

Assessment of Thiopurine Methyltransferase Activity in Patients Prescribed Azathioprine or Other Thiopurine-based Drugs

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to **epc@ahrq.gov**.

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Structured Abstract

Objectives: To examine whether pretreatment determination of thiopurine methyltransferase (TPMT) enzymatic activity (phenotyping) or TPMT genotype, to guide thiopurine therapy in chronic autoimmune disease patients, reduces treatment harms. Other objectives included assessing: preanalytic, analytic, and postanalytic requirements for TPMT testing; diagnostic accuracy of TPMT genotyping versus phenotyping; association of thiopurine toxicity with TPMT genotypic or phenotypic status; and costs of testing, care, and treating drug-associated complications.

Data Sources: MEDLINE®, EMBASE®, and Healthstar were searched from inception to May 2010; the Cochrane Library® to October 2009; and BIOSIS®, Genetics Abstracts, and EconLit™ to May 2009, for English language records.

Review Methods: A reviewer screened records, and a second reviewer verified exclusions and subsequent selection of relevant studies. Studies in patients with leukemia and organ transplant were excluded. Additionally, laboratories that provide TPMT analytical services were surveyed to assess means of TPMT testing in practice. Where possible, risk of bias was assessed using standard criteria. Meta-analyses estimated diagnostic sensitivity, and specificity; and odds ratios of associations.

Results: 1790 titles or abstracts, and 538 full text records were screened. 114 observational studies and one RCT were included. Majority of studies were rated fair quality, except for diagnostic studies with 37 percent of studies rated poor. In general, there were few patients who were homozygous (or compound heterozygous) for TPMT variant alleles in the included studies limiting applicability.

There is insufficient evidence examining effectiveness of pretesting in terms of reduction in clinical adverse events.

Sufficient preanalytical data were available regarding preferred specimen collection, stability and storage conditions for TPMT testing. There was no clinically significant effect of age, gender, various coadministered drugs, or most morbidities (with the exception of renal failure and dialysis). TPMT phenotyping methods had coefficients of variation generally below 10 percent. TPMT genotyping reproducibility is generally between 95-100 percent.

The sensitivity of genotyping to identify patients with low or intermediate TPMT enzymatic activity is imprecise, ranging from 70.70 to 82.10 percent (95 percent CI, lower bound range 37.90 to 54.00 percent; upper bound range 84.60 to 96.90 percent). Sensitivity of homozygous TPMT genotype to correctly identify patients with low to absent enzymatic activity was 87.10 percent (95 percent CI 44.30 to 98.30 percent). Genotyping specificity approached 100 percent.

Leukopenia was significantly associated with low and intermediate enzymatic activity (low activity OR 80.00, 95 percent CI 11.5 to 559; and intermediate activity OR 2.96, 95 percent CI 1.18 to 7.42), and homozygous and heterozygous TPMT variant allele genotype (OR 18.60, 95 percent CI 4.12 to 83.60; and 4.62, 95 percent CI 2.34 to 9.16, respectively). In general, TPMT phenotyping costs less than genotyping, although estimates across studies are quite heterogeneous.

Conclusions: There is insufficient direct evidence regarding the effectiveness of pretesting of TPMT status in patients with chronic autoimmune diseases. Indirect evidence confirms strong association of leukopenia with lower levels of TPMT activity and carrier genotype already established in the literature.

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Appendixes and Evidence Tables for this report are provided electronically at
<http://www.ahrq.gov/downloads/pub/evidence/pdf/tpmt/tpmt.pdf>.

Executive Summary

Background

Thiopurine drugs are used to treat chronic autoimmune inflammatory conditions and hematological malignancies, and to prevent organ transplant rejection. The present study focuses on populations with autoimmune disease.

Thiopurine drugs are associated with various toxic adverse effects, including myelosuppression, hepatotoxicity, pancreatitis, and flu-like symptoms. One of the most serious dose-dependent reactions is severe myelosuppression that is thought to be caused by the active metabolite, deoxy-6-thioguanosine 5' triphosphate (6-tGN). Excessive levels of 6-tGN may arise not only due to overdosing, but also because of decreased inactivation of the drug.

