A pragmatic randomized controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study

Aim: To conduct a pragmatic, randomized controlled trial to assess whether thiopurine methyltransferase (TPMT) genotyping prior to azathioprine reduces adverse drug reactions (ADRs). Methods: A total of 333 participants were randomized 1:1 to undergo TPMT genotyping prior to azathioprine or to commence treatment without genotyping. Results: There was no difference in the primary outcome of stopping azathioprine due to an adverse reaction (ADR, p = 0.59) between the two study arms. ADRs were more common in older patients (p = 0.01). There was no increase in stopping azathioprine due to ADRs in TPMT heterozygotes compared with wild-type individuals. The single individual with TPMT variant homozygosity experienced severe neutropenia. Conclusion: Our work supports the strong evidence that individuals with TPMT variant homozygosity are at high risk of severe neutropenia, whereas TPMT heterozygotes are not at increased risk of ADRs at standard doses of azathioprine.

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KEYWORDS: azathioprine   pharmacogenetics   randomized controlled trial   thiopurine methyltransferase   TPMT

Adoption of pharmacogenetic tests into clinical practice has been limited for a number of reasons [1], including a lack of robust evidence and the need for randomized controlled trials (RCT) to demonstrate clinical utility [2].

Azathioprine is a thiopurine immuno-suppressant widely used for the treatment of a range of inflammatory disorders, including inflammatory bowel disease. Its use is limited by a range of common side effects, including nausea, vomiting and rashes, which leads to discontinuation of therapy in up to a third of patients [3]. Bone marrow suppression and especially neutropenia, is an important adverse reaction (ADR) [4]. Individuals deficient in thiopurine methyltransferase (TPMT), an important rate-limiting enzyme in the conversion of azathioprine to its active metabolite, 6-thioguanine nucleotide (6-TGN), are at high risk of early-onset profound neutropenia [5,6]. Approximately one in 300 individuals have a complete deficiency of this enzyme activity [5,7]. In the majority of cases, across all ethnic groups, enzyme deficiency can be accounted for by carriage of two copies of the common variant alleles termed TPMT*2, TPMT*3A and TPMT*3A [8]. A number of case reports [9,10] and retrospective series [6,11–13] of patients treated with azathioprine supported an association between low TPMT activity and profound, early-onset neutropenia. This led to recommendations for the adoption of either genetic or enzyme testing prior to azathioprine use to reduce the incidence of neutropenia [14], relabeling of the drug package insert in the USA, advice in the British National Formulary and the approval of a diagnostic test by the US FDA in 2004 [15]. However, testing for TPMT status has limitations in that it does not predict all individuals at risk of thiopurine-induced neutropenia [11] and only has a weak association with the other side effects associated with this medication. In addition, 10% of individuals have intermediate TPMT activity [5,7], due to heterozygosity for a deficient allele. This reduces enzyme activity by 50% and reports have emerged regarding both the increased rate of ADRs in this group [16,17] and a potential increase in drug efficacy due to increased conversion to 6-TGNs [18]. Adoption of clinical TPMT testing by 2005 in the UK was relatively sparse [19] and clinical practice guidelines did not recommend clinicians to use it. Notably, the British Society of Gastroenterologists stated: “It [TPMT testing] cannot yet be recommended as a prerequisite to therapy, because decades of experience has shown clinical aza( thioprine) to be safe in ulcerative colitis or Crohn’s disease” [20].

Importantly, no large RCT has established the clinical utility of TPMT testing. Therefore, we designed and undertook a pragmatic RCT, aiming to reflect real-life clinical practice in patients with a range of inflammatory diseases, to determine if TPMT genotyping could reduce the incidence of ADRs.


*Author for correspondence: Department of Medical Genetics, Manchester Academic Health Science Centre (MAHSC), St Mary’s Hospital, University of Manchester, Manchester M13 0JH, UK Tel.: +44 161 276 6264 Fax: +44 161 276 6345 willian.newman@manchester.ac.uk
†Authors contributed equally
For a full list of affiliations please see the back page
Methods & patients

■ Design overview
This was a pragmatic RCT (ISRCTN30748308) to establish if the use of TPMT genotyping would reduce ADRs associated with azathioprine. The study was approved by the ethical review committee (05/MRE12/5) and each patient provided written informed consent.

