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TITLE

RANDOMISED CLINICAL TRIAL: EFFICACY, SAFETY AND DOSAGE OF ADJUNCTIVE ALLOPURINOL IN AZATHIOPRINE/MERCAPTOPURINE NON-RESPONDERS TO OPTIMISE 6-THIOGUANINE NUCLEOTIDE PRODUCTION (AAA Study)

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STATEMENT OF INTERESTS:

1. Dr Antony B. Friedman has received speaker fees from AbbVie, Janssen – Cilag, Shire and Takeda. He has received research funding for investigator-driven studies from AbbVie.
2. Dr Steven J. Brown has received speaker fees from AbbVie and Janssen – Cilag.
3. A/Prof Peter Bampton has served as a served as consultant or advisory board member for AbbVie. He has received research grants for investigator-driven studies from National Health and Medical Research Council Australia and the Beat Cancer Project (South Australia).
4. Prof Murray L. Barclay has no interests to declare.
5. Dr Alvin Chung has no interests to declare.
6. Prof Finlay A. Macrae has served as a served as consultant or advisory board member for Pfizer. He has received research funding from Cancer Australia, NSW Cancer Council (Aus), National Institute of Health (USA), Cancer Research UK, multiple pharmaceutical trials for study coordinator support and the World Cancer Research Fund.
7. Ms Jo McKenzie has no interests to declare.
8. A/Prof John Reynolds has received research funding from AbbVie, Novartis, Celgene, Janssen – Cilag, Bristol – Myers Squibb, Takeda, National Health and Medical Research Council Australia, Victorian Cancer Agency, Leukemia & Lymphoma Society (USA). He owns stocks in Novartis AG.
9. Professor Peter R. Gibson has served as consultant or advisory board member for AbbVie, Ferring, Janssen, Merck Sharp & Dohme, Nestle Health Science, Danone, Allergan, Pfizer, Celgene and

Takeda. His institution has received speaking honoraria from AbbVie, Janssen, Ferring, Takeda, Mylan, Danone and Pfizer. He has received research grants for investigator-driven studies from AbbVie, Janssen, Danone and A2 Milk Company. His Department financially benefits from the sales of a digital application and booklets on the low FODMAP diet. He has published an educational/recipe book on diet.

10. Prof Stephen B. Hanauer has received consulting fees from Prometheus Laboratories Inc prior to 2014.
11. A/Prof Miles P. Sparrow has received speaker fees from Janssen, AbbVie, Ferring, Takeda and Hospira. He has served as consultant or advisory board member for Janssen, Takeda, Pfizer, Celgene, AbbVie and Merck Sharp & Dohme.

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Author Contributions:

1. **Antony B Friedman:** study concept and design; recruitment of patients, acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content and study supervision
2. Dr Steven J. Brown: recruitment of patients, acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content and study supervision
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10. Prof Stephen B. Hanauer: study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content and study supervision
11. A/Prof Miles P. Sparrow: study concept and design; obtained funding, recruitment of patients, acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content and study supervision

ABSTRACT

Background: Thiopurine hypermethylation towards 6-methylmercaptopurine (6MMP) instead of 6-thioguanine nucleotides (6TGN) is associated with inefficacy in patients with inflammatory bowel disease (IBD). Allopurinol reverses such hypermethylation.

Aims: To prospectively determine efficacy of allopurinol-thiopurine combination and to compare two doses of allopurinol.

Design: In a multicentre, double-blind trial, patients with clinically active or steroid-dependent IBD and thiopurine shunting were randomised to 50 or 100 mg/d allopurinol and 25% of their screening thiopurine dose, which was subsequently optimised, aiming for 6TGN of 260–500 pmol/8x10^8RBCs. The primary endpoint was steroid-free clinical remission at 24 weeks.

Results: Of 73 patients, 39 [53%(95% CI 42–65)] achieved steroid-free remission, (54% with 50 mg/d and 53% with 100 mg/d). 81% were able to discontinue steroids. Therapeutic 6TGN levels were achieved in both groups. Final thiopurine doses were lower with 100 mg/d allopurinol ($p<0.005$). 6MMP:6TGN ratio decreased from mean 64 to 4 ($p<0.001$), being higher with 50 mg/d (6±1.83) than for 100 mg/day [(1±0.16), $p=0.003$]. Three patients on 50 mg/d failed to sustain low ratios at 24 weeks. Toxicity was minimal; three patients on 50 mg/d allopurinol developed transient leukopenia. Alanine aminotransferase concentrations decreased ($p<0.001$) similarly in both arms. Faecal calprotectin levels at study end were lower in patients who achieved the primary endpoint [median 171(85-541) vs 821(110-5892) ug/g, $p=0.03$].

Conclusions: Low-dose allopurinol-thiopurine combination safely reverses shunting and optimises 6TGN with associated improvement in disease activity. 100 mg/d allopurinol is preferable due to greater metabolite profile stability and lower thiopurine dose without additional toxicity.

Australian and New Zealand Clinical Trials Registry, http://www.anzctr.org.au (ID:ACTRN12611000363987)

Keywords: thiopurine metabolites, thiopurine hypermethylation, allopurinol AND inflammatory bowel disease

INTRODUCTION

The aims of medical therapy for patients with inflammatory bowel disease (IBD) are to eliminate inflammatory symptoms and heal the bowel to prevent complications and disability.[1-3] Azathioprine and mercaptopurine are thiopurines with well-established, steroid-sparing effects in IBD treatment

algorithms.[4,5] However, up to 60% of patients do not respond to conventional (weight-based) thiopurine doses and up to 40% will experience an adverse event leading to drug cessation,[6-8] such that alternative strategies are required to optimise efficacy and safety.

Thiopurines are inactive pro-drugs. Of the two major metabolites, 6-thioguanine nucleotides (6TGN) are responsible for therapeutic efficacy and risk of myelotoxicity. Levels above 235-260 pmol/8x10^8 red blood cells (RBC) are associated with clinical remissions of IBD. In contrast, methylated metabolites, principally 6-methylmercaptopurine (6MMP), show no correlation with efficacy and are associated with hepatic transaminitis when serum concentrations are above 5,700 pmol/8x10^8 RBCs.[9] Weight-based dosing of thiopurines poorly predicts metabolite levels.[9,10] Up to 20% of patients preferentially metabolise thiopurines to produce high levels of 6MMP and low levels of 6TGN.[11] These so-called "thiopurine hypermethylators" or "shunters" are usually refractory to standard doses of thiopurines.[12]

Combining allopurinol with reduced doses of thiopurines favours production of 6TGN over 6MMP, leading to optimisation of 6TGN concentrations and improved clinical benefits in hypermethylators. However, all data are derived from retrospective case series using a variety of allopurinol and thiopurine dose reduction strategies.[13-19] Both drugs independently carry hypersensitivity and dose-related toxicities. Higher allopurinol doses are associated with increased rates of upper respiratory tract infection, back pain, headache and hypersensitivity reactions.[20-24]

The aims of the AAA study were to determine the efficacy, safety and optimal dosing strategies for combining thiopurines with allopurinol. We therefore conducted the largest prospective, randomised trial of combination thiopurines and allopurinol in thiopurine refractory IBD patients.

