Assessment of Thiopurine S-Methyltransferase Activity in Patients Prescribed Thiopurines: A Systematic Review

Ronald A. Booth, PhD; Mohammed T. Ansari, MBBS, MMEdSc, MPhil; Evelin Loit, PhD; Andrea C. Tricco, PhD; Laura Weeks, PhD; Steve Doucette, MSc; Becky Skidmore, MLS; Margaret Sears, PhD; Richmond Sy, MD; and Jacob Karsh, MD, CM

Background: The evidence for testing thiopurine S-methyl transferase (TPMT) enzymatic activity or genotype before starting therapy with thiopurine-based drugs is unclear.

Purpose: To examine the sensitivity and specificity of TPMT genotyping for TPMT enzymatic activity, reducing harm from thiopurine by pretesting, and the association of thiopurine toxicity with TPMT status in adults and children with chronic inflammatory diseases.

Data Sources: MEDLINE, EMBASE, the Cochrane Library, and Ovid HealthSTAR (from inception to December 2010) and BIOSIS and Genetics Abstracts (to May 2009).

Study Selection: Two reviewers screened records and identified relevant studies in English.

Data Extraction: Data on patient characteristics, outcomes, and risk for bias were extracted by one reviewer and independently identified by another.

Data Synthesis: 54 observational studies and 1 randomized, controlled trial were included. Insufficient evidence addressed the effectiveness of pretesting. Genotyping sensitivity to identify patients with low to absent enzymatic activity ranged from 70.33% to 86.15% (lower-bound 95% CI, 54.52% to 70.88%; upper-bound CI, 78.50% to 96.33%). Sparse data precluded estimation of genotype sensitivity to identify patients with low to absent enzymatic activity. Genotyping specificity approached 100%. Compared with noncarriers, heterozygous and homozygous genotypes were both associated with leukopenia (odds ratios, 4.29 [CI, 2.67 to 6.89] and 20.84 [CI, 3.42 to 126.89], respectively). Compared with intermediate or normal activity, low TPMT enzymatic activity was significantly associated with myelotoxicity and leukopenia.

Limitation: Available evidence was not rigorous and was underpowered to detect a difference in outcomes.

Conclusion: Insufficient evidence addresses the effectiveness of TPMT pretesting in patients with chronic inflammatory diseases. Estimates of the sensitivity of genotyping are imprecise. Evidence confirms the known associations of leukopenia or myelotoxicity with reduced TPMT activity or variant genotype.

Primary Funding Source: Agency for Healthcare Research and Quality.

Ann Intern Med. 2011;154:814-823.

For author affiliations, see end of text.
Various clinical guidelines suggest measuring TPMT enzymatic activity or screening for common variant TPMT alleles before initiating thiopurine therapy. The drug monograph for azathioprine approved by the U.S. Food and Drug Administration (FDA) also recommends pretesting but does not mandate it. The evidence base for this recommendation is unclear, particularly the crucial evidence that pretherapy TPMT testing decreases myelotoxicity-specific mortality (14, 15). In addition, regular performance of complete blood count is recommended and routinely practiced for the duration of therapy (16). Measurement of TPMT activity may not be of additional benefit, because regular monitoring may be sufficient to identify adverse events.

The status of a patient’s TPMT enzymatic activity can be assessed directly, by activity testing (phenotyping), or indirectly, by genotyping for variant alleles. No recommendation exists for which testing method should be used. However, enzymatic analysis should identify most patients at risk, with the exception of those with a recent blood transfusion (17). Whether genotyping is sufficiently sensitive for routine use in clinical practice is also unclear, because most laboratories identify only the most common variant alleles and would miss rare variants.

In light of these uncertainties, we systematically reviewed evidence addressing several key questions related to TPMT testing before initiating thiopurine therapy in chronic inflammatory disease populations. This research topic was nominated by the American Association for Clinical Chemistry and commissioned by the Agency for Healthcare Research and Quality. We examined the evidence on the sensitivity and specificity of TPMT genotyping as a replacement for assessment of TPMT activity; investigated whether previous assessment of TPMT status (by genotyping or phenotyping) compared with no pretesting to guide thiopurine therapy leads to changes in management and reduction in harms; and sought evidence of an association between TPMT status and thiopurine toxicity. This report summarizes our findings.

**Context**

Some experts recommend routine measurement of thiopurine S-methyltransferase (TPMT) enzymatic activity before initiating thiopurine therapy.

**Contribution**

This systematic review found insufficient evidence that TPMT pretesting guides appropriate prescribing or improves patient outcomes (such as by limiting toxicity) compared with routine blood count monitoring in patients receiving thiopurine therapy. Limited-quality evidence suggested imperfect and imprecise sensitivity but near-perfect specificity of genotyping for identifying patients with low and intermediate TPMT enzymatic activity.

**Implication**

We urgently need more high-quality evidence regarding the utility and costs of routine TPMT pretesting.

---

The Editors

Healthcare Research and Quality. We examined the evidence on the sensitivity and specificity of TPMT genotyping as a replacement for assessment of TPMT activity; investigated whether previous assessment of TPMT status (by genotyping or phenotyping) compared with no pretesting to guide thiopurine therapy leads to changes in management and reduction in harms; and sought evidence of an association between TPMT status and thiopurine toxicity. This report summarizes our findings.

**Figure 1. Metabolization of thiopurine-based drugs.**

Azathiopurine, 6-MP, and 6-TG are enzymatically modified to their active compound, 6-tGN. Decreased TPMT activity increases the amount of prodrug available for conversion to the active compound, thus increasing the risk for toxic levels of 6-tGN. Thiopurine S-methyltransferase also plays a minor role in the inactivation of 6-TG. AO = aldehyde oxidase; AZA = azathiopurine; GD = guanine deaminase; HGPRT = hypoxanthine guanine phosphoribosyltransferase; IMPDH = inosine monophosphate dehydrogenase; MP = mercaptopurine; TG = thioguanine; tGMP = thioguanosine acid; tGN = thioguanine nucleotides; tIMP = thiomercaptopurine; TMPT = thiopurine S-methyltransferase; tXMP = thiooxanthosine; XO = xanthine oxidase.
**Methods**

We followed a prespecified and peer-reviewed study protocol. The full evidence report, including search strategies and a detailed list of a priori outcomes, risk for bias assessment, and detailed evidence tables, is available at www.ahrq.gov/clinic/epcindex.htm.

**Data Sources and Searches**

Using a peer-reviewed strategy, we searched MEDLINE (1950 to week 3 of December 2010), the Cochrane Library (fourth quarter of 2010), EMBASE (1980 to week 52 of 2010), Ovid HealthSTAR (1966 to December 2010), and BIOSIS and Genetics Abstracts (to May 2009), with no language restrictions. We also searched for unpublished studies.