■ Setting & participants
Patients were recruited from clinics at 19 participating study centres, predominantly based in the North West of England (Supplementary Table 1, www.futuremedicine.com/doi/suppl/10.2217/pgs.11.32). Patients with inflammatory disease aged ≥16 years, being considered for azathioprine treatment were eligible for the study (Supplementary Table 2). Patients were excluded if they had previously stopped azathioprine because of pancreatitis or severe neutropenia (neutrophil count <1 x 10^7/l), had a current neutrophil count of <1 x 10^7/l, impaired liver function (alanine transaminase > twice the upper limit of normal), or impaired renal function (glomerular filtration rate <20 ml/min) at baseline. Patients who were pregnant or breastfeeding, had a hypersensitivity to azathioprine, or who had previously had TPMT enzyme testing were also excluded. Patients with a coprescription of allopurinol were excluded due to the increased risk of thiopurine toxicity through xanthine oxidase inhibition. The first patient was recruited on 18/10/2005 and the final recruit on 31/12/2007.

■ Randomization & interventions
Study patients were randomized 1:1 to receive standard care based on individual clinician practice with routine hematological and biochemical monitoring (nongenotyping arm) or to undergo TPMT genotyping.

Blood samples were collected from all patients at recruitment. Following trial entry and baseline assessment, patients were allocated randomly to the genotyping or nongenotyping arms on receipt of the pretreatment blood sample at the genetics laboratory and the treating clinician being informed. Randomization was stratified by study center and speciality, using computer-generated lists with a variable block-size prepared by the study statistician. The laboratory staff had no knowledge of patient status except for name, date of birth and referring clinician and had no involvement with patient recruitment or treatment. Individuals allocated to the genotyping arm had TPMT genetic testing undertaken. Results were generated within 1 week and the referring clinician was informed of the result prior to the commencement of azathioprine. In the nongenotyping arm, the blood sample was stored and TPMT genotyping was undertaken at completion of the study. Study clinicians were not blinded to the status of the participants, as the study was reliant on the study physician prescribing azathioprine in light of the TPMT test result in the genotyped arm.

Genotyping was performed by Taqman® Drug Metabolism Genotyping Assays (C_12091552_30, C_30634116_20 and C_19567_20, for TPMT*2 and TPMT*3(A, B and C) alleles (Applied Biosystems, Warrington, UK) in an accredited clinical laboratory. The variant allele TPMT*2 is defined by a single missense change c.238G>C (p.Ala80Pro, rs1800462). TPMT*3A is defined by a haplotype of two SNPs (c.460G>A, rs1800460 and c.719A>G, rs1142345) resulting in nonsynonymous changes p.Ala154Thr and p.Tyr240Cys, respectively, whereas TPMT*3C is characterized by c.719A>G, without the c.460G>A variant. Individuals were defined as wild-type if they did not possess one or more of these three variant alleles. For quality assurance, assays were run with previously sequenced positive controls and results were confirmed retrospectively with 100% concordance by pyrosequencing (Qiagen) and iPLEX (Sequenom) genotyping techniques. With the clinical report, recruiting clinicians were given three forms of advice: for individuals with a wild-type TPMT genotype, they were advised to start a maintenance dose of azathioprine (i.e., 1.5–3 mg/kg/day); for individuals with a heterozygous TPMT genotype, they were advised to start azathioprine at a low dose (i.e., 25–50 mg/day) and titrate to the maintenance dose; for individuals homozygous for TPMT variant alleles, they were advised not to start azathioprine, but to use an alternative treatment. However, all final treatment decisions were at the discretion of the treating clinicians.

Phenotype was determined by measuring red blood cell TPMT activity measurements using a modified version of the liquid chromatography-fluorescence assay [21]. Briefly, TPMT metabolism of the substrate 6-thioguanine was quantified by measuring the rate of formation of the fluorescence metabolite 6-methylthioguanine, expressed relative to red blood cell lysate hemoglobin content.

■ Outcomes & follow-up
Baseline clinical data were collected at enrolment. Standardized outcome data were collected at 4 months from multiple sources including case
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notes, patient diaries and case report forms. Case note review was undertaken at 12 months on all patients still taking azathioprine at 4 months to establish later onset ADRs. ADRs were reported by patients, treating physician and verified by the trial manager, using laboratory test results, where appropriate. Laboratory monitoring tests, including full blood counts and liver function tests, were undertaken according to local guidance. Hepatotoxicity was defined as alanine transaminase ≥ two times upper limit of normal range; neutropenia (severe <1.0 × 10^9/l and moderate 1.0–1.5 × 10^9/l) and pancreatitis as serum amylase ≥ two times upper limit of normal range.