METHODS

PROTOCOL

The AAA study was a prospective, multicentre, double-blind, dose-ranging, parallel-group, randomised trial conducted over 24 weeks. Patients with IBD were randomised in a 1:1 fashion to receive a blinded daily dose of either 50 or 100 mg allopurinol in combination with reduced-dose thiopurine via a computer-generated randomisation schedule. Corticosteroids were tapered as per standard of care in both allopurinol groups. Oral or rectal 5-aminosalicylates could not be introduced during the trial. All other IBD medications including maintenance doses of tumour necrosis factor (TNF) antagonists were continued at stable doses. Adherence to study medication was assessed by pill-counts of returned medications. Written informed consent was obtained prior to enrolment. The protocol was approved by ethics committees at each participating site. The study was funded by an unrestricted grant from the Broad Medical Research Program (Los Angeles, USA). The study was registered with Australian and New Zealand Clinical Trials

Registry (Trial ID:ACTRN12611000363987). All authors had access to the study data and reviewed and approved the final manuscript.

STUDY POPULATION

Patients with Crohn's disease or ulcerative colitis were enrolled if aged at least 16 years, taking azathioprine or mercaptopurine for at least the preceding eight weeks and being thiopurine shunters [6TGN levels <260 pmol/8x10^8 RBC and 6MMP >4,500 pmol/8x10^8 RBC or 6MMP:6TGN ratio ≥20 or hepatotoxicity (ALT ≥2x upper limit of normal)]. Patients must have had clinically active disease, comprising Harvey Bradshaw Index (HBI) ≥5 for Crohn's disease, Simple Clinical Colitis Activity Index (SCCAI) ≥3 for ulcerative colitis[25,26], or been corticosteroid-dependent or refractory; and have a white blood cell count ≥3.5x10^9/L. Corticosteroid dependence was defined as patients being unable to reduce steroids below prednisolone 10 mg/day (or budesonide below 3 mg/day) within 3 months of starting steroids, without recurrent active disease.[27] Corticosteroid refractoriness was defined as patients who have active disease despite prednisolone of up to 0.75 mg/kg/day over a period of 4 weeks.[27] Exclusions were suspected or known intolerance to allopurinol, concomitant salvage or new induction therapy (cyclosporine used within 4 weeks from screening visit, TNF antagonists within 8 weeks or any investigational drug within 4 weeks), pregnancy, breastfeeding, TPMT deficiency (TPMT phenotype <0.1 IU/mL), or inability to obtain informed consent.

STUDY MEDICATIONS

Allopurinol was supplied as 100 or 50 mg (half 100mg tablet and inert filler) tablets, each encased in identical capsules (Capsugel Coni-Cap Capsules, Pharmaceutical Packaging Professionals, Port Melbourne, Australia). Patients were commenced at 25% of their screening thiopurine dose when randomised. Thiopurine doses were subsequently modified based on thiopurine metabolite levels measured at weeks 4, 12 and 18 to achieve therapeutic 6TGN levels above 260 pmol/8x10^8 RBCs.

OUTCOME MEASURES

The primary outcome was the proportion of patients in steroid-free clinical remission at 24 weeks in each group. Remission was defined as HBI ≤4 for Crohn's disease and SCCAI ≤2 for ulcerative colitis patients. Patients without a 24-week assessment were deemed not to be in steroid-free clinical remission. Pre-specified secondary endpoints included the proportion of patients in steroid-free remission at 12 weeks, tolerability and safety of allopurinol-thiopurine combination, comparison of thiopurine metabolites, disease activity indices, corticosteroid dosages, leucocyte counts, liver function tests, C-reactive protein (CRP) and faecal calprotectin before and after 24 weeks of allopurinol co-therapy.

STATISTICAL ANALYSES

Assuming that up to 15% of IBD patients achieve steroid-free remission at 24 weeks without intervention[28], a sample size of 34 subjects in each group was selected to allow detection, with 80% power, of a clinically significant change of 20% (i.e., from 15% to 35%) in either group (using a two-sided, one-sample, exact binomial test at a significance level of 0.05). Intention-to-treat and per-protocol analyses were performed, including all patients withdrawn after randomisation. Withdrawn patients were treated as non-responders. Continuous variables were assessed using mixed-model analyses and categorical variables were assessed using chi-squared tests and McNemar's test. For the analyses of repeated measurements of continuous variables, predicted means, standard errors of the means (SEMs) and standard errors of the differences (SEDs) of the means are reported. Where appropriate, p-values for the F-tests of the main effects of group and time, and their two-way interaction, are reported. In the analyses of (binary) responses, patients with missing data for any reason, including those who were lost to follow up, were deemed to be non-responders. Where required, variance-stabilising techniques including log-transformation and square-root transformation were employed in order to conduct conventional parametric tests (t-tests and F-tests); p-values refer to analyses on the transformed scale but means and SEDs are reported on the original scale (in particular for analyses of 6TGN, 6MMP).

RESULTS

PATIENTS

Of 73 patients enrolled (May 2011 to January 2014), 37 were randomised to 50 mg/day allopurinol of whom three withdrew, and 36 patients to 100 mg allopurinol of whom nine withdrew prior to the end of the study (Figure 1). In the entire cohort, there were more female (63%) participants and more with Crohn's disease (63%) than ulcerative colitis (37%). Overall, the groups were well-matched (Table 1), in particular, with respect to thiopurine dosage at randomisation, proportion taking concurrent corticosteroids and the dose used, and proportion receiving TNF antagonists. 39 patients were taking azathioprine and 34 patients received mercaptopurine. Patients had already been on thiopurines for an extended period of time with mean (standard error) time on thiopurines being 108 (20) weeks prior to commencement of allopurinol. At enrolment the thiopurine metabolite profiles of patients were as follows: 63 patients (86%) had 6MMP levels above 4,500 pmol/8x10^8RBCs (range: 4,518 – 31,154 pmol/8x10^8RBCs), all of whom had 6MMP:6TGN ratios of at least 20 (range: 20 – 313). Nine patients (12%) had 6TGN levels less than 260 pmol/8x10^8RBCs (range: 37 – 193 pmol/8x10^8RBCs) with 6MMP:6TGN ratios of at least 21 (range: 21 – 66). One patient had a 6TGN of 220 pmol/8x10^8RBCs, 6MMP of 2701 pmol/8x10^8RBCs and a ratio of 12. His ALT was persistently elevated at 111 U/L and by study end, it had normalised to 32 U/L.