**Study Selection**

To assess the effectiveness of TPMT testing before thiopurine therapy, at least 1 study group had to have had thiopurine dose adjustment or drug replacement guided by pretherapy TPMT genotyping or phenotyping. To determine the association between TPMT status and drug toxicity, thiopurine therapy should not have been guided by the results of TPMT testing. All study designs were included except for effectiveness of pretesting, for which we limited eligibility to experimental, cohort, and case–control studies. Non–English language records, editorials, reviews, commentaries, letters, and news or case reports were excluded.

One reviewer screened titles and abstracts for potential relevance, and a second reviewer verified exclusions at this level. Two independent reviewers assessed the full publication of potentially relevant studies, and discrepancies were resolved by consensus.

**Data Extraction and Quality Assessment**

Data were extracted by using standardized forms and subsequently verified. Patients who tested negative for any of the single-nucleotide polymorphisms were considered noncarriers (wild-type homozygous), whereas carriers were either heterozygous or homozygous for a variant allele. Homozygous carriers (those with one of the same variant alleles on each of the paired chromosomes) were not differentiated from compound heterozygous carriers (those with different variant alleles on each of the paired chromosomes); both states were considered to be homozygous. Patients with normal and high TPMT activity were also grouped together; most studies used these terms interchangeably.

Two reviewers assessed the risk for bias and rated the strength of the evidence by consensus. For studies of test performance, a modified QUADAS (Quality Assessment of Diagnostic Accuracy Studies) tool with additional enquiries about Hardy–Weinberg equilibrium was used (18). For other studies, risk for bias was evaluated by using generic items that assessed selection, performance, detection, and attrition bias, as well as confounding and potential for financial conflict of interest. Each study was given an overall risk-for-bias assessment of good (low risk), fair, or poor (high risk).

We rated the strength of the evidence for the outcomes of death, serious adverse events, myelotoxicity, and health-related quality of life across the domains of risk for bias, consistency, directness, and precision, as per published guidance (19). Other outcomes of interest were patients who required thiopurine dose reduction or a switch to nonthiopurine therapy, number of monitoring tests, infection, hospitalization, withdrawal due to adverse events, leukopenia, neutropenia, thrombocytopenia, anemia, hepatotoxicity, and pancreatitis.

**Data Synthesis and Analysis**

We assessed performance characteristics of genotyping compared with phenotyping and estimated test sensitivity and specificity. When meta-analysis was considered inappropriate, evidence was synthesized qualitatively. Heterogeneity in investigator categorizations of enzymatic activity was not explored, because the numerical values generated by the various methods (even by similar methods in different laboratories) are often not comparable. We used a codominant model to pool data associated with noncarrier, heterozygous carrier, and homozygous carrier states when estimating the magnitude of association between genotype and thiopurine toxicity. Similarly, 3 categories of enzymatic activities were defined (high or normal, intermediate, and low or absent). In separate analyses, we compared toxicity rates in each genotypic state and TPMT category with the others.

Our primary analyses of genotype–toxicity association pooled studies that tested at least TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C, regardless of whether they tested additional variants. In contrast, for primary analyses of test performance, we pooled studies that genotyped identical sets of TPMT mutations. This different meta-analytic approach between the genotype–toxicity association and the test performance studies was based on our understanding that estimates of strength of association between TPMT genotype or phenotype and thiopurine drug toxicity are largely indirect and hypothesis-generating, whereas estimates of sensitivity and specificity of TPMT genotyping could directly affect management decisions involving thiopurine dosing. This necessitated estimating test performance specifically for the particular set of alleles genotyped. For both the performance of genotyping and the genotypic association with thiopurine toxicity, we considered additional meta-analyses by pooling studies as long as they genotyped for all ethnicity-specific mutations with a known prevalence greater than 1%.

When appropriate, sensitivity and specificity estimates were pooled by first transforming proportions into the Freeman–Tukey variant of the arcsine square root transformed proportion (20). The pooled proportion was calculated as the back-transformation of the weighted mean of the transformed proportions. Data were pooled by using a
fixed-effects inverse variance weighted average. Odds ratios (ORs) were pooled by using the fixed-effects Mantel–Haenszel method without continuity correction (21).

Pooled estimates of sensitivity and ORs and their 95% CIs were calculated by using StatsDirect, version 2.7.8 (StatsDirect, Cheshire, United Kingdom). We tested for statistical heterogeneity (but not for sensitivity and specificity meta-analyses) by using the Cochran Q test, to be reported when substantial ($P$ for chi-square test of heterogeneity <0.10 and $I^2 \geq 50\%$).

Role of the Funding Source
The Agency for Healthcare Research and Quality supported this study but had no role in formulating study questions, conducting the systematic review, or approving the manuscript for submission and publication.

RESULTS
We screened 1890 records and included 118 unique studies in the full report (Figure 2). Of these, 55 (22–
Assessment of Thiopurine S-Methyltransferase Activity

76] addressed the 3 key questions. Most studies (>75%) were rated as fair, whereas a substantial proportion of the studies of test performance (37%) were of poor design. Three studies were restricted to pediatric patients (38, 58, 67).

Test Performance of TPMT Genotyping

Nineteen studies, mostly of cross-sectional and prospective observational design, contributed evidence. Most of these were not designed to assess test performance but provided parallel genotypic and phenotypic data, a limitation that was captured in the quality assessment of the studies. Approximately 70% of studies included patients with inflammatory bowel disease. Among 1735 total patients, 184 were heterozygous and 16 were homozygous for variant alleles. In most studies, all or most study patients were white, except for 2 studies (43, 51) that were restricted to Japanese and South Asian patients. In the 11 studies that reported concomitant medications, 5-aminosalicylic acid and steroids were commonly used. One study (45) reported recent transfusion as an exclusion criterion. A common study limitation was lack of clarity about whether the determination of either enzymatic activity level or patient genotype was influenced by previous knowledge of the other value.

The sensitivity of the carrier genotype (heterozygous or homozygous) for correctly identifying patients with subnormal (intermediate or low) enzymatic activity was imprecise, ranging from a pooled estimate of 70.33% to 86.15% (lower-bound 95% CI, 54.52% to 70.88%; upper-bound CI, 78.50% to 96.33%) across the subgroups of alleles tested (Figure 3). Small, single studies of poor to fair quality provided limited evidence for 3 of the subgroups. Meta-analysis of the 19 studies that genotyped all ethnicity-specific mutations with a known prevalence established in more than 90% of studies. The studies comprised 2211 patients, including 357 with intermediate and 74 with low enzymatic activity. The odds of leukopenia were significantly greater with low TPMT enzymatic activity than with intermediate (OR, 1.54 to 4.86; 3 studies, 257 patients, and 91 events) or normal (OR, 19.12 [CI, 4.56 to 80.24]; 3 studies, 403 patients, and 29 events) activity. The odds of leukopenia were significantly greater with low TPMT activity than with intermediate (OR, 2.74 [CI, 1.54 to 4.86]; 4 studies, 257 patients, and 91 events) or normal (OR, 2.56 [CI, 1.41 to 4.67]; 4 studies, 397 patients, and 81 events) enzymatic activity. Because of the few events and small study sizes, nonsignificant pooled ORs with wide CIs were noted for other comparisons. Evidence was absent for the outcomes of death, hospitalization, serious adverse events, or quality of life.