In addition, at 4-month review, a blood sample was assayed for TPMT activity and the azathioprine metabolites, 6-TGNs and 6-methylmercaptopurines. Metabolites were assayed using an adapted liquid chromatography–UV assay [22]. In the Crohn’s disease patients, the Harvey–Bradshaw index (HBI) was used to assess disease activity at recruitment and at 4 months follow-up [24].

Statistical analysis
Analyses were conducted on an intention-to-treat basis. The primary end point was initially defined as severe hematological ADRs that required the dose of azathioprine to be reduced, or the treatment stopped, in the first 4 months of therapy. The study was designed to have 80% power, (two-sided 5% significance level) to detect a change in the incidence of the primary endpoint from 14 to 8%, based on the available literature at the time [24]. This required 500 patients in each arm. A preplanned, blinded, interim review of event rates in the first 100 patients established that rates of neutropenia were considerably lower than initially predicted (1.3%). A revised primary end point was agreed by the independent steering committee and defined as stopping azathioprine due to any ADR in the first 4 months of treatment. This was based on evidence that individuals with reduced TPMT activity were at increased risk of ADRs requiring treatment cessation [30]. The study was resized to have 80% power to detect a 40% reduction in stopping azathioprine due to occurrence of an ADR (the revised primary end point – a composite of the original primary and a secondary end point) with a total of 330 patients. Predefined secondary end points included moderate neutropenia (neutrophil count 1–1.5 × 10^9/l) in the first 4 months of azathioprine treatment, and drug efficacy. The odds ratios (ORs) for overall ADR rates were estimated with mixed logistic regression models adjusting for specialty and age as fixed effects and center as a random effect as prespecified. As the number of events for individual ADRs was small, these were compared using unadjusted Fisher’s exact tests. Dose levels were compared between trial arms and genotypes using Mann–Whitney U tests.

Results
The trial profile is illustrated in Figure 1. In total, 336 subjects were recruited, of whom three were excluded (duplicate sample, age <16 years, coprescription of allopurinol). The baseline characteristics of the study patients are summarized in Table 1. The demographic profiles and baseline clinical characteristics of the genotyping and nongenotyping arms were similar. There were no differences in TPMT variant genotype frequencies between the two study arms. The overall TPMT variant genotype frequencies were one homozygote in 333 individuals [0.3, 95% confidence interval [CI]: 0.02–1.7 and 34 heterozygotes [10.2, 95% CI: 7.4–13.9], similar to those reported previously in the UK [7]. No rare TPMT variant alleles were identified in any patient by a screen of all previously reported TPMT variants. As the trial was pragmatic in nature, we were not prescriptive about the type or frequency of blood monitoring in the two study arms. We observed a modest, but nonsignificant, excess of blood draws (including full blood count, electrolytes and liver function tests) in the nongenotyping arm of 6.7 versus 5.8 mean blood draws in the genotyping arm (p = 0.26, Mann–Whitney U test).

TPMT genotyping does not reduce azathioprine-related ADRs
At 4 months, of the 333 eligible individuals recruited 322 had provided outcome data (Figure 1). Of the 322 individuals, 91 (28.3%) patients had stopped azathioprine due to an ADR. In addition, four individuals had died, none due to azathioprine-related toxicity (Supplementary Table 3); five had stopped due to inefficacy and 13 had never started azathioprine. With regard to the primary end point, there was a trend to stopping azathioprine with increasing age due to ADRs (OR: 1.3; 95% CI: 1.1–1.5 per decade; p = 0.01) (Supplementary Figure 1), but there was no significant association between speciality (OR: 0.49; 95% CI: 0.20–1.2; p = 0.12) or gender (OR: 0.98; 95% CI: 0.60–1.6; p = 0.95) and the number of ADRs within 4 months.

