)</td>
</tr>
<tr>
<td>Difference compared to week 0 (p-value)</td>
<td>–</td>
</tr>
</tbody>
</table>

^a Median (range); 6-TGN and 6-MMPR were not normally distributed; p-value <0.05 was considered as statistically significant by comparison to thiopurine levels of weeks 2, 4, 6 and 12 with baseline (week 0: n = 12); weeks 4, 6 and 12: n = 11.
Recent studies showed that AZA and infliximab combination therapy is more effective than when either is used as monotherapy, at least in naïve patients with moderate-to-severe active CD and UC. In line with this, results from a recent retrospective study may be interpreted that early combination of adalimumab and immunosuppressive therapy may lead to a decrease of flares and a lower risk of adalimumab failure. Combined anti-TNF-α and thiopurine therapy, started shortly after diagnosis, seems to have additional beneficial therapeutic effects in the treatment of active disease, although it is still debated for how long the combination should preferably be continued.

The presented results concerning adalimumab in CD patients treated with AZA or 6-MP are in contrast with the earlier reported effect of infliximab on the pharmacokinetics of AZA. An increase of 6-TGN concentration was shown within 1–3 weeks after the first infusion in CD patients who received one (week 0; n = 17) or three infliximab infusions (weeks 0, 2 and 6; n = 15). Three months after the first infusion 6-TGN concentrations were similar to the concentrations before infliximab treatment in both patient groups. 6-MMPR thiopurine metabolite concentrations were not reported and the pharmacological mechanism underlying this reversible pharmacokinetic interaction remained elusive. Since these patients received only one or three infusions and the follow-up was only the first three months, the clinical relevance of this interaction for the long-term clinical outcome is still unknown. We do not have an adequate explanation why infliximab affects 6-TGN formation and adalimumab does not, at least in our study cohort. However, in almost two-third of our patient group subtherapeutic 6-TGN concentrations were found, which is in contrast with the patients of the study population of Roblin et al. of whom in 90% 6-TGN concentrations higher than 250 pmol/8 × 10⁸ RBC were found before infliximab infusion. Although unlikely, we cannot exclude that addition of adalimumab to patients with higher or therapeutic thiopurine metabolites may lead to an increase of 6-TGN. Differences in anti-TNF-α plasma concentrations during infliximab and adalimumab therapy may also be an explanation, although this should be elucidated in future investigations. In a recent study of 81 patients performed by Teichgräber et al., a trend toward higher 6-TGN concentrations in patients on AZA and infliximab combination therapy did not reach statistical significance compared to patients on AZA monotherapy, which in fact is not in line with the findings of Roblin and colleagues. Interestingly, their results did reveal that infliximab co-treatment resulted in a higher 6-thioguanine triphosphate (6-TGTP) concentration, the 6-thioguanine nucleotide which in particular is considered to contribute to the immunosuppressive effect of thiopurines. In the present study only the sum of the 6-thioguanine nucleotides was assessed, so that the influence of adalimumab on the individual 6-thioguanine monophosphate, 6-thioguanine diphosphate and 6-thioguanine triphosphate formation remains elusive.

Addition of adalimumab to thiopurine monotherapy resulted in a decrease in leukocyte count at week 12 in our patients, which was also described 1–3 weeks after infliximab was added to AZA. Two patients (17%) showed clinically relevant myelotoxicity within 2–4 weeks on combination therapy, which in one patient eventually led to discontinuation of 6-MP and adalimumab, conceivably due to an autoimmune thrombocytopenia.

Myelotoxicity is one of the dose-dependent adverse events of thiopurines, mostly associated with elevated 6-TGN above 490 pmol/8 × 10⁸ RBC. The reported rate of clinically relevant leukocytopenia in IBD patients varies between 2 and 11%, depending on the definition of leukocytopenia. Haematologic adverse events are also frequently reported for adalimumab. Leukocytopenia occurs in more than 10% of patients treated with adalimumab and thrombocytopenia in 1–10%. Since our patients developed myelotoxicity after addition of adalimumab and given the fact that in both patients low therapeutic 6-TGN concentrations were found, it indicates that combination

![Figure 3](image)

**Figure 3** 6-TGN concentration at baseline and 2, 4, 6 and 12 weeks after initiation of concurrent adalimumab therapy.

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**Table 3** Median TPMT, ITPase and HGPRT enzyme activity at baseline and 4 weeks after adalimumab combination therapy.