Association Between TPMT Status and Thiopurine Toxicity

Enzymatic Activity

Of the 17 eligible studies, 24% (most of which had observational designs) were rated as poor and the rest were judged as fair. Comparability of prognostic factors across groups, double-blinded outcomes assessment, and genotype or phenotype determination could not be clearly established in more than 90% of studies. The studies comprised 2211 patients, including 357 with intermediate and 74 with low enzymatic activity. Greater odds of myelotoxicity were noted with low TPMT enzymatic activity than with intermediate (OR, 14.53 [CI, 2.78 to 76.01]; 3 studies, 92 patients, and 10 events) or normal (OR, 19.12 [CI, 4.56 to 80.24]; 3 studies, 403 patients, and 29 events) activity. The odds of leukopenia were significantly greater with low TPMT activity than with intermediate (OR, 2.74 [CI, 1.54 to 4.86]; 4 studies, 257 patients, and 91 events) or normal (OR, 2.56 [CI, 1.41 to 4.67]; 4 studies, 397 patients, and 81 events) enzymatic activity. Because of the few events and small study sizes, nonsignificant pooled ORs with wide CIs were noted for other comparisons.

Genotype

Thirty-one of 34 studies, most of which were observational, contributed to the quantitative synthesis. Of the 3638 patients across studies, 260 were heterozygous and 19 were homozygous for variant alleles. Most of the studies...
were of fair quality and included patients with inflammatory bowel disease. Comparability of prognostic factors across groups, double-blinded outcomes assessment, and genotype or phenotype determination could not be clearly established in more than 85% of studies. Compared with noncarriers, heterozygotes had pooled odds of 4.29 (CI, 2.67 to 6.89) for leukopenia (Appendix Figure 2, available at www.annals.org). Meta-analysis of 5 studies that compared 7 homozygotes with 475 noncarriers demonstrated greater but very imprecise odds of leukopenia (OR, 20.84 [CI, 3.42 to 126.89]). For all other outcomes, evidence either was absent or lacked the power to demonstrate significant differences between heterozygous and homozygous carriers compared with noncarriers or between themselves. Broadening the meta-analyses to studies that genotyped all ethnicity-specific mutations with a known prevalence greater than 1% did not improve the precision of the estimates. Of note, withdrawals due to adverse events were

![Forest plot of the sensitivity of carrier genotype (heterozygous or homozygous) for correctly identifying patients with subnormal (intermediate or low) enzymatic activity.](http://www.annals.org/)

| Study, Year (Reference) | TPMT*2, *3A, and *3C | Sensitivity (95% CI) | Specificity (Exact 95% CI) |
|-------------------------|-----------------------|----------------------|---------------------------|
| Ansari et al, 2002 (53) | 10 0 0 30             | 100.00 (69.15–100.00) | 100.00 (88.43–100.00)     |
| Marinaki et al, 2003 (51)| 5 0 0 80              | 100.00 (47.82–100.00) | 100.00 (95.49–100.00)     |
| Gardiner et al, 2008 (27)| 5 2 4 57             | 55.56 (21.20–86.30)  | 96.61 (88.29–99.59)       |
| Stocco et al, 2004 (64) | 4 0 0 23              | 100.00 (39.76–100.00) | 100.00 (85.18–100.00)     |
| von Ahsen et al, 2005 (42)| 5 1 0 50              | 23.81 (8.22–47.17)   | 100.00 (92.89–100.00)     |
| Okada et al, 2005 (43)  | 3 11 10 41           | 23.08 (5.04–53.61)   | 97.62 (87.43–99.94)       |
| Stocco et al, 2005 (41) | 3 0 0 25              | 100.00 (29.24–100.00) | 100.00 (86.28–100.00)     |
| Winter et al, 2007 (29) | 11 0 6 113            | 64.71 (38.33–85.79)  | 100.00 (96.79–100.00)     |
| Stassen et al, 2009 (22) | 7 0 0 101              | 100.00 (59.04–100.00) | 100.00 (96.41–100.00)     |
| Newman et al, 2011 (75)  | 35 0 2 296            | 94.59 (81.81–99.34)  | 100.00 (98.76–100.00)     |
| Snow and Gibson, 1995 (62)t | 5 0 0 21             | 100.00 (47.82–100.00) | 100.00 (83.89–100.00)     |
| Langley et al, 2002 (54) | 6 4 3 40              | 66.67 (29.93–92.51)  | 90.91 (78.33–97.47)       |
| Ansari et al, 2008 (23) | 17 1 6 168            | 73.91 (51.59–89.77)  | 99.41 (96.75–99.99)       |
| Hindorf et al, 2004 (45) | 5 0 0 50              | 100.00 (47.82–100.00) | 100.00 (92.89–100.00)     |
| Schwab et al, 2002 (55) | 5 0 0 85              | 100.00 (47.82–100.00) | 96.59 (90.36–99.29)       |
| Haglund et al, 2004 (48) | 21 0 0 9               | 100.00 (83.89–100.00) | 100.00 (66.37–100.00)     |

FN = false negative; FP = false positive; TN = true negative; TP = true positive; TPMT = thiopurine S-methyltransferase.

tTested alleles were not reported, so those we assumed most likely to have been tested in 1993 (when the study was conducted) are reported.
significant higher among heterozygotes than noncarriers (OR, 6.54 [CI, 2.53 to 16.91]; 4 studies, 27 heterozygotes and 330 noncarriers, and 121 events).

**DISCUSSION**

To our knowledge, ours is the first comprehensive systematic review to investigate the ability of TPMT genotyping to correctly identify TPMT activity status, and the utility of determining TPMT status of patients by genotyping or phenotyping before initiating thiopurine therapy. We also investigated indirect evidence linking TPMT status with thiopurine toxicity.

Little good-quality primary research addresses these questions. Limited-quality evidence indicates that the estimates of genotyping sensitivity are imprecise, despite near-perfect specificity, for identifying subnormal enzymatic activities. Evidence is currently insufficient to address the utility of TPMT testing before initiating thiopurine therapy compared with routine blood count monitoring. Whether pretesting guides appropriate prescribing is also unclear. Indirect evidence confirms previously known strong associations between thiopurine-related leukopenia and either low levels of TPMT enzymatic activity or the presence of TPMT allelic polymorphisms (77). This was reflected in significant associations between low levels of enzymatic activity and myelotoxicity.