There was no difference in the frequency of stopping azathioprine due to an ADR at 4 months in the two study arms (genotyping 47/163
[28.8%] vs nongenotyping 44/159 [27.7%]; OR: 1.1 [0.66–1.8]; adj p = 0.74). Furthermore, there was no difference in the likelihood of stopping azathioprine between TPMT wild-type individuals (81/287, 28.2%) or heterozygotes (9/34, 26.5%) for TPMT variants (OR: 0.95; 95% CI: 0.41–2.2; p = 0.90) (Supplementary Table 4).

The frequencies of azathioprine-related ADRs in the two study arms are summarized in Table 2. In total, 116 out of 322 (36.0%) patients experienced one or more ADR in the first 4 months of follow-up. Hepatotoxicity was more common in the genotyping arm, although this difference would not be considered significant after allowing for the number of ADRs tested, and the numbers are small, so such comparisons have limited statistical power. The one individual in the nongenotyped arm who was homozygous for TPMT variant alleles experienced severe, early-onset nonfatal neutropenia after azathioprine treatment. Two other cases of moderate neutropenia (1–1.5 × 10^9/l) occurred in patients with wild-type TPMT in the genotyped arm. These patients had no defined cause for neutropenia, which occurred at 75 and 91 days, respectively.

Case note review was undertaken at 12 months in the 181 patients who were still taking azathioprine at 4 months. Six cases of moderate neutropenia were recorded. Of these, one individual was a TPMT heterozygote and the others were TPMT wild-type. No obvious precipitants for the neutropenia were recorded and there were no striking differences in the concomitant medications in the two study arms (Supplementary Table 5).

Effect of TPMT genotyping on the starting & maintenance dose of azathioprine

The trial was pragmatic in design and the only intervention was the TPMT genotype result and associated advice regarding appropriate azathioprine prescription. Therefore, we attempted to establish whether TPMT genotyping altered
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In the nongenotyping arm, when clinicians did not have the patients’ TPMT status, the average starting dose was similar between patients wild-type or heterozygous for TPMT variants (Table 3). In the genotyping arm, when the TPMT status was available, the average starting dose of azathioprine was lower in the TPMT heterozygotes than wild-type individuals (p = 0.007). This indicated that, overall, the clinicians were initiating treatment at a lower dose for TPMT heterozygotes as advised by the genotype result. However, the average starting dose in the TPMT wild-type individuals was similar in the two arms (Table 3). At 4 months, the mean azathioprine dose across both arms was 1.68 mg/kg/day. There was no difference in dose at 4 months between the two study arms (p = 0.25) or between individuals heterozygous or wild-type for variant TPMT alleles (p = 0.98). Furthermore, the prescribing dose patterns of azathioprine between initiation and at 4 months were not different between the two arms.

TPMT genotyping does not improve azathioprine efficacy

At baseline, there was no difference in HBI scores between the Crohn’s disease patients in the two arms. The mean HBI of >5 in both study arms at recruitment indicated active disease [23]. At 4 months, despite only two-thirds of patients continuing with azathioprine, there was a modest improvement in clinical symptoms in both the study arms (mean HBI of <5), with no significant differences between the arms (Table 4). At baseline, 52 out of 113 CD patients had a HBI of <5, whereas at 4 months of azathioprine treatment, 75 out of 112 CD patients had a HBI <5 (p = 0.002).

TPMT genotype-phenotype correlations

All individuals heterozygous or homozygous for TPMT variant alleles had either intermediate or negligible TPMT enzyme activity, respectively. All individuals wild-type for the three tested TPMT variant alleles had normal/high TPMT activity, except for two with borderline intermediate levels. DNA sequencing of the entire TPMT open reading frame did not detect a variation in these two individuals that would explain their lower enzyme levels (Figure 2).

In 13 (3.9%) of the patients, there had been a blood transfusion in the 3 months prior to recruitment to the study (Supplementary Table 6). There was no discordance between the TPMT genotype and enzyme activity in these individuals.