STEROID-FREE CLINICAL REMISSION AT WEEK 24

39 of the 73 patients, 53% [95% CI 41%–65%] achieved the primary endpoint of steroid-free clinical remission after 24 weeks of therapy [54% (37%–71%) in those receiving 50mg allopurinol and 53% (35%–70%) for those receiving 100mg/day] on intention-to-treat analysis. There was no difference between allopurinol groups (p=0.91; Figure 2). When stratified by disease, no differences were seen in the remission rates (Crohn's disease 59% and ulcerative colitis 45%; p=0.24). There were no differences between allopurinol groups when stratified by disease for either Crohn's disease (p=0.77) or ulcerative colitis (p=0.86). On per-protocol analysis, 70% (95% CI: 56% - 81%) of the entire cohort achieved steroid free clinical remission at week 24 (SFR24). In the 50mg allopurinol arm, 67% achieved SFR24 and in the 100mg allopurinol arm 73% achieved SFR24 (p = 0.602 for comparison between groups). When stratified by disease type, 79% of Crohn's disease patients achieved SFR24 compared to only 55% of ulcerative colitis patients (p = 0.049).

CLINICAL OUTCOMES

Across the entire cohort, 37 patients (51%) achieved steroid-free clinical remission after 12 weeks of therapy without differences between allopurinol groups (p=0.91) or disease types (p=0.19). Of the 46 patients with Crohn's disease, mean HBI fell from 7.25 at baseline to 2.06 by week 24 [-5.19(SED=0.46), p<0.001], with no difference in changes over time between allopurinol groups (p=0.27). Of the 27 patients with ulcerative colitis, SCCAI fell from 6.18 at baseline to 2.99 by week 24 [-3.20 (0.64), p<0.001], with no difference between allopurinol groups (p=0.93). The clinical benefit of allopurinol and achievement of clinical remission was seen early in the study. Mean HBI scores for Crohn's disease patients dropped below 4.0 in both groups after only four weeks of treatment. For ulcerative colitis patients, significant reductions were seen as early as week two, with mean SCCAI dropping to 5.4 (p <0.001). These clinical improvements corresponded to significant rises in 6TGN levels at these timepoints.

THIOPURINE METABOLITE CHANGES

Across the entire cohort, mean 6TGN levels increased from a mean 177 (SE±16) at screening to 402±18 pmol/8x10^8RBCs at week 24 [+225(60), p<0.001]. As shown in Figure 3, 6TGN levels rose faster in the 100 mg allopurinol arm than those in the 50 mg arm (p=0.03). By study end, there was no difference in 6TGN levels between allopurinol groups (p=0.72). Across the entire cohort, 6MMP levels decreased from a mean of 9,949 to 1141 pmol/8x10^8RBCs at week 24 [-8,800(2700) p<0.001]. Reductions occurred early (after four weeks) in therapy in both groups (Figure 3) with 6MMP levels dropping to below 500 pmol/8x10^8RBCs. However, as thiopurine doses were escalated through the study to achieve therapeutic 6TGN levels, 6MMP rose significantly in the 50 mg allopurinol arm, so that by week 24, 6MMP levels were higher in the 50 mg compared to 100 mg allopurinol group (1837 and 445 pmol/8x10^8RBCs, respectively, p=0.02). This is also reflected in the changes in 6MMP:6TGN ratios. Across the entire cohort, the mean 6MMP:6TGN ratio fell by

60(5) from 64 at baseline to 4 at week 24 (p<0.001). However differences were seen between allopurinol groups. At week 4, no significant differences in ratios were seen between groups, with the 100mg allopurinol arm having a mean ratio of 1 compared a ratio of 2 in the 50mg allopurinol arm (p = 0.546). However, as per the 6MMP results, as thiopurine doses were escalated, so 6MMP:6TGN ratios rose in the 50 mg allopurinol group, such that by week 24, patients in the 50 mg group had a mean ratio of 5. In contrast, mean 6MMP:6TGN ratios in patients in the 100 mg allopurinol group remained unchanged with a mean of 1 at study end (p<0.001).

Seven (10%) patients during the study "re-shunted" and developed 6MMP levels >5,000 pmol/8x10^8RBCs, six in the 50 mg allopurinol group. This only occurred from week 12 onwards, once thiopurine doses were escalated to achieve therapeutic 6TGN levels. All seven patients were adherent to study medications, as assessed by pill counts. The re-shunting was transient in four patients and resolved by week 24 without a reduction in thiopurine dose. Only three patients remained with elevated 6MMP levels at study end (range 7,081–10,228 pmol/8x10^8RBCs). All three were female, had an elevated ALT at week 24 (range 45–89, normal ≤36 U/L) and were in the 50 mg allopurinol group. Of these three, two still had subtherapeutic 6TGN levels (215 and 223 pmol/8x10^8RBCs) and one had a therapeutic 6TGN of 307 pmol/8x10^8RBCs.

THIOPURINE DOSAGE CHANGES

Final azathioprine doses (0.8mg/kg/day) were on average 36% (SEM=2.50) of original dose (2.2mg/kg/day) in the 100mg allopurinol group compared with 48% (1.0mg/kg/day, SEM=2.85) of original dose (2.2mg/kg/day) in the 50mg allopurinol group (p=0.004). Final mercaptopurine doses (0.4mg/kg/day) were 37% (SEM=7.28) of original dose (1.1mg/kg/day) in the 100 mg allopurinol group compared with 62% (0.7mg/kg/day, SEM=6.09) of original dose (1.1mg/kg/day) in the 50 mg allopurinol group (p=0.01).

INFLAMMATORY BIOMARKER CHANGES

The total white cell count (WCC) decreased from a mean of 7.1(SEM=0.3)x10^9/L at baseline to 5.8(0.3)x10^9/L by week 24 across the entire cohort (p<0.001). No differences were seen between allopurinol groups (p=0.48). Three patients developed a mild leucopenia (lowest count 2.6x10^9/L), but this recovered with thiopurine dose reduction by the end of follow-up. All three were in the 50 mg allopurinol arm. In one patient, leucopenia occurred after 12 weeks of therapy and was ascribed to high 6MMP levels (6MMP = 25,954pmol/8x10^8RBCs, 6TGN = 250pmol/8x10^8RBCs). The second patient developed supra-therapeutic 6TGN levels (6TGN = 604pmol/8x10^8RBCs, 6MMP = 508pmol/8x10^8RBCs) after 24 weeks of treatment. The third patient developed leucopenia after 12 weeks of therapy and had sub-therapeutic 6TGN levels of 196pmol/8x10^8RBCs and low 6MMP levels of 756pmol/8x10^8RBCs. These three patients all

had normal TPMT activity (mean 9.8 IU/mL vs 10.1 for entire cohort) and none developed any complication related to transient leucopenia.