The most extensively characterized enzyme in the metabolism of thiopurines is thiopurine methyltransferase (TPMT). TPMT inactivates the active forms of two commonly used thiopurine drugs, azathioprine (AZA) and 6-mercaptopurine (6-MP), by methylation. Multiple studies have shown that lower TPMT enzymatic activity is correlated with higher levels of the active drug metabolites and increased thiopurine toxicity. Genetic polymorphisms associated with lower TPMT enzymatic activity are similarly correlated.

Approximately 0.3 percent of the population with chronic autoimmune disease that could potentially benefit from thiopurine treatment is homozygous for a variant TPMT allele expressed as low or even absent TPMT activity. These patients are at greatest risk of myelosuppression. Approximately 15 percent of the patient population is heterozygous for variant alleles; they are likely to have intermediate TPMT enzymatic activity, with moderate risk of myelosuppression with thiopurine therapy. Four common variant alleles (TPMT*2, *3A, *3B, and *3C) account for 80 percent to 95 percent of individuals with below normal TPMT activity; however the frequency of these alleles varies among Caucasians, Asians, and Africans.

Until recently, the recommended starting dose of either AZA or 6-MP did not take into account patients with very low or absent TPMT activity. The initial doses range from 1.0 to 2.5 mg/kg/day for AZA, and 0.75 to 1.25 mg/kg/day for 6-MP. It has been proposed that patients with either intermediate or low to absent TPMT activity may benefit from lower initial doses.

Various clinical guidelines recommend measuring TPMT enzymatic activity or screening for TPMT alleles before starting patients on thiopurine drugs. However, the evidence base for these recommendations is unclear. It is also unclear whether one or both of the tests, TPMT genotyping or enzymatic activity (phenotyping) should be used to determine TPMT status before thiopurine treatment initiation. As such, there is a need to review the current literature regarding the assessment of TPMT status prior to administration of thiopurine drugs, to determine if pretreatment TPMT testing reduces drug-related toxicity. The population of interest was restricted to those with chronic autoimmune disease, as patients with malignancy or organ transplant frequently require concomitant treatments of similar toxicity profile.

This evidence report was commissioned by the Agency for Healthcare Research and Quality (AHRQ) to address the following of questions about TPMT genotypic and phenotypic testing methodology, their comparative diagnostic accuracy, effectiveness of pretreatment testing, association with drug toxicity, and costs involved.

Key Questions

KQ1. In terms of the analytical performance characteristics of enzymatic measurement of TPMT activity and determination of TPMT allelic polymorphisms:

- a) What are the preanalytical requirements for enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms? (e.g. specimen types and collection procedures, lab transportation, interference of coadministered drugs, patient preparation and identification etc.)
- b) What are the within and between laboratory precision and reproducibility of the available methods of enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms (proficiency testing)?
- c) What is the diagnostic sensitivity and specificity of TPMT allelic polymorphism measurement compared to the measurement of TPMT enzymatic activity in correctly identifying chronic autoimmune disease patients eligible for thiopurine therapy with low or absent TPMT enzymatic activity? How do effect modifiers (e.g. underlying disease prevalence and severity, different activity thresholds, Hardy-Weinberg equilibrium, number and types of alleles tested) explain any observed heterogeneity in sensitivity and specificity?
- d) Are there any postanalytical requirements specific to measurement of TPMT enzymatic activity or TPMT allelic polymorphism measurement? (e.g. timely reporting of data, reference intervals, immediate or reporting within a time-frame, highlighting of extreme results)

KQ2. Does the measurement of TPMT enzymatic activity or determination of TPMT allelic polymorphisms change the management of patients with chronic autoimmune disease when compared with no determination of TPMT status?

KQ3. In chronic autoimmune disease patients prescribed thiopurine-based drugs (AZA or 6-MP), does the assessment of TPMT status to guide therapy, when compared with no pretreatment assessment, lead to:

- a) reduction in rates of mortality, infection, hospitalization, withdrawal due to adverse events (WDAE), serious adverse events (SAE) and improvement in health-related quality of life?
- b) reduction in rates of myelotoxicity, liver toxicity, and pancreatitis?
- c) In the absence or inconclusiveness of evidence answering key question 3a and/or 3b above, is there an association between TPMT status (as determined by TPMT enzymatic activity and/or TPMT allelic determination) and/or the following amongst chronic autoimmune disease patients treated with thiopurines?
 - i. the clinical outcomes of mortality, infections, hospitalization, WDAE, SAE and health-related quality of life?
 - ii. surrogate outcomes of myelotoxicity, liver toxicity, and pancreatitis?