High concordance between TPMT genotype and enzymatic activity (phenotype) has been reported in healthy populations, leading to frequent replacement of TPMT phenotyping with genotyping (78). Because TPMT enzymatic activity (absent or low, intermediate, or normal or high) actually defines the TPMT status that guides thiopurine dosing, comparing the test performance of genotyping with phenotyping in diseased populations was considered important. Estimates of the sensitivity of genotyping to identify either low or low-to-intermediate enzymatic activity were generally imprecise and lower than specificity estimates (which approached 100%). When we broadened the meta-analysis to pool sensitivity data across the 19 studies that genotyped all ethnicity-specific mutations with a known prevalence greater than 1%, the pooled sensitivity of the carrier genotype (heterozygous or homozygous) to correctly identify patients with subnormal (intermediate or low) enzymatic activity varied (CI, 75% to 85%). This range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. The range was derived by using a fixed-effects meta-analytic model that does not account for between-study heterogeneity. Thus, the range may be considered no more than a model that does not account for between-study heterogeneity. Therefore, the observed scarcity of evidence.

Our finding that TPMT testing before initiating thiopurine therapy is of indeterminate utility seems at odds with previously published economic evaluations that recommend such testing. However, those evaluations have been criticized for incorporating clinical data from retrospective studies and expert opinion instead of prospective empirical evidence; the latter, as our review shows, is lacking (83). Approximately 5% to 15% of patients are heterozygous, whereas approximately 0.3% are homozygous (5, 6, 8). Available evidence of associations with thiopurine toxicity was limited by few heterozygotes (or those with intermediate activity), occasional homozygotes (or those with low or absent activity), and low event rates among the study populations. The findings therefore lacked power to rule in or rule out significant associations between TPMT status and most outcomes of thiopurine toxicity. Our review was restricted to English-language literature; however, it is unclear how much this restriction might have contributed to the observed scarcity of evidence.

Higgs and colleagues’ recent systematic review (79) aimed to quantify the associations between leukopenia and intermediate TPMT activity or heterozygous genotype, compared with normal activity or noncarrier genotype. The authors pooled both the TPMT activity and genotype data. No disease restrictions were used; thus, patient populations were broadened to include recipients of transplanted organs and patients with cancer. The pooled odds ratio for leukopenia was 4.19 (CI, 3.20 to 5.48), almost identical to our meta-analytic estimate of 4.29 (CI, 2.67 to 6.89) when heterozygotes were compared with noncarriers.
Higgs and colleagues wisely questioned the importance of modest decreases in leukocyte counts and argued that modest leukopenia may reflect effective treatment with thiouarine-based drugs rather than the undesired adverse event of myelosuppression.

Various recent guidelines, as well as the FDA-approved product monograph for azathioprine, have advocated determining TPMT status before initiating treatment with thiouarine (14, 84). The proposition that knowledge of TPMT status before therapy would lead to decreased rates of dose-dependent toxicity is rational and based on evidence of strong genotypic and phenotypic associations in observational studies. However, from an evidence-based perspective, guideline recommendations of pretreatment TPMT testing are premature for several reasons. First, the direct evidence base for these recommendations is lacking, especially the crucial evidence that TPMT testing before thiouarine therapy decreases myelotoxicity-specific mortality. Second, patients who receive thiouarine-based drugs must have complete blood counts measured on a regular basis to prevent severe myelotoxicity by early detection. Third, azathioprine and 6-mercaptopurine had been used successfully for several years before TPMT testing was available, and present management (testing or no testing before therapy) varies across clinical specialties. Fourth, thiouarine-related toxicities are also partially explained by mutations in other enzymes, drug interactions, concurrent infections, and immune-mediated drug reactions. Fifth, the extremely low prevalence of homozygotes means that available studies are severely underpowered to provide direct evidence of the effectiveness of pretesting in the subpopulation believed to be most at risk (5, 6, 8). Finally, the use of TPMT status to guide treatment has the potential to reduce the efficacy of thiouarine drugs if physicians are overzealous in reducing thiouarine dosages. The 2004 guidelines from the British Society of Gastroenterology (85) recognized this, stating, “It cannot yet be recommended as a prerequisite to therapy, because decades of experience have shown clinical [azathioprine] to be safe in [ulcerative colitis] or [Crohn disease].”

In conclusion, the utility of pretesting for TPMT status before initiating thiouarine treatment remains in question, because insufficient evidence demonstrates that this strategy is effective to reduce harm or is superior to the established clinical standard of hematologic monitoring.

From the Ottawa Hospital, Ottawa Hospital Research Institute, and University of Ottawa, Ottawa, and St. Michael’s Hospital, Toronto, Ontario, Canada.

Disclaimer: The authors of this report are responsible for its content. Statements in the report should not be construed as endorsements by the Agency for Healthcare Research and Quality, the National Center for Complementary and Alternative Medicine, National Institutes of Health, or the U.S. Department of Health and Human Services.

Acknowledgment: The authors thank Mr. Raymond Daniel, Dr. James Galipeau, Dr. Jeff Hoch, Ms. Suja Mani, Dr. Alex Tsertsavzadz, Ms. Sophia Tsourou, Ms. Fatemeh Yazdi, and Mr. Alex Yurkiewich for their assistance in data extraction, quality assessment, and the preparation of the original evidence report.

Grant Support: By contract HHSAG290-2007-10059-I (EP[CIII]) from the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services.

Potential Conflicts of Interest: Dr. Booth: Grant (money to institution): Agency for Healthcare Research and Quality; Consultancy: Agency for Healthcare Research and Quality; Dr. Ansari: Grant (money to institution): Agency for Healthcare Research and Quality; Consultancy: Health Canada; Payment for lectures including service on speakers bureaus: Hoffman-La Roche, Abbott, Amgen, Bristol-Myers Squibb, Merck. Dr. Tricco: Consultancy: Agency for Healthcare Research and Quality; Payment for writing or reviewing the manuscript: Agency for Healthcare Research and Quality; Other: GlaxoSmithKline. Ms. Skidmore: Consulting fee or honorarium: Knowledge Synthesis Group, Ottawa Methods Centre. Dr. Sears: Fees for participation in review activities, such as data monitoring boards, statistical analysis, end point committees, and the like: University of Ottawa Evidence-based Practice Center; Payment for writing or reviewing the manuscripts: University of Ottawa Evidence-based Practice Center; Payment for manuscript preparation: University of Ottawa Evidence-based Practice Center. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M10-2516.

Requests for Single Reprints: Mohammed T. Ansari, MBBS, MMedSc, MPhil, University of Ottawa Evidence-based Practice Center, Ottawa Methods Centre, Clinical Epidemiology Program, Ottawa Hospital Research Institute, Box 208, 501 Smyth Road, Ottawa, Ontario K1H 8L6, Canada; e-mail, moansari@ohri.ca.

Current author addresses and author contributions are available at www.annals.org.