26 IBD patients entered the study with an elevated CRP (>3mg/L). Of these, 8 patients (31%) were able to normalise their CRP by study end. The mean CRP fell by 45% from 11 mg/L to 6mg/L at week 24 (p<0.001). In patients with ulcerative colitis, CRP fell by 41% from 6.1 to 3.6 mg/L at week 24 (p=0.019). In contrast, for patients with Crohn's disease, there was no significant reduction in CRP, with mean levels falling from 4.3 mg/L at baseline to 4.0mg/L by week 24 (p = 0.751).

CHANGES IN FAECAL CALPROTECTIN

42 patients submitted paired samples at both entry and exit. There was no difference in faecal calprotectin levels at baseline between those patients who achieved the primary endpoint of steroid free clinical remission [median 251 (interquartile range: 117–636) ug/g] and those who still had active disease at study end [median 299 (172–924) ug/g, p=0.498]. However, patients who achieved the primary endpoint (steroid-free clinical remission) had significantly lower faecal calprotectin levels at study end [median 171(85-541) ug/g], compared to patients who still had active disease [median 821(110-5892) ug/g, p=0.03]. In addition, 88% (21 of 24) of patients whose faecal calprotectin reduced by study end were able to achieve steroid-free remission by 24 weeks. In contrast, for those who failed the intervention, faecal calprotectin levels tended to rise from a median 299(172–924) ug/g at baseline to 821(110–5892) ug/g by study end (p=0.39). Upon sub-group analysis, steroid-dependent or refractory Crohn's disease patients significantly reduced their faecal calprotectin from a median of 300(138 – 1740) μg/g at baseline to 121 (51 – 278) μg/g at week 24 (p = 0.043). However this reduction was not seen in ulcerative colitis patients (p = 0.195).

CORTICOSTEROID REDUCTIONS

At study entry, 24 (33%) patients were steroid-dependent and this reduced to 4 (5%) by week 24 (p <0.001). Four of the 13 patients randomised to the 50 mg/d allopurinol group remained on prednisolone, but all 11 were able to withdraw from steroids in the 100 mg/d group. Across all 24 patients, total steroid requirements significantly reduced from a mean of 21 (SD 1) mg/d to 4 (1) mg/d at study end (p <0.001). The prednisolone dosage fell in both groups (50 mg/d: mean 18 to 6 mg/d; 100 mg/d: 24 to 0 mg/d; both p <0.001), and there was no difference between the groups.

Of 6 patients (8%) requiring budesonide at study entry, five were randomised to 100 mg/d and 1 to 50 mg/d allopurinol. By week 24, no patient remained on budesonide with mean doses reducing from 7 mg at baseline to 0 mg by week 24 (p <0.001).

All patients who were steroid free by study end (week 24) had been able to cease steroids by week 12 of the study.

BIOCHEMICAL CHANGES

Improvements in liver biochemistry were seen, with mean ALT improving from 52(6) U/L at baseline to 27(6) U/L at study end (p<0.001) with no differences associated with allopurinol dosage. Aspartate aminotransferase (AST) also fell from 30(2) U/L over the course of study to 22(2) U/L (p=0.004). 28 patients began the study with an elevated ALT [16 female (ALT ≥36 U/L), 12 male (ALT ≥52 U/L)]. After 24 weeks, 23 of 26 (88%) achieved a mean reduction of 76 U/L in ALT levels (p<0.001), with normalization in 18 (69%). There were no differences in the reductions in ALT between allopurinol groups [-56U/L in 50 mg allopurinol group and -74 U/L in 100 mg group, p=0.32]

Sixteen patients at study baseline had mildly elevated serum bilirubin concentrations (normal values: male ≤23 µmol/L, female ≤14 µmol/L). In men (n=5), the mean (standard deviation) bilirubin was 31(8) µmol/L. In women (n=11), the bilirubin was 23(10) µmol/L. Of the 10 patients in the 100 mg allopurinol group, 6 had normalised bilirubin by study end (p = 0.031), with mean bilirubin levels tending to fall from 26(10) µmol/L to 15(10) µmol/L (p = 0.071). Furthermore, there was a trend for the serum bilirubin for all patients treated with 100 mg allopurinol to fall, with mean levels reducing by 2(1) µmol/L by study end (p = 0.071). This was in contrast to the patients in the 50 mg allopurinol group, where none of the five patients (0%, p = 0.125) with elevated bilirubin levels [26(10) µmol/L] at baseline normalised by study end [26(7) µmol/L, p = 0.182]. Across all patients in this group, there was a numerical increase in mean bilirubin levels of 1(1) µmol/L (p = 0.538). In comparing the two allopurinol groups, the changes seen in bilirubin levels across the study favour the 100mg allopurinol group (p = 0.069)

TPMT activity (normal range: 7.0 – 14.5 U/mL) was available for analysis in 68 patients at screening and for 54 patients at study end. All five patients who did not have TPMT activity available at baseline had previously been well established on thiopurines and none were leucopenic at enrolment. Interestingly, a significant increase in TPMT activity was seen over the course of the study, mean TPMT activity increasing from 9.73 (SD 0.28) U/mL to 10.60 (0.33) U/mL (p = 0.006). This rise was similar in each of the allopurinol groups (p = 0.727).

SAFETY

A total of 89 adverse events occurred across 132 patient-episodes affecting 42 patients. The most frequently observed adverse events (at least one AE per patient) were: infection (15 vs 9), flare of IBD (8 vs 3), GI upset (5 vs 5) and dermatological condition (5 vs 4) in the 50 vs 100 mg/d arms respectively (Table 4). There were no differences in the number of patients experiencing at least one of the adverse events listed

in Table 4 (p=0.30). 15 serious adverse events requiring hospitalisation occurred in 14 patients (Table 3). Six patients developed a flare of IBD. Four patients developed the following infections: cellulitis, pneumonia, viral gastroenteritis and dental abscess after wisdom tooth extraction. None of these patients were leucopenic at the time of infection. Two patients developed nephrolithiasis and one a pulmonary embolism (after four weeks in the study with underlying active IBD). One patient was admitted twice due to psychological disturbances. No serious adverse events were graded by investigators as being related to combination therapy. One patient randomised to 50mg/d allopurinol developed an urticarial rash and was withdrawn from the study; the rash resolved with allopurinol cessation. Seven additional patients developed adverse events (nausea, vomiting and leucopenia) that were assessed by clinicians as probably due to the combination therapy. All resolved with conservative management and all patients remained in the study.