KQ4. What are the costs of determining TPMT enzyme activity and/or genotyping for patients with chronic autoimmune disease being considered for thiopurine-based therapy (e.g., costs of testing, costs of care, and costs of treating drug-associated complications)?

Methods

Search Strategy

The following databases were searched: Ovid MEDLINE® 1950 to May Week 3 2010; The Cochrane Library® (CLIB 2009 3) including CENTRAL, CDSR, DARE, HTA, and NHSEED; BIOSIS® May 6 2009; EMBASE® 1980 to 2010 Week 21; Genetics Abstracts: May 7 2009; and Ovid Healthstar 1966 to April 2010. EconLit™ was searched May 7 2009 for the economic question (Key Question 4).

Study Selection

English language records of any study design in chronic autoimmune disease populations were included. Effectiveness studies of testing prior to treatment were restricted to comparative experimental or observational designs.

Outcomes included determinants of preanalytic variability and proficiency of TPMT genotypic and phenotypic (enzymatic activity) testing; diagnostic accuracy of genotypic testing compared with the enzymatic assay; clinical and laboratory measures of drug toxicity; and costs of both testing and drug-associated complications.

One reviewer screened abstracts to include studies, and a second reviewer independently verified exclusions. Two reviewers independently screened full-text reports, with conflicts resolved by consensus or third party adjudication. Data were extracted in standardized forms.

Risk of Bias Assessment

Standard criteria were used to assess risk of bias of individual studies, except for studies eligible for questions pertaining to TPMT testing methods (KQ 1a and 1b) and costs (KQ4), for which no assessment scales exist. Studies were assessed as good, fair or poor.

Evidence Synthesis

Evidence was synthesized qualitatively for key questions 1a, 1b and 4. Data synthesis was not possible for key questions 1d, 2, 3a and 3b due to scarcity of evidence. We therefore examined associations between thiopurine toxicity and TPMT genotype and phenotype (KQ3c). For key question 4, costing data were converted to U.S. dollars (2009) using purchasing power parities, inflated to reflect 2009 values using the consumer price index for U.S. medical care for all urban consumers.

Quantitative syntheses were undertaken with the underlying assumption that given similar doses of the drugs, differences in outcomes of thiopurine toxicity arise from differences in TPMT enzymatic activities. Because enzymatic activity or genotype are the main determinant of thiopurine toxicity, we assumed that the underlying autoimmune disease, method of genotyping or phenotyping (enzymatic activity testing), population demographics, and study design did not give rise to substantial diversity in effect estimates. We, therefore, pooled studies across these covariates to estimate diagnostic accuracy and strength of association with adverse events related to TPMT testing and status, respectively. Individual study estimates (odds ratios, or sensitivity and specificity) were pooled using DerSimonian and Laird's random-effects model, with

weighting by individual study variance and the estimated between-study heterogeneity. Data were pooled when two or more studies were in a given analysis for an outcome. Pooled estimates of diagnostic sensitivity and specificity, and odds ratios and their 95 percent confidence intervals (CIs) were calculated using CMA software (version 2.2.046). With small numbers of studies in most analyses, we could explore clinical and/or methodological diversity for very few of the preidentified covariates. When feasible, statistical heterogeneity was tested using Cochran's Q, and reported when found to be substantial (p value for chi-squared test of heterogeneity below 0.10, and I² above 50 percent).