| Table 1. Patient demographic and baseline clinical characteristics by study arm. |
|---------------------------------------------|---------------------------------------------|
| n (%) or mean (SD) | Nongenotyping (n = 166) | Genotyping (n = 167) |
| Specialty | Gastroenterology | 145 (87.3) | 149 (89.2) |
| | Rheumatology | 21 (12.7) | 18 (10.8) |
| Age | Years | 43.2 (17.0) | 41.0 (16.2) |
| Gender | Female | 84 (50.6) | 84 (50.3) |
| | Male | 82 (49.4) | 83 (49.7) |
| Ethnicity | White | 153 (92.2) | 150 (89.8) |
| | South Asian | 8 (4.8) | 12 (7.2) |
| | Black | 1 (0.6) | 5 (3.0) |
| | Mixed/other | 4 (2.4) | 0 (0.0) |
| Weight† | kg | 72.2 (15.4) | 75.9 (17.0) |
| Diagnosis | IBD | 142 (85.5) | 141 (84.4) |
| Thiopurine methyltransferase genotype | Wild-type | 150 (90.4) | 148 (88.6) |
| | Heterozygous | 15 (9.0) | 19 (11.4) |
| | Homozygous variant | 1 (0.6) | 0 (0.0) |

*Two missing, one per arm.
IBD: Inflammatory bowel disease; SD: Standard deviation.

6-thioguanine nucleotide and 6-methylmercaptopurines metabolite levels at 4 months indicated that eight out of 129 (6%) patients, who were still recorded as on azathioprine and on

| Table 2. Frequency of adverse reactions within 4 months by study arm and TMPT genotype in patients with 4 months follow-up. |
|---------------------------------------------|---------------------------------------------|
| Adverse reactions | Nongenotyping (n = 159) | Genotyping (n = 163) |
| | TPMT Wt | TPMT variant | TPMT Wt | TPMT variant |
| Adverse reactions before 4 months leading to withdrawal of AZA | 41 | 3’ | 40 | 7 |
| Any adverse reaction before 4 months | 48 | 3’ | 57 | 8 |
| Severe neutropenia (<1 × 10⁹/l) | 0 | 1’ | 0 | 0 |
| Moderate neutropenia (1–1.5 × 10⁹/l) | 0 | 0 | 2 | 0 |
| Pancreatitis | 4 | 0 | 0 | 1 |
| Hepatotoxicity | 8 | 0 | 19 | 0 |
| Rash | 3 | 0 | 4 | 2 |
| Oral ulcers | 2 | 0 | 4 | 0 |
| Bruising | 1 | 0 | 3 | 1 |
| Sore throat | 0 | 1 | 5 | 0 |
| Nausea and vomiting | 24 | 2 | 24 | 1 |
| Diarrhea | 3 | 0 | 7 | 1 |
| Malaise | 8 | 1 | 14 | 0 |
| Myalgia | 7 | 0 | 13 | 2 |
| Other | 20 | 2 | 22 | 5 |

In total, 116 of 322 individuals experienced adverse reactions with some patients experiencing multiple adverse reactions.
*One individual was a TPMT variant homozygote.
AZA: Azathioprine; TPMT: Thiopurine methyltransferase; Wt: Wild-type.
Table 3. Doses of azathioprine by study arm and TMPT genotype.

<table>
<thead>
<tr>
<th>Azathioprine doses mean (SD) [n]</th>
<th>Nongenotyping (n = 166)</th>
<th>Genotyping (n = 167)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt</td>
<td>Het</td>
</tr>
<tr>
<td>Starting dose mg/kg/day*</td>
<td>0.86 (0.53) [141]</td>
<td>0.93 (0.64) [15]</td>
</tr>
<tr>
<td>Dose at 4 months mg/kg/day‡</td>
<td>1.74 (0.50) [78]</td>
<td>1.62 (0.56) [10]</td>
</tr>
</tbody>
</table>

*The single TPMT variant homozygote started on a dose of 0.6 mg/kg/day.
‡A total of 13 never started azathioprine and three withdrew early and no dose information was available.