DISCUSSION

In recent years, the efficacy of thiopurines has been questioned with two, large, randomised controlled trials reporting that rates of steroid-free clinical remission were not improved when utilising conventional doses of thiopurines.[29,30] In contrast, the AAA Study documents clinical outcomes of thiopurines when dosing is individualised according to a patient's thiopurine metabolite results rather than their weight. While there was no placebo control (considered not ethical when the study was devised), it is reasonable to say that the findings of the present prospective study represent both clinical and biochemical efficacy of the addition of low-dose allopurinol to dose-reduced thiopurines, in a cohort of patients who had failed thiopurine monotherapy due to shunting.

Clinical response rates were seen as early as week two and one half of patients achieved the primary endpoint of steroid-free clinical remission in both Crohn's disease and ulcerative colitis. Similar clinical outcomes were associated with both allopurinol groups. We believe this early clinical response may relate to the rapid rise in 6TGN levels seen, rather than it simply being an effect of longer time on thiopurines as on average, patients had been on thiopurines for two years prior to enrolment. However, due to the complexities of thiopurine metabolism, there may be other unidentified factors involved that explain the rapid clinical benefit. Clinical benefit was supported by improvement in objective markers of systemic inflammation (CRP and faecal calprotectin). These findings, combined with marked reduction of steroid requirement, confirmed a reduction in the inflammatory burden of disease for patients. While response rates in the AAA study were lower than in previous retrospective case series, it is important to remember that the endpoints of a randomised clinical trial are far more rigorous and difficult to achieve in comparison.

The study addressed the issue of allopurinol dosage, with the premise that minimising the dose will reduce adverse events. Previous studies have used a variety of allopurinol doses and it has been reported that doses of allopurinol as low as 50 mg/d is sufficient to reverse hypermethylation in adults.[31] Indeed, the low dose of 50 mg/d was sufficient to initially reverse hypermethylation in all of the patients. However, metabolite profiles were less stable in the 50 mg/d group as thiopurine doses were subsequently escalated to achieve a therapeutic 6TGN level. These effects were presumably related. Breakthrough shunting occurred in the 50 mg allopurinol arm with 6MMP levels rising above 5,700 pmol/8x10^8RBCs with 6MMP:6TGN ratios above 20. Furthermore, the degree of change in the 6MMP:6TGN ratio in the 50 mg allopurinol arm was less than that achieved for 100 mg/d. Nonetheless, therapeutic 6TGN were achieved levels in both groups. The final thiopurine dose was lower in the 100 mg allopurinol arm, being approximately one third of the screening thiopurine dose compared with 50% of the initial thiopurine dose in the 50 mg allopurinol arm. The thiopurine dose reductions seen in this study were lower in magnitude than previously reported.[32] These findings indicate that the blockade of 6MMP production is dose-dependent in the range of allopurinol used in the present study. However in this study, thiopurine metabolite profiles with 100 mg allopurinol were superior, refuting previously published research that suggests that 50 mg allopurinol is sufficient to overcome hypermethylation in adults.[31]

Reduction of thiopurine-related adverse events is another benefit of co-therapy.[33] High concentrations of 6MMP are associated with hepatotoxicity [9], and as expected, significant reductions in ALT and AST levels were seen across the entire cohort, especially in patients with elevated transaminases at baseline. Interestingly, differences in response of the serum bilirubin concentrations were significantly different between the 100 and 50 mg/d allopurinol groups with falls being observed in the former. While this observation per se is unlikely to be of any clinical relevance, it provides further weight to the contention that the 100 mg/d dose is preferable due to reduced toxicity, presumably from lower 6MMP concentrations. Altering the dosage of thiopurines with allopurinol co-therapy has additional benefits, such as reducing thiopurine-induced adverse events[8,16,34,35]. However, the present study was not designed to address this specific issue.

The risks of allopurinol–thiopurine co-therapy require special attention.[36] First, leucopenia and subsequent sepsis have been longstanding concerns due to higher 6TGN concentrations when the combination is used without reduction of thiopurine dosage. Clinically-insignificant reductions in leucocyte count were observed in the present study, and only three patients developed a mild transient leucopenia without clinical consequence. All counts recovered with thiopurine dose reduction without complications. Interestingly, all occurred in the 50 mg/d allopurinol group. The first case related to supratherapeutic 6TGN levels. In the second case, leucopenia was related to thiopurine re-shunting and toxic 6MMP levels, consistent with

previous reports, suggesting that leucopenia does not only involve 6TGN.[37] Although infections leading to hospitalisation did occur in four patients, none of these patients were leucopenic.

The second risk is that introducing a new drug can be associated with an array of adverse events and allopurinol is not free from toxicity. In the present study, allopurinol appeared to be well tolerated, the only allopurinol-related adverse event being a mild urticarial rash in one patient on 50 mg/d. While adverse events were not uncommon, they were consistent with outcomes seen in IBD studies. While the adverse events did not seem to be specifically related to the thiopurine–allopurinol combination, given there was no placebo arm, this is not definitive. The majority of adverse events were mild and self-limiting in the setting of close monitoring within the clinical trial. However, due to the relatively short duration of the trial, we are unable to comment on the potential for increased side effects in the long term.

This study has several limitations. The population enrolled is heterogeneous with regards to their IBD and treatment. Due to the inherent funding constraints of investigator initiated research, endoscopy was unable to be included in the study protocol, so the inclusion criteria were based on clinical disease activity indices without any objective confirmation of disease activity prior to study entry. Therefore, outcomes may have been affected by the placebo effect; however, this is mitigated by reductions in objective markers of disease activity such as CRP and faecal calprotectin. While a control group was not included, it would not be possible to blind the placebo group for several reasons. First, because of the need for regular dose adjustments in order to achieve therapeutic 6TGN levels, patients in the placebo arm would have realised when their pill burden was not changed. Secondly, as dose adjustments were predicated on thiopurine metabolite results, it would have been obvious to clinicians which patients were in the placebo arm as their metabolite profile would not have changed and these patients would have remained hypermethylators. Furthermore, it was also considered unethical to randomise known hypermethylators into a placebo group, knowing that these patients will never achieve the full benefits of thiopurines. However, given the magnitude of response seen, along with the reduction in objective markers of disease activity and steroid requirements, we believe this does not compromise the study.