References
1. Prefontaine E, Sutherland LR, Macdonald JK, Cepoiu M. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn’s disease. Cochrane Database Syst Rev. 2009:CD000067. [PMID: 19160175]
2. Gibert JP, Gomollon F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease. a review. Am J Gastroenterol. 2008;103:1783-800. [PMID: 18557712]
3. Meggitt SJ, Reynolds NJ. Azathioprine for atopic dermatitis. Clin Exp Dermatol. 2001;26:369-75. [PMID: 11488818]
4. Swann PF, Waters TR, Moulton DC, Xu YZ, Zheng Q, Edwards M, et al. Role of postreplicative DNA mismatch repair in the cytotoxic action of thioguanine. Science. 1996;273:1109-11. [PMID: 8688098]
5. Collie-Duguid ES, Pritchard SC, Powrie RH, Sludden J, Collier DA, Li T, et al. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. Pharmacogenetics. 1999;9:37-42. [PMID: 10208641]
6. Engen RM, Marsh S, Van Booven DJ, McLeod HL. Ethnic differences in pharmacogenetically relevant genes. Curr Drug Targets. 2006;7:1641-8. [PMID: 17168839]
7. Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine S-methyltransferase gene in African-Americans. Hum Mol Genet. 1999;8:371-6. [PMID: 9931346]
8. Ameyaw MM, Collie-Duguid ES, Powrie RH, Ofori-Adjei D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations. Hum Mol Genet. 1999;8:367-70. [PMID: 9931345]
9. Efrati E, Adler L, Krivoy N, Sprecher E. Distribution of TPMT risk alleles for thiopurine [correction of thiopurine] toxicity in the Israeli population. Eur J Clin Pharmacol. 2009;65:257-62. [PMID: 19048244]
10. Weyer N, Kröpflin T, Fricke L, Iven H. Human thiopurine S-
Assessment of Thiopurine S-Methyltransferase Activity and efficacy. Aliment Pharmacol Ther. 2007;26:737-45. [PMID: 17692707]

29. Winter JW, Gaffney D, Shapiro D, Spooner RJ, Marinski AM, Sanderson JD, et al. Assessment of thiopurine methyltransferase enzyme activity is superior to genotype in predicting myelosuppression following azathioprine therapy in patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2007;25:1069-77. [PMID: 17439508]

30. Tamori A, Shinzaki M, Kosaka S, Hayashi T, Iwai S, Enomoto M, et al. Thiopurine S-methyltransferase gene polymorphism in Japanese patients with autoimmune liver diseases. Liver Int. 2007;27:95-100. [PMID: 17241387]

31. Stocco G, Martelossi S, Barabino A, Decorti G, Bartoli F, Monico M, et al. Glutathione-S-transferase genotypes and the adverse effects of azathioprine in young patients with inflammatory bowel disease. Inflamm Bowel Dis. 2007: 13:57-64. [PMID: 17206640]

32. Gibert JP, Niño P, Rodríguez L, Cara C, Guijarro LG. Thiopurine methyltransferase (TPMT) activity and adverse effects of azathioprine in inflammatory bowel disease: long-term follow-up study of 394 patients. Am J Gastroenterol. 2006;101:2769-76. [PMID: 17026564]

33. Lindqvist M, Hindorf U, Alser M, Söderkvist P, Ström M, Hjortswang H, et al. No induction of thiopurine methyltransferase during thiopurine treatment in inflammatory bowel disease. Nucleosides Nucleotides Nucleic Acids. 2006:25:1033-7. [PMID: 17065925]

34. Banerjee S, Bishop WP. Evolution of thiopurine use in pediatric inflammatory bowel disease in an academic center. J Pediatr Gastroenterol Nutr. 2006;43:324-30. [PMID: 16959454]

35. Schedel J, Gödde A, Schütz E, Bongartz TA, Lang B, Schölerich J, et al. Impact of thiopurine methyltransferase activity and 6-thioguanine nucleotide concentrations in patients with chronic inflammatory diseases. Ann N Y Acad Sci. 2006;1069:477-91. [PMID: 16851576]

36. Hindorf U, Lindqvist M, Hildebrand H, Fagerberg U, Alser M. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2006;24:331-42. [PMID: 16842860]

37. Ohno A, Carpenter HA. Thiopurine methyltransferase deficiency and azathioprine intolerance in autoimmune hepatitis. Dig Dis Sci. 2006;51:968-75. [PMID: 16773433]

38. De Ridder L, Van Dieren JM, Van Deventer HJ, Stokkers PC, Van der Woude JC, Van Vuuren AJ, et al. Pharmacogenetics of thiopurine therapy in paediatric IBD patients. Aliment Pharmacol Ther. 2006;23:1137-41. [PMID: 16611274]

39. Zelinkova Z, Derijks LJ, Stokkers PC, Voskens EW, van Kampen AH, Curvers WL, et al. Inosine triphosphate pyrophosphatase and thiopurine s-methyltransferase genotypes relationship to azathioprine-induced myelosuppression. Clin Gastroenterol Hepatol. 2006;4:44-9. [PMID: 16431049]

40. Jun JB, Cho DY, Kang C, Bae SG. Thiopurine S-methyltransferase polymorphisms and the relationship between the mutant alleles and the adverse effects in systemic lupus erythematosus patients taking azathioprine. Clin Exp Rheumatol. 2005;23:873-6. [PMID: 16396707]

41. Stocco G, Martelossi S, Barabino A, Fontana M, Lionetti P, Decorti G, et al. TPMT genotype and the use of thiopurines in paediatric inflammatory bowel disease. Dig Liver Dis. 2005;37:929-36. [PMID: 16214825]

42. von Ahesen N, Armstrong VW, Behrens C, von Tipiritz C, Stallmach A, Herfarth H, et al. Association of inosine triphosphate 94C>C and thiopurine S-methyltransferase deficiency with adverse events and study drop-outs under azathioprine therapy in a prospective Crohn disease study. Clin Chem. 2005;51: 2283-9. [PMID: 16214825]

43. Okada Y, Nakamura K, Kodama T, Ueki K, Tsukada Y, Maekawa A, et al. Thiopurine methyltransferase genotype and phenotype status in Japanese patients with systemic lupus erythematosus. Biol Pharm Bull. 2005;28:2117-9. [PMID: 16272700]

44. Gearry RB, Roberts RL, Barclay ML, Kennedy MA. Lack of association between the ITPA 94C>C polymorphism and adverse effects from azathioprine. Pharmacogenetics. 2004;14:779-81. [PMID: 15564886]

45. Hindorf U, Lyrenås E, Nilsson A, Schmiegelow K. Monitoring of long-term thiopurine therapy among adults with inflammatory bowel disease. Scand J Gastroenterol. 2004;39:1105-12. [PMID: 15545169]

46. Derijks LJ, Gilissen LP, Engels LG, Bos LP, Bus PJ, Lohman JJ, et al. Pharmacokinetics of 6-mercaptopurine in patients with inflammatory bowel disease: implications for therapy. Ther Drug Monit. 2004;26:311-8. [PMID: 15167634]

47. Marinski AM, Ansari A, Duley JA, Arenas M, Sumi S, Lewis CM, et al.
Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). Pharmacogenetics. 2004;14:181-7. [PMID: 15167706]

64. Kolorz M, Bartosova L, Hosek J, Dvorackova D, Chylkova A, Zboril V, et al. Importance of thiopurine S-methyltransferase gene polymorphisms for prediction of azathioprine toxicity. Neuro Endocrinol Lett. 2009;30 Suppl 1:137-43. [PMID: 19473575]

65. Newman W, Payne K, Tricker K, Roberts S, Fargher E, Pushpakom S, et al. A pragmatic randomised controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. Pharmacogenomics. 2011. [Forthcoming].