# Discussion

We present the findings of the first large randomized controlled pharmacogenetics study conducted to establish the clinical utility of TPMT testing prior to azathioprine dosing. Despite only one patient in this study being deficient for TPMT, consistent with retrospective series and case reports of TPMT deficient patients, our trial reinforces that such individuals are at a significantly increased risk of azathioprine-induced profound neutropenia and azathioprine should therefore be avoided or used very cautiously in this patient group. However, contrary to other studies, our report provides no evidence to indicate that TPMT heterozygotes (i.e., individuals with intermediate enzyme activity) are at increased risk of neutropenia. This difference may be explained by a later onset of neutropenia in TPMT heterozygotes, beyond the 4-month follow-up cut-off used in our study. However, analysis in the extended follow-up group did not indicate an association between TPMT heterozygosity and neutropenia. Likewise, our study does not indicate that TPMT heterozygotes are at increased risk of stopping azathioprine due to ADRs, contrasting with a prospective study, which found that 79% of TPMT heterozygotes had stopped azathioprine at 6 months compared with 35% with wild-type TPMT. Again, this difference, may in part, be explained by the difference in the length of study follow-up and the set dose of 2 mg/kg/day for all patients recruited to the previous study. However, our study indicates that in clinical practice lower doses of azathioprine are used than recommended and in this situation TPMT testing did not predict the two patients who experienced moderate neutropenia within 4 months or in the six patients who experienced neutropenia by 12-month follow-up. These findings are consistent with Colombel et al., in that azathioprine-related neutropenia can have multiple causes, but contrasts with results in children with acute lymphoblastic leukemia where toxicity to mercaptopurine increases with time in individuals with intermediate TPMT activity. Therefore, our study does not support a reduction in postprescription full blood count monitoring in patients on azathioprine. Over the past 5 years there has been an enormous increase in TPMT enzyme testing in UK clinical practice, from 1–2000 tests per year in 2003 to >50,000 in 2008. This increase reflects increased availability of testing; increased knowledge and a change in clinical guidelines, which now recommend testing. However, the basis upon which these recommendations were made was from “evidence obtained from expert committee reports or opinions, and/or clinical experience of respected authorities … [with] an absence of directly applicable studies of good quality”. Therefore, there was a need for a trial to establish the role of TPMT testing. However, the increased uptake of TPMT testing coincided with the conduct of this trial and severely impacted on recruitment, as some centers felt that allocation to not undertake TPMT testing would be against professional guidance. Our study is considerably larger than the two previous RCTs of 29 and 63 patients, which have

Table 4. Harvey–Bradshaw index at baseline (n = 113) and 4 months (n = 112) in patients with Crohn’s disease.

<table>
<thead>
<tr>
<th>Harvey–Bradshaw index mean (SD)</th>
<th>Nongenotyping (n = 56)</th>
<th>Genotyping (n = 54)</th>
<th>p (Wilcoxon test between arms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At baseline</td>
<td>5.8 (4.5)</td>
<td>6.2 (4.9)</td>
<td>0.87</td>
</tr>
<tr>
<td>At 4 months</td>
<td>4.5 (5.9)</td>
<td>4.5 (5.6)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

SD: Standard deviation.
considered TPMT testing prior to azathioprine treatment [37,38]. Neither study was powered to establish the role of TPMT in predicting toxicity.

The increased likelihood of ADRs in elderly patients has been well documented [39]. However, we believe our study is the first formal demonstration of this for azathioprine and indicates that clinicians should be especially vigilant in monitoring elderly patients on azathioprine.

Our study does not demonstrate any advantage or disadvantage of TPMT genotyping compared with phenotyping. However, importantly, nearly 4% of patients had a blood transfusion in the 3 months prior to recruitment. Patients deficient in TPMT have been misclassified if they have had a recent transfusion due to the activity in the donor erythrocytes [40]. The tight correlation between the two methods indicates that phenotyping, supplemented with genotyping in specific circumstances (e.g., after recent blood transfusion) is entirely appropriate [41–43].

There is no published actual cost data that directly compare the resource use associated with TPMT genotyping, phenotyping or no testing when prescribing azathioprine and impact on subsequent treatment pathways. Existing cost data, used in economic modelling studies, are from estimates of resource use, using expert opinion, comparing testing with current practice [44]. Further research is required to establish the relative cost and outcomes of TPMT genotyping, phenotyping or no testing.

The pragmatic design of this study allowed us to assess physician practice in the interpretation of TPMT genotype results. Physicians started TPMT heterozygotes on a lower dose of azathioprine, consistent with the guidance on the clinical report. However, overall they did not start known TPMT wild-type individuals on the recommended maintenance dose, but preferred to use a lower starting dose. Despite the fact that individuals with normal TPMT activity are at decreased risk of azathioprine-induced neutropenia, the implicit concern that TPMT testing does not predict other side effects, including nausea and myalgia, which commonly occur after treatment initiation and lead to intolerance, means that introduction of TPMT testing will not lead to more rapid treatment induction.