Another limitation is that the AAA study only examines the utility and tolerability of allopurinol in patients already tolerating thiopurines. However, this study was not designed to examine the ability of allopurinol to overcome non-specific thiopurine related adverse events such as nausea, abdominal pain, fever and/or arthralgias. Hence the study gives a representation of allopurinol intolerance but does not examine thiopurine or co-therapy intolerance. In addition, by limiting the use of allopurinol co-therapy only to patients with documented hypermethylation, this may lead to an unnecessary restriction of allopurinol usage in a broader population of patients. With regards to metabolite testing, due to the complexities of commercial metabolite assays and indirect measurements used, significant intra- and interassay variability

occurs with results varying by as much as 10%.[38] Furthermore, metabolite testing measures thiopurine metabolites in red cells, not the target of thiopurine therapy, the leucocyte. Given the limited correlation between leucocyte and red cell metabolite levels, extrapolation of these indirect measurements has potential pitfalls.[39] Additionally, due to the complexities of thiopurine metabolism, involving a number of different enzymes and metabolic products, it is possible that TGNs are not the only metabolite that contributes to the efficacy of thiopurines in IBD, making it potentially erroneous to rely solely on metabolite testing results to guide therapy.[40] Similarly, the sensitivity and specificity of 6MMP levels and adverse events is only modest; a significant proportion of patients with thiopurine-related hepatotoxicity do not have high 6MMP levels. Clinically, large case series demonstrate that thiopurine related-adverse events including hepatotoxicity can be overcome in approximately 70% of patients, including with the use of allopurinol without metabolite testing.[34,35,41]

In conclusion, this prospective study confirms the efficacy, safety and tolerance of allopurinol in combination with reduced-dose thiopurines. Greater stability of thiopurine metabolites without any additional toxicity was observed with the 100 mg/d dose of allopurinol. This advance in clinical pharmacology applied to practice has significant implications for physicians who use thiopurines in the management of patients with inflammatory bowel disease.

Australian and New Zealand Clinical Trials Registry, http://www.anzctr.org.au (ID:ACTRN12611000363987)

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FIGURE LEGENDS

Figure 1. Patient flow (CONSORT diagram)

Figure 2. Proportion of patients achieving steroid-free remission at week 24 for **(a)** the entire cohort and according to allopurinol dose; and **(b)** according to allopurinol dose and IBD subtype. No statistically significant differences were seen between the groups on intention-to-treat analysis.

Figure 3. Comparison of changes of (a) 6TGN concentrations, (b) 6MMP concentrations and (c) 6MMP:6TGN ratios over time between 100 mg/d allopurinol and 50 mg/d allopurinol groups at each time point during the study in patients with Crohn's disease and ulcerative colitis. The results are shown as mean (standard error). No significant differences were seen at study entry and study end. Metabolite concentrations rose faster in the 100 mg allopurinol group compared to the 50 mg allopurinol group. Ratios subsequently rose in the 50 mg, but not in the 100 mg allopurinol group.

Figure 1.

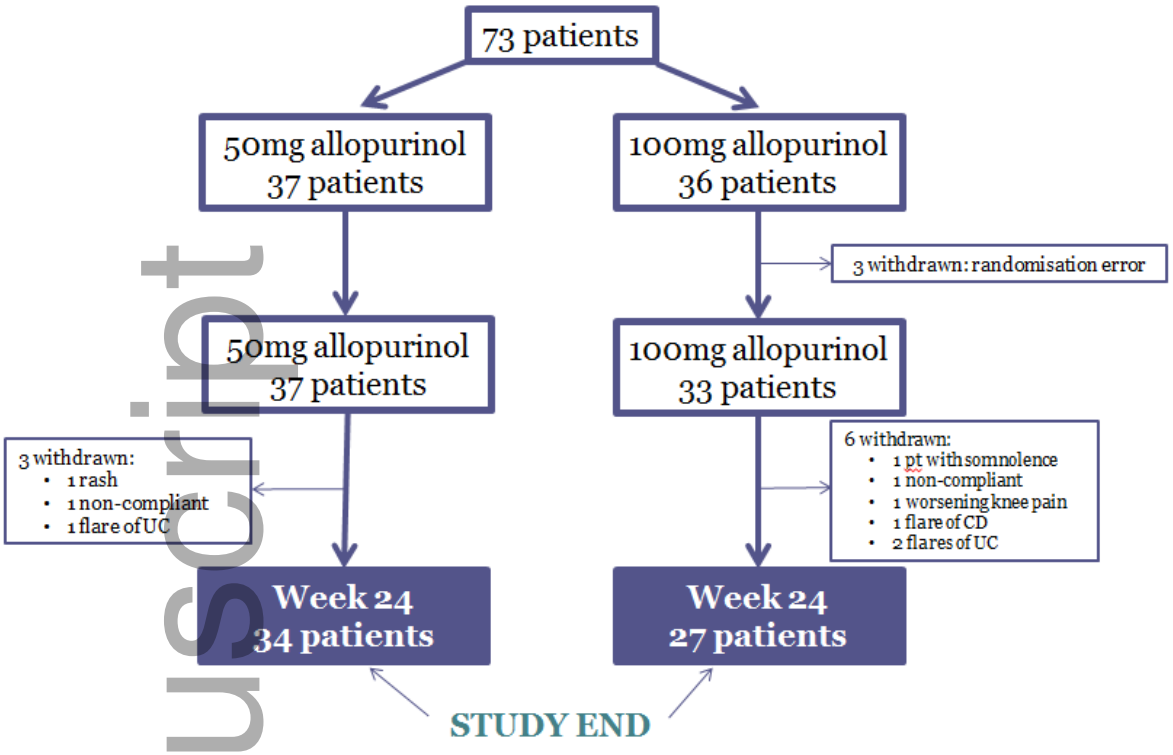


Figure 2a.

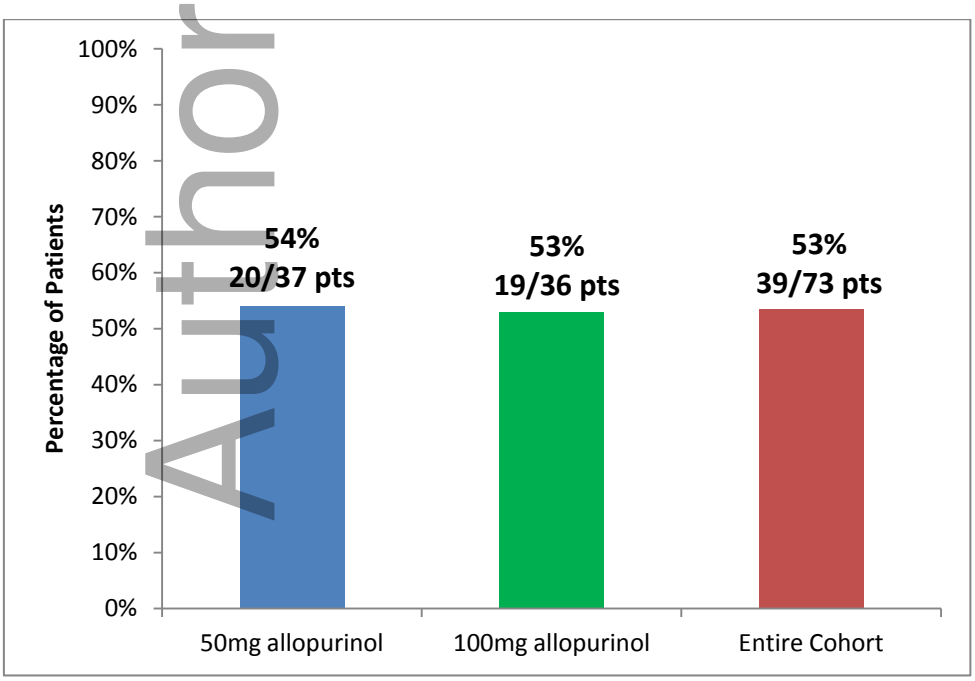


Figure 2b.