66. Schmeling H, Abdelmalek M, Benseler S, Tyrell P, Silverman E. Role of TPMT genotyping and azathioprine metabolites in predicting toxicity of azathioprine in PSLE patients. Arthritis Rheum. 2007;56:4265-6.

67. Lopez AJ, Schwab M, Witt H, Ansari A, Dejaco C, Schaeffeler E, et al. The impact of genetic variants in TPMT, TTPA, MTHFR, SPINK1 and PRSS1 on azathioprine-induced pancreatitis. Gastroenterology. 2006;130(4 Suppl 2).

68. van Dieren J, van Vuuren H, Kuipers E, Kusters H, Nieuwenhuis E, van der Woude J. Heterozygous polymorphisms in the genes encoding TTPA and TPMT are not predictive for the development of adverse effects of azathioprine treatment in IBD patients. Gastroenterology. 2005;128(4 Suppl 2).

69. van der Woude J. Heterozygous polymorphisms in the genes encoding TTPA and TPMT are not predictive for the development of adverse effects of azathioprine treatment in IBD patients. Gastroenterology. 2005;128(4 Suppl 2).

70. Bodea M, Teixier F, Ferrari N, Cortot A, Libera C, Brody F, et al. Thiopurine S-methyltransferase genotyping does not predict azathioprine-induced myelosuppression in Crohn’s disease. AAGA Abstract S1311. Digestive Disease Week, Orlando, Florida, 18–21 May 2003.

71. Tani C, Mosca M, Colucci R, Gori G, d’Ascanio A, Ghisu N, et al. Genetic polymorphisms of thiopurine S-methyltransferase in a cohort of patients with systemic autoimmune diseases. Clin Exp Rheumatol. 2009;27:321-4. [PMID: 19473575]

72. Hindorf U, Jahed K, Bergquist A, Verbaan H, Prytz H, Wallerstedt S, et al. Characterisation and utility of thiopurine methyltransferase and thiopurine metabolite measurements in autoimmune hepatitis. J Hepatol. 2010;52:106-11. [PMID: 19906459]

73. Vestergaard T, Bygum A. An audit of thiopurine methyltransferase genotyping and phenotyping before intended azathioprine treatment for dermatological conditions. Clin Exp Dermatol. 2010;35:140-4. [PMID: 19663853]

74. Kolorz M, Bartosova L, Hosek J, Dvorackova D, Chylkova A, Zboril V, et al. Importance of thiopurine S-Methyltransferase gene polymorphisms for prediction of azathioprine toxicity. Neuro Endocrinol Lett. 2009;30 Suppl 1:137-43. [PMID: 19473575]

75. Newman W, Payne K, Tricker K, Roberts S, Fargher E, Pushpakom S, et al. A pragmatic randomised controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. Pharmacogenomics. 2011. [Forthcoming].

76. Kim JH, Cheon JH, Hong SS, Eun CS, Byeon JS, Hong SY, et al. Influences of thiopurine methyltransferase genotype and activity on thiopurine-induced leukopenia in Korean patients with inflammatory bowel disease: a retrospective cohort study. J Clin Gastroenterol. 2010;44:242-8. [PMID: 20308917]

77. Saaharanman S, Howard D, Roy S. Clinical pharmacology and pharmacogenomics of thiopurines. Eur J Clin Pharmacol. 2008;64:753-67. [PMID: 18506437]

78. Schaeffeler E, Fischer C, Brockmeier D, Wernet D, Moerike K, Eichelbaum M, et al. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics. 2004;14:407-17. [PMID: 15226673]

79. Higgs JE, Payne K, Roberts C, Newman WG. Are patients with intermediate TPMT activity at increased risk of myelosuppression when taking thiopurine medications? Pharmacogenomics. 2010;11:177-88. [PMID: 20136357]

80. Ford I, Kampanis P, Berg J. Thiopurine S-methyltransferase genotype-phenotype concordance: used as a quality assurance tool to help control the phenotype assay. Ann Clin Biochem. 2009;46:152-4. [PMID: 19164342]

81. Sanderson J, Ansari A, Mariniak T, Duley J. Thiopurine methyltransferase: should it be measured before commencing thiopurine drug therapy? Ann Clin Biochem. 2004;41:294-302. [PMID: 15298741]

82. Ford LT, Berg JD. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment: a pharmacogenomic test whose time has come. J Clin Pathol. 2010;63:288-95. [PMID: 20354201]

83. Payne K, Newman WG, Gurwitz D, Ibarreta D, Phillips KA. TPMT testing in azathioprine: A cost-effective use of healthcare resources? Personal Med. 2009;6:103-13. [PMID: 19064713]

84. Chakravarty K, McDonald H, Pullar T, Taggart A, Chalmers R, Oliver S, et al. British Society for Rheumatology, British Health Professionals in Rheumatology Standards, Guidelines and Audit Working Group. BSR/BHR guide-line for disease-modifying anti-rheumatic drug (DMARD) therapy: in consultation with the British Association of Dermatologists. Rheumatology (Oxford). 2008;47:924-5. [PMID: 16904035]

85. Carter MJ, Lobo AJ, Travis SP; IBD Section, British Society of Gastroenterology. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2004;53 Suppl 5:V1-16. [PMID: 15306560]
Author Contributions: Conception and design: R.A. Booth, M.T. Ansari, E. Loit, A.C. Tricco, L. Weeks, B. Skidmore. Analysis and interpretation of the data: R.A. Booth, M.T. Ansari, A.C. Tricco, L. Weeks, S. Doucette, M. Sears, R. Sy. Drafting of the article: R.A. Booth, M.T. Ansari, A.C. Tricco, S. Doucette, B. Skidmore, M. Sears, R. Sy. Critical revision of the article for important intellectual content: R.A. Booth, M.T. Ansari, M. Sears, R. Sy, J. Karsh. Final approval of the article: R.A. Booth, M.T. Ansari, E. Loit, A.C. Tricco, L. Weeks. Statistical expertise: M.T. Ansari, S. Doucette. Administrative, technical, or logistic support: M.T. Ansari, B. Skidmore, M. Sears. Collection and assembly of data: M.T. Ansari, E. Loit, A.C. Tricco, L. Weeks, B. Skidmore.