<table>
<thead>
<tr>
<th></th>
<th>TPMT (μmol 6MMP/(mmol Hb × h))</th>
<th>ITPase (mmol IMP/(mmol Hb × h))</th>
<th>HGPRT (mmol IMP/(mmol Hb × h))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (week 0) (range)</td>
<td>0.54 (0.28–0.78)</td>
<td>3.96 (0.03–6.23)</td>
<td>2.55 (2.25–3.24)</td>
</tr>
<tr>
<td>Week 4 (range)</td>
<td>0.60 (0.33–0.95)</td>
<td>4.73 (0.01–11.3)</td>
<td>2.51 (2.25–3.43)</td>
</tr>
<tr>
<td>Patient number (n)</td>
<td>11</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Difference compared to week 0 (p-value)</td>
<td>p = 0.142</td>
<td>p = 0.142</td>
<td>p = 0.799</td>
</tr>
</tbody>
</table>
therapy may lead to an increased risk of myelotoxicity, which is not attributable to elevated toxic 6-TGN concentrations, but rather to adalimumab. Alternatively, the myelotoxic effect of thiopurines may be potentiated by an additive myelotoxic effect of adalimumab, as has been documented before with concomitant use of mesalazine.17 Obviously, the observed decrease of leukocyte count at week 12 in the whole study population could also be attributed to a decrease of inflammatory disease activity resulting from combination therapy.

Our study has some limitations that should be mentioned. First, our study population of twelve patients is relatively small compared to previous relevant pharmacokinetic interaction studies on thiopurines.15,28,29 Second, there are some missing thiopurine metabolite concentration and enzyme activity data. Because of stability issues, strict storage and the shipping time applied to these blood samples, we had to exclude the samples from analysis, as these were inappropriate for reliable measurement resulting from long shipping time. Third, international IBD guidelines advise a standard daily dose regimen of AZA and 6-MP of 2–2.5 mg/kg and 1–1.5 mg/kg, respectively.10,13 The median daily thiopurine dose in our study was relatively low (1.78 mg AZA/kg and 0.77 mg 6-MP/kg), which suggests suboptimal dosing. Patients treated with AZA or 6-MP who relapse or show poor response should be evaluated for adherence to therapy or under-dosing.6,10 In poorly responding patients with low 6-TGN and low 6-MMPR concentrations due to underdosing, thiopurine dosage should be optimised before considering a change to methotrexate or costly anti-TNF-α therapy.10,13

In conclusion, this prospective study in CD patients does not provide evidence for a pharmacokinetic interaction between adalimumab and the conventional thiopurines AZA and 6-MP. Clinicians should be aware of an increased risk for myelotoxicity when combining these immunosuppressive drugs.

Conflict of interest
None.

Statement of authorship
Specific author contributions are listed below.

DW led the study design and coordination, contributed to acquisition of data, took care of the thiopurine measurements and a part of data analysis and drafted a great part of the manuscript.

MP contributed to acquisition of data, carried out a great part of the data analysis and helped in drafting the manuscript.

MS contributed to acquisition of data and critically revised and helped in drafting the manuscript.

AB contributed to the acquisition of data, helped to interpret the data, provided the main authors with significant advice and revised the manuscript critically for important intellectual content.

LG participated in study design, contributed to acquisition of data and helped in drafting the manuscript.

PB contributed to acquisition of data and helped in drafting the manuscript.

JB participated in study design regarding TPMT, ITPase and HGPRT enzyme assays, took care of the enzyme activity measurements and helped in drafting the manuscript.

AM provided the main authors with significant advice concerning intellectual content and helped in drafting the manuscript.

CN helped in interpreting the data and helped in drafting the manuscript.

LE participated in study design, contributed to acquisition of data, helped in drafting the manuscript and provided the main authors with significant advice concerning intellectual content.

PH led the study design, helped in drafting the manuscript and provided the main authors with significant advice concerning intellectual content.

All authors approved the final version of the manuscript, including the authorship list.

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