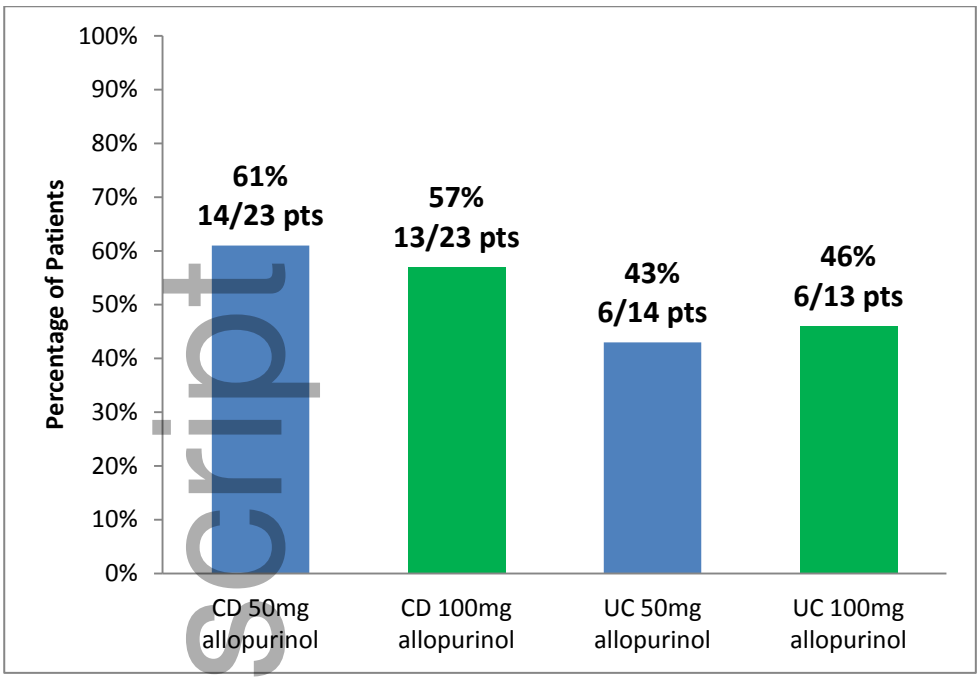
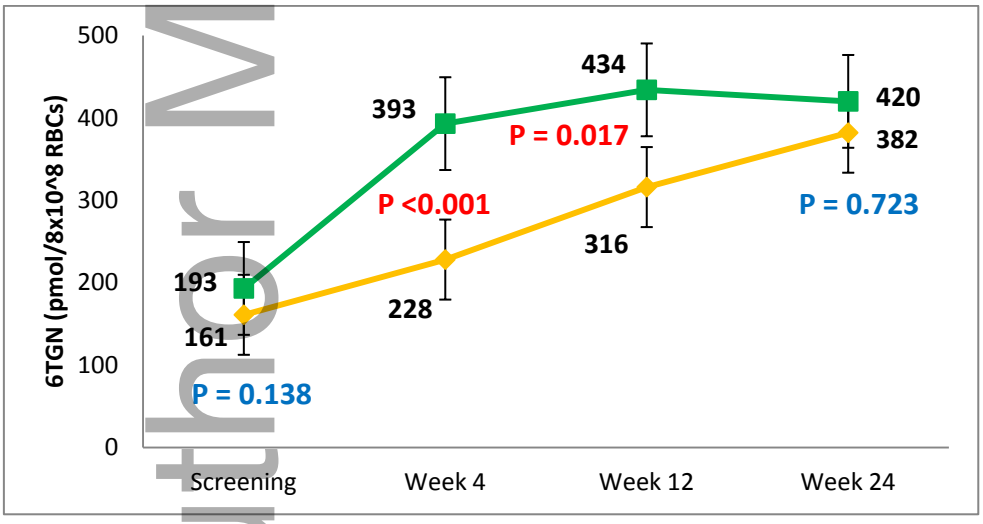
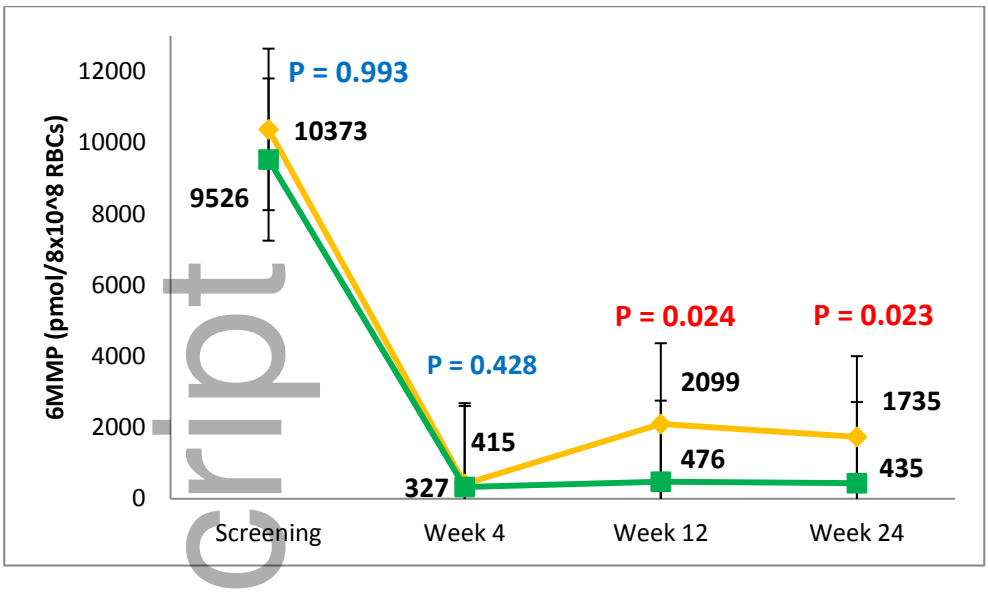
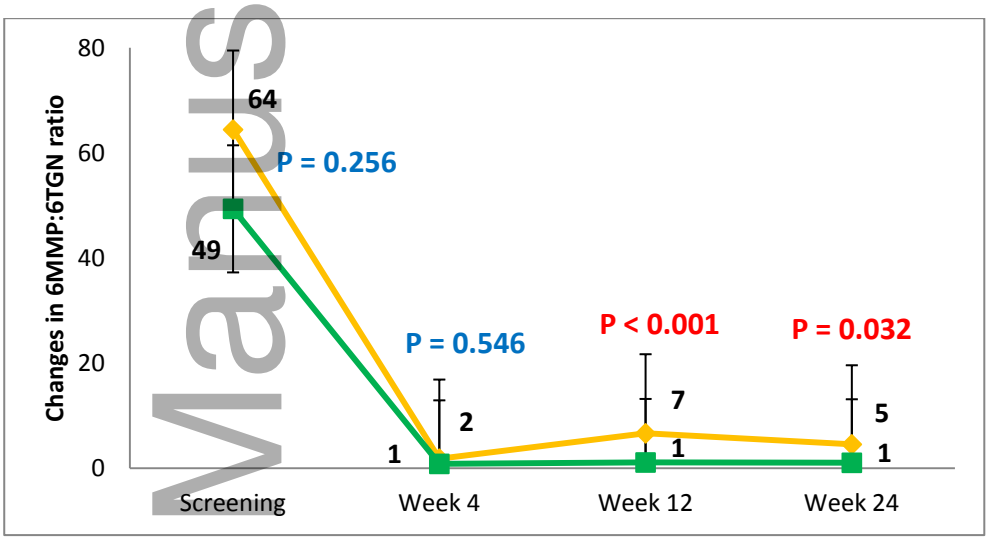