86. Wang L, Pellemounter L, Weinshalbourn R, Johnson JA, Hebert JM, Altman RB, et al. Very important pharmacogene summary: thiopurine S-methyltransferase. Pharmacogenet Genomics. 2010;20:401-5. [PMID: 20154640]

87. Arenas M, Duley J, Sumi S, Sanderson J, Marinaki A. The ITPA c.94C>A and g.IVS2 + 21A>C sequence variants contribute to missplicing of the ITPA gene. Biochim Biophys Acta. 2007;1772:96-102. [PMID: 17113761]
88. Salavaggione OE, Wang L, Wiespert M, Yee VC, Weinshalbourn RM. Thiopurine S-methyltransferase pharmacogenetics: variant allele functional and comparative genomics. Pharmacogenet Genomics. 2005;15:801-15. [PMID: 16200112]
89. Ujie S, Sasaki T, Mizugaki M, Ishikawa M, Hiratsuka M. Functional characterization of 23 allelic variants of thiopurine S-methyltransferase gene (TPMT*2~*24). Pharmacogenet Genomics. 2008;18:887-93. [PMID: 18708949]
90. Otterness D, Szumlanski C, Lennard L, Klemetsdal B, Aarbakke J, Park-Hah JO, et al. Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. Clin Pharmacol Ther. 1997;62:60-73. [PMID: 9246020]
91. Hamdan-Khalil R, Allorge D, Lo-Guidice JM, Cauffiez C, Chevalier D, Spire C, et al. In vitro characterization of four novel non-functional variants of the thiopurine S-methyltransferase. Biochem Biophys Res Commun. 2003;309:1005-10. [PMID: 13679074]
92. Hamdan-Khalil R, Gala JL, Allorge D, Lo-Guidice JM, Horsmans Y, Houdret N, et al. Identification and functional analysis of two rare allelic variants of the thiopurine S-methyltransferase gene, TPMT*16 and TPMT*19. Biochem Pharmacol. 2005;69:525-9. [PMID: 15652248]
93. Schaeffeler E, Eichelbaum M, Reinisch W, Zanger UM, Schwab M. Three novel thiopurine S-methyltransferase allelic variants (TPMT*20, *21, *22)—association with decreased enzyme function. Hum Mutat. 2006;27:976. [PMID: 16917910]
94. Sasaki T, Goto E, Konno Y, Hiratsuka M, Mizugaki M. Three novel single nucleotide polymorphisms of the human thiopurine S-methyltransferase gene in Japanese individuals. Drug Metab Pharmacokinet. 2006;21:332-6. [PMID: 16946561]
95. Lindqvist M, Skoglund K, Karlgren A, Soderkvist P, Peterson C, Kidhall I, et al. Explaining TPMT genotype/phenotype discrepancy by haplotyping of TPMT*3A and identification of a novel sequence variant, TPMT*23. Pharmacogenet Genomics. 2007;17:891-5. [PMID: 17885628]
96. Garat A, Cauffiez C, Renault N, Lo-Guidice JM, Allorge D, Chevalier D, et al. Characterisation of novel defective thiopurine S-methyltransferase allelic variants. Biochem Pharmacol. 2008;76:404-15. [PMID: 18660285]
97. Kham SK, Suh CK, Aw DC, Yeoh AE. TPMT*26 (208F—>L), a novel mutation detected in a Chinese. Br J Clin Pharmacol. 2009;68:120-3. [PMID: 19600100]
98. Feng Q, Vannapasraht S, Peng Y, Anguthum S, Avihingsanon Y, Yee VC, et al. Thiopurine S-methyltransferase pharmacogenetics: functional characterization of a novel rapidly degraded variant allozyme. Biochem Pharmacol. 2010;79:1053-61. [PMID: 19945438]
## Appendix Table 1. TPMT Polymorphisms

| Allele      | Nucleotide | Amino Acid Substitution            | Enzyme Activity                          | Allele Frequencies, % | Reference |
|-------------|------------|-----------------------------------|------------------------------------------|-----------------------|-----------|
|             |            |                                   |                                          | White | African | Asian |          |
| TPMT*1      | WT         | Wild-type                         |                                          |        |         |       |          |
| TPMT*2      | 277G→C     | 80Alanine→proline                 | Low                                      | 0-0.7 | 0-0.4   | 0     | 77, 86, 87 |
| TPMT*3A     | 460G→A     | 154Alanine→threonine              | Low                                      | 2.24-8.6 | 0-0.8 | 0-0.3 | 77, 86, 87 |
| TPMT*3B     | 460G→A     | 154Alanine→threonine              | Significant decrease on in vitro assay   | 0-0.13 | 0       | 0     | 77, 86, 87 |
| TPMT*3C     | 719A→G     | 240Tyrosine→cysteine              | Decrease on in vitro assay              | 0.1-0.8 | 2.4-10.1 | 0.6-4.75 | 77, 86, 87 |
| TPMT*3D     | 460G→A     | 154Alanine→threonine              | Intermediate                            | <0.1   |         |       | 78, 86   |
| TPMT*4      | -1G→A (intron 9) | Splicing defect                    | Low                                      | 0.002  |         |       | 78, 86   |
| TPMT*5      | 146T→C     | 49Leucine→serine                  | Decrease on in vitro assay              | 0.0018† |         |       | 88-90    |
| TPMT*6      | 539A→T     | 180Tyrosine→phenylalanine         | Low                                      | 0.2    |         |       | 86, 87   |
| TPMT*7      | 681T→G     | 227Histidine→glutamine            | Intermediate                            | 0.3    |         |       | 86, 87   |
| TPMT*8      | 644G→A     | 95Arginine→histidine              | Intermediate                            | 0.2    |         |       | 86, 87   |
| TPMT*9      | 356A→C     | 119Lysine→threonine               | Intermediate or normal                   | 0.2    |         |       | 78, 86   |
| TPMT*10     | 430G→C     | 144Glycine→arginine               | Decrease on in vitro assay              | 1 patient (1 in 41 screened) |         |       | 13, 86, 88, 91 |
| TPMT*11     | 395G→A     | 132Cysteine→tyrosine              | Low                                      | 1 patient |         |       | 86       |
| TPMT*12     | 374C→T     | 125Serine→leucine                 | Decrease on in vitro assay              | 1 patient† |         |       | 86       |
| TPMT*13     | 83A→T      | 28Glutamic acid→valine            | Decrease on in vitro assay              | 1 patient† |         |       | 86, 88, 91 |
| TPMT*14     | 1A→G       | 1Methionine→valine                | Low                                      | 1 patient |         |       | 86       |
| TPMT*15     | -1G→A (intron 7) | Splicing defect                   | Low                                      | 1 patient |         |       | 86       |
| TPMT*16     | 488G→A     | 163Arginine→histidine             | Intermediate                            | 0.1    | <0.1    |       | 78, 86, 92 |
| TPMT*17     | 124C→G     | 42Glutamine→glutamic acid         | Intermediate                            | 0.1    |         |       | 78, 86   |
| TPMT*18     | 211G→A     | 71Glycine→arginine                | Intermediate                            | 0.1    |         |       | 78, 86   |
| TPMT*19     | 365A→C     | 122Lysine→threonine               | Normal                                  | <0.1   |         |       | 86, 92   |
| TPMT*20     | 712A→G     | 277Lysine→glutamic acid           | Intermediate                            | <0.1   |         |       | 86, 93   |
| TPMT*20†    | 106G→A     | 36Glycine→serine                  | Significant decrease on in vitro assay  | 0.003  |         |       | 86, 94   |
| TPMT*21     | 205C→G     | 69Leucine→valine                  | Intermediate                            | <0.1   |         |       | 86, 93   |
| TPMT*22     | 488G→C     | 163Arginine→proline               | Intermediate                            | <0.1   |         |       | 86, 93   |
| TPMT*23     | 500C→G     | 167Alanine→glycine                | Low                                     | 1 patient (but none in 200 screened) |         |       | 86, 95   |
| TPMT*24     | 537G→T     | 179Glycine→histidine              | Intermediate                            | <0.1   |         |       | 86, 96   |
| TPMT*25     | 634T→C     | 212Cysteine→arginine              | Intermediate                            | <0.1   |         |       | 86, 96   |
| TPMT*26     | 117T→C     | 208Phenylalanine→leucine          | Intermediate                            | 1 patient |         |       | 97       |
| TPMT*27     | 19T→G      | 107Tyrosine→aspartic acid         | Intermediate                            | 1 patient (but none in 220 screened) |         |       | 98       |