Our study supports previous work that azathioprine is an effective treatment in achieving or maintaining remission in the subset of patients with Crohn’s disease by lowering of the mean HBI to <5 [3]. This improvement was achieved at an average maintenance dose of 1.68 mg/kg/day, similar to the effective dose achieved in a randomized study of azathioprine use in atopic eczema [29] and lower than the dose used in some previous studies [30]. Interestingly, we found no difference in the rates of remission achieved between the genotyped and nongenotyped arms and no evidence that TPMT heterozygotes achieved remission with lower doses of azathioprine.

In conclusion, previous reports indicate that pharmacogenetic testing for TPMT status is important to identify variant homozygote (~1/300) individuals who are at high risk of severe neutropenia with standard dose azathioprine treatment. This is supported by the severe neutropenia experienced by the single variant homozygote in our study. Unfortunately, our study was not adequately powered to formally establish this important relationship. However,
our study cautions against the over-interpretation of TPMT results and provides no evidence of an increased risk of ADRs in individuals with intermediate TPMT activity.

**Future perspective**

Randomized controlled trials are considered the optimum way to generate robust data to inform clinical decision-making. To date, few prospective RCT studies have been conducted in pharmacogenomics, the study on HLA-B*5701 and abacavir is a notable successful exception [46]. However, retrospective pharmacogenetic analyses are increasingly being undertaken on RCTs where the primary outcome was not related to pharmacogenetics, for example with clopidogrel [46,47]. Sometimes the adverse event is so rare or the strength of the association is so strong that undertaking an RCT would be unfeasible or inappropriate [34]. Despite the expense, time and effort, undertaking RCTs in pharmacogenomics can generate valuable information and pragmatic studies that assess utility in real-life clinical settings are especially relevant.

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No writing assistance was utilized in the production of this manuscript.

**Ethical conduct of research**

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

**Executive summary**

- Our study supports the role of thiopurine methyltransferase (TPMT) testing in the identification of individuals at risk of severe neutropenia from azathioprine.
- Individuals with intermediate TPMT activity (10%) are not at increased risk of adverse reactions.
- Adverse reactions to azathioprine were more common in older patients.

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** of considerable interest


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Thiopurine methyltransferase genotyping prior to azathioprine treatment


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* Relationship between TPMT status and adverse drug reactions in patients with inflammatory bowel disease.


* First randomized controlled trial of TPMT status and azathioprine toxicity.


Affiliations

- **William G Newman**
  Genetic Medicine, Manchester Academic Health Science Centre (MAHSC), University of Manchester and Central Manchester NHS Foundation Trust, UK

- **Katherine Payne**
  Health Sciences – Methodology, School of Community Based Medicine, University of Manchester, UK

- **Karen Tricker**
  Genetic Medicine, Manchester Academic Health Science Centre (MAHSC), University of Manchester and Central Manchester NHS Foundation Trust, UK

- **Julie Andrews**
  School of Pharmacy and Pharmaceutical Sciences, University of Manchester, University of Manchester, UK

- **J Brian Houston**
  School of Pharmacy and Pharmaceutical Sciences, University of Manchester, University of Manchester, UK

- **Faeiza Qasim**
  Department of Medicine, Central Manchester NHS Foundation Trust, UK

- **Jon Shaffer**
  Department of Gastroenterology, Salford Royal NHS Foundation Trust, UK

- **Christopher EM Griffiths**
  Department of Dermatology, School of Translational Medicine, University of Manchester, UK

- **David W Ray**
  Endocrine Sciences Research Group, University of Manchester, UK

- **Jan Bruce**
  Department of Rheumatology, Central Manchester NHS Foundation Trust and Arthritis Research Council Epidemiology Unit (arc-eu), University of Manchester, UK

- **William ER Ollier**
  Centre for Integrated Genomic Medical Research (CIGMR), University of Manchester, UK

**TARGET study recruitment team**

- **Y Ahmed**
  Central Manchester University Hospitals Foundation Trust, UK

- **S Ahmed**
  Blackpool, Fylde and Wyre Hospitals NHS Foundation Trust, UK

- **I Ahmed**
  Warrington and Halton Hospitals NHS Foundation Trust, UK

- **C Babbs**
  Salford Royal NHS Foundation Trust, UK

- **J Bartlett**
  Tameside Hospital NHS Foundation Trust, UK

- **A Barton**
  East Cheshire NHS Trust, UK

- **A Bassi**
  St Helens and Knowsley Hospitals NHS Trust, UK

- **RM Bernstein**
  Central Manchester University Hospitals Foundation Trust, UK