Figure 3.







50mg allopurinol
100mg allopurinol

Table 1. Baseline demographics of study participants according to Montreal Classification.

Baseline Characteristics	**50 mg Allopurinol**	**100 mg Allopurinol**	**P-value**
Total enrolment	37 patients	36 patients	
Gender			0.034[a]
Female	22 (59%)	24 (67%)	
Male	15 (41%)	12 (33%)	0.524[b]
Ethnicity			0.800
Caucasian	31 (84%)	30 (83%)	
Asian	1 (3%)	2	
Other	5 (13%)	4	
Mean body mass index	27.6 kg/m^2	27.1 kg/m^2	0.719

Smoking Status			0.897
Never smoked	22 (60%)	20 (55%)	
Previous smoker	9 (24%)	10 (28%)	
Current smoker	6 (16%)	6 (17%)	
Crohn's disease (phenotype)	23 (62%)	23 (64%)	0.034
Inflammatory	14 (61%)	12 (52%)	
Stricturing	7 (30%)	3 (13%)	
Penetrating	2 (9%)	8 (35%)	0.069
Crohn's disease mean age at diagnosis (range)	28.0 (9 – 62)	28.4 (6 – 54)	0.898
Crohn's disease mean years since diagnosis (range)	8.0 (0 – 32)	13.9 (0 – 47)	0.085
Mean HBI (SE)	6.7 ± 0.4	7.8 ± 0.4	0.437
Ulcerative colitis (disease extent)	14 (38%)	13 (36%)	0.465
Extensive	3 (21%)	5 (38%)	
Left sided	8 (57%)	7 (54%)	
Proctitis	3 (21%)	1 (8%)	
ulcerative colitis mean age at diagnosis (range)	35.7 (17 – 64)	29.5 (4 – 55)	0.292
ulcerative colitis mean years since diagnosis (range)	6.3 (0 – 27)	6.3 (0 – 18)	0.993
Mean SCCAI (SE)	6.3 ± 0.5	6.1 ± 0.5	0.802

[a]entire cohort; [b]group differences

Table 2. Baseline medications of study participants.

Medications	**50 mg Allopurinol**	**100 mg Allopurinol**	**P-value**
Steroids	14 (38%)	17 (47%)	
Prednisolone	13 (35%)	12 (34%)	0.940
Budesonide	1 (3%)	5 (14%)	0.082
TNFα antagonists	5 (14%)	3 (8%)	
Infliximab	3 (8%)	3 (8%)	0.972
Adalimumab	2 (5%)	0 (0%)	0.157
Prior TNFα antagonist exposure	10 (27%)	8 (22%)	0.634
Aminosalicylates	16 (43%)	17 (47%)	
Sulfasalazine	4 (11%)	6 (17%)	0.467

Mesalazine	12 (32%)	11 (31%)	0.863
Antibiotics			
Metronidazole	1 (3%)	1 (3%)	0.984
Ciprofloxacin	0 (0%)	1 (3%)	0.307

Table 3. Serious Adverse Events. No statistically significant differences were observed between the groups

Type of Serious Adverse Event	**50 mg Allopurinol**		**100 mg Allopurinol**	
	Patients	**Episodes**	**Patients**	**Episodes**
Flare of IBD	1	1	5	5
Infection	4	4	0	0
Nephrolithiasis	0	0	2	2
Psychological disturbance	1	2	0	0
Pulmonary Embolism	1	1	0	0
Total	**7**	**8**	**7**	**7**

Table 4. Number and nature of the adverse events. No statistically significant differences were observed between the groups.

Type of Adverse Event	**Allopurinol 50 mg/d**		**Allopurinol 100 mg/d**	
	Patients	**Episodes**	**Patients**	**Episodes**
Flare of IBD	8	13	3	4
Infection	15	22	9	16
Nausea	4	5	1	4
Gastrointestinal upset	5	12	5	8
Dermatological condition	5	5	4	8
Systemic symptoms (fatigue, sweats, hot flushes)	1	1	3	5
Musculoskeletal injury	1	1	3	4
Haematological disorder (pulmonary embolism, elevated international normalised ratio)	1	1	1	3
Psychological disturbance	0	0	3	4
Elective non-IBD surgery	0	0	3	3

Non-study drug allergic reaction	2	3	0	0
Serious infection (methicillin resistant staphylococcus aureus, pneumonia, post wisdom tooth abscess)	2	3	0	0
Myelosuppression (leucopenia)	1	1	1	1
Neurological disturbance (headache, vertigo)	1	1	1	1
Nephrolithiasis	2	2	0	0
Minor rectal bleeding	1	1	1	1
Hepatobiliary disorder (primary sclerosing cholangitis)	0	0	1	1
Endocrine disorder (goitre)	1	1	0	0
Total	**50**	**72**	**39**	**63**