TPMT = thiopurine S-methyltransferase.
† Ethnicity not determined.
‡ Originally called TPMT*20 (94), but another paper by the same group (89) refers to this allele as TPMT*24.
### Appendix Figure 1. Forest plot of sensitivity of homozygous genotype for correctly identifying patients with low enzymatic activity.

| Study, Year (Reference) | TPFP FN TN | Sensitivity (95% CI) | Specificity (95% CI) |
|-------------------------|------------|----------------------|---------------------|
| **TPMT*2, *3A, and *3C** |            |                     |                     |
| Ansari et al, 2002 (53) | 0 0 0 40   | NA                  | 100.00 (91.19–100.00) |
| Marinaki et al, 2003 (51)| 0 0 0 85   | NA                  | 100.00 (95.75–100.00) |
| Gardiner et al, 2008 (27)| 0 0 0 68   | NA                  | 100.00 (94.72–100.00) |
| **TPMT*2, *3A, *3B, and *3C** |          |                     |                     |
| Stocco et al, 2004 (64) | 0 0 0 27   | NA                  | 100.00 (87.23–100.00) |
| von Ahsen et al, 2005 (42)| 0 0 0 71   | NA                  | 100.00 (94.94–100.00) |
| Okada et al, 2005 (43)  | 0 0 0 55   | NA                  | 100.00 (93.51–100.00) |
| Stocco et al, 2005 (41) | 0 0 0 28   | NA                  | 100.00 (87.66–100.00) |
| Winter et al, 2007 (29) | 0 0 1 129  | 0.00 (0.00–97.50)   | 100.00 (97.18–100.00) |
| Stassen et al, 2009 (22) | 0 0 0 108  | NA                  | 100.00 (96.64–100.00) |
| Newman et al, 2011 (75) | 1 0 0 332  | 100.00 (2.50–100.00) | 100.00 (98.90–100.00) |
| **TPMT*3A, *3B, and *3C** |          |                     |                     |
| Snow and Gibson, 1995 (62)† | 0 0 0 26   | NA                  | 100.00 (86.77–100.00) |
| Langley et al, 2002 (54) | 0 0 1 52   | 0.00 (0.00–97.50)   | 100.00 (93.15–100.00) |
| Ansari et al, 2008 (23) | 0 0 0 192  | NA                  | 100.00 (98.10–100.00) |
| **TPMT*3A, *3B, *3C, and *3D** |       |                     |                     |
| Hindorf et al, 2004 (45) | 1 0 0 54   | 100.00 (2.50–100.00) | 100.00 (93.40–100.00) |
| **TPMT*2, *3A, *3B, *3C, and *3D** |     |                     |                     |
| Schwab et al, 2002 (55) | 1 0 0 92   | 100.00 (2.50–100.00) | 100.00 (96.07–100.00) |
| **TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, and *15** | |                     |                     |
| Haglund et al, 2004 (48) | 6 0 2 22   | 75.00 (34.91–96.81) | 100.00 (84.56–100.00) |
| Hindorf et al, 2006 (36) | 6 0 0 46   | 100.00 (54.07–100.00) | 100.00 (92.29–100.00) |
| Lindqvist et al, 2006 (33) | 1 0 0 59 | 100.00 (2.50–100.00) | 100.00 (93.94–100.00) |
| Hindorf et al, 2010 (72) | 0 0 0 229  | NA                  | 100.00 (98.40–100.00) |

FN = false negative; FP = false positive; NA = not applicable; TN = true negative; TP = true positive; TPMT = thiopurine S-methyltransferase. † Tested alleles were not reported, so those we assumed most likely to have been tested in 1993 (when the study was conducted) are reported.
Appendix Table 2. Pretreatment Genotyping to Guide Thiopurine Treatment Versus Thiopurine Treatment Without Pretesting

| Variable                        | Studies, n | Patients, n | Domains Pertaining to Strength of Evidence | Odds Ratio (95% CI) | Strength of Evidence |
|---------------------------------|------------|-------------|---------------------------------------------|---------------------|----------------------|
|                                 |            |             | Risk for Bias | Consistency | Directness | Precision |                         |                     |                      |
| Mortality                       | 1 RCT      | 333         | Medium        | Unknown     | Direct     | Imprecise | 0.33 (0.03–3.18)         | Insufficient        |
| Serious adverse events          | 1 RCT      | 333         | Medium        | Unknown     | Direct     | Imprecise | 0.48 (0.14–1.64)         | Insufficient        |
| Health-related quality of life  | 0          | –           | –             | –          | –         | –         | –                        | Insufficient        |
| Myelotoxicity                   | 0          | –           | –             | –          | –         | –         | –                        | Insufficient        |

Applicability of evidence is limited for the outcomes of death and serious adverse events because the patients, most of whom had inflammatory bowel disease, were observed for only 4 months and contained only 1 homozygous carrier of a TPMT variant allele. Also, patients who were more likely to experience adverse events were excluded during the screening phase.

RCT = randomized, controlled trial; TPMT = thiopurine S-methyltransferase.

Appendix Figure 2. Forest plot of odds ratios for leukopenia.