- **K Bodger**
  Aintree University Hospitals NHS Foundation Trust, UK
Thiopurine methyltransferase genotyping prior to azathioprine treatment

A Bohan
University Hospital of North Staffordshire
NHS Trust, UK

M Bray
Taunton and Somerset NHS Foundation
Trust, UK

E Brown
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Foundation Trust, UK

D Burke
North Cumbria University Hospitals NHS
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GP Butcher
Southport and Ormskirk Hospital NHS
Trust, UK

S Campbell
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S Christy-KüIlner
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NHS Foundation Trust, UK

J Collum
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JR Crampton
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NHS Foundation Trust, UK

E Darling
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V Edge
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P Foster
East Cheshire NHS Trust, UK

C Francis
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Foundation Trust, UK

A Garstang
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Foundation Trust, UK

RG Glass
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NHS Trust, UK

L Gray
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Foundation Trust, UK

J Green
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V Hall
Lancashire Teaching Hospitals NHS
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R Hammonds
Penne Acute Hospitals NHS Trust, UK

R Harris
Countess of Chester Hospital NHS
Foundation, UK

K Hartigan
Salford Royal NHS Foundation Trust,
UK

MT Hendrickse
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Foundation Trust, UK

A Hulme
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UK

K Hyrich
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Foundation Trust, UK

M Ibbrik
Tameside Hospital NHS Foundation Trust,
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P Isaacs
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Foundation Trust, UK

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N Kapoor
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K Kemp
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Foundation Trust, UK

HJ Klass
Penne Acute Hospitals NHS Trust, UK

SM Knight
East Cheshire NHS Trust, UK

K Koss
East Cheshire NHS Trust, UK

A Koulouzidis
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Foundation Trust, UK

S Lal
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Foundation Trust, UK

F Leslie
University Hospital of North Staffordshire
NHS Trust, UK

B Linaker
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M Macaskill
Penne Acute Hospitals NHS Trust, UK

C Macdonald
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AJ Makin
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Foundation Trust, UK

J Mason
Trafford Healthcare NHS Trus, UK

J McKay
Mid Cheshire Hospitals NHS Foundation
Trust, UK

J McLaren
Penne Acute Hospitals NHS Trust, UK

J McLaughlin
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J McLindon
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Foundation Trust, UK

D McSorland
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E Meecham
East Cheshire NHS Trust, UK

E Nelson
East Cheshire NHS Trust, UK

P O’Toole
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Foundation Trust, UK

B Pandya
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Foundation Trust, UK

S Postill
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Foundation Trust, UK

J Preston
Central Manchester University Hospitals
Foundation Trust, UK

M Prince
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Foundation Trust, UK

JM Puleston
Central Manchester University Hospitals
Foundation Trust, UK

A Reddy
Gateshead Health NHS Foundation Trust,
UK

W Rees
Salford Royal NHS Foundation Trust,
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A Robinson
Salford Royal NHS Foundation Trust,
UK

A Rowlinson
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NHS Trust, UK

A Saeed
Gateshead Health NHS Foundation Trust,
UK

P Sanders
University Hospital of South Manchester
NHS Foundation Trust, UK

S Sarkar
Aintree University Hospitals NHS
Foundation Trust, UK
S Sen
University Hospital of North Staffordshire NHS Trust, UK

M Sephton
Aintree University Hospitals NHS Foundation Trust, UK

N Shaath
Tameside Hospital NHS Foundation Trust, UK

J Sherlmerdine
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N Snowden
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R Sturgess
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C Summerton
Trafford Healthcare NHS Trust, UK

DPM Symmons
East Cheshire NHS Trust, UK

V Tabern
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WC Tan
Warrington and Halton Hospitals NHS Foundation Trust, UK

J Thompson
Southport and Ormskirk Hospital NHS Trust, UK

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Gateshead Health NHS Foundation Trust, UK

T Wardle
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R Warner
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G Watts
University Hospital of South Manchester NHS Foundation Trust, UK

G Whatley
Tameside Hospital NHS Foundation Trust, UK

I White
Salford Royal NHS Foundation Trust, UK