For quantitative syntheses of evidence of genetic association studies for drug toxicity outcomes, a codominant model was used to pool estimates associated with noncarrier, heterozygous carrier and homozygous carrier states. *Noncarrier* state indicated absence of tested TPMT polymorphisms. *Heterozygous* carrier state indicated presence of one variant TPMT allele on one of the paired chromosomes; *homozygous* carrier state implied presence of one of the identical TPMT variant alleles on each one of the paired chromosomes, or presence of two different variant alleles each on one of the two paired chromosomes (the latter is also called a compound heterozygous state). Similarly, three categories of enzymatic activities were defined (high/normal, intermediate and low/absent). We compared each state with the other two genotypic or phenotypic states. The TPMT enzymatic activity assay was considered to be the reference, for the index test of genotyping of the different single nucleotide polymorphisms (SNPs). With a dichotomous index test, i.e. the presence or absence of variant alleles, investigation of implicit or explicit cut-off threshold effects was ruled out by design. Therefore, we pooled for the outcomes of test sensitivity and specificity for each set of variant TPMT alleles tested.

Rating the Strength of Evidence

Evidence of comparative effectiveness of TPMT pretesting versus no testing for the critical and important outcomes of mortality (critical), serious adverse events (critical), myelotoxicity (important), and health-related quality of life (important) was rated across the domains of risk of bias, consistency, directness and precision as high, moderate, low or insufficient.

Laboratory Survey

To augment the limited published literature to answer key questions 1a, 1b, 1c, and 1d, further data regarding the preanalytical and postanalytical requirements and performance characteristics of TPMT laboratory analyses were collected. With advice from the Technical Expert Panel, the review team decided to survey English speaking laboratories that provide TPMT analytical services. Seven laboratories were contacted. An 11-item questionnaire addressing TPMT analytical methods (e.g., sample type and handling), preanalytical requirements (e.g., specimen stability), quality control procedures, and reporting of results was administered via Survey Monkey™.

Results

We screened 1790 titles or abstracts and 538 full text records. One hundred and fifteen unique studies and their 21 companion reports were included. One randomized controlled trial

was included; all other studies were of observational design. The majority (greater than 75 percent) of studies were rated as fair, while a substantial (37 percent) percentage of diagnostic studies were of poor design. No evidence was found to answer Key Question 1d. Sparse evidence answered Key Questions 2, 3a and 3b. In general, there were few patients who were homozygous (or compound heterozygous) for TPMT variant alleles in the included populations.

Six of the seven laboratories invited to participate in the survey returned responses; three from Canada and three from the United Kingdom. Among the responses, yearly TPMT testing volumes ranged from 50 to 1500 allelic determinations and 600 to 19,000 enzymatic determinations.

KQ 1a: Preanalytical Requirements for TPMT Enzymatic Activity and allelic Polymorphisms Measurements

Storage conditions and study designs varied widely across 13 studies assessing the influence of storage on TPMT activity. Temperatures ranging from -85°C to room temperature, and time periods ranging from a few hours to 16 months were studied. TPMT is a stable enzyme and its activity remained constant during storage at room temperature for five days or at -20°C for three months. Storage at -80°C resulted in 15 percent of TPMT activity decrease after 16 months. All surveyed laboratories analyzed specimens of blood with EDTA anticoagulant, stored for up to eight days, at 4°C or room temperature before analysis. Other factors noted prior to testing, such as gender, age, and race did not significantly affect the TPMT enzyme activity.

Nineteen different drugs studied to date in patients being treated for autoimmune conditions had no clinically relevant inhibiting effect on TPMT activity (see the main Results section for a list of drugs). Studies showing potentially clinically significant effects were conducted in vitro, and therefore their in vivo influence on TPMT activity remains unknown.

Research suggests that younger red blood cells (RBCs) have higher TPMT activity than older RBCs, but these differences are not clinically relevant and can be avoided if the TPMT activity is expressed per grams of hemoglobin or per milliliter of packed RBCs. However, these two reporting units are not identical and results are not directly comparable.

Two studies investigated the effect of comorbid conditions on TPMT activity, including inflammatory bowel disease, autoimmune hepatitis, multiple sclerosis, myasthenia gravis, pemphigus and chronic renal failure. They reported clinically insignificant differences in TPMT activity for all diseases, except for patients with the chronic renal failure. These patients' TPMT activity predialysis was 50 percent higher than healthy controls, but postdialysis levels dropped to levels comparable with the controls'.

No evidence was reported for patient preparation or identification. Also, no evidence was found regarding preanalytical factors influencing TPMT genotyping. However, since preanalytical requirements are commonly understood for genetic testing, previously published guidelines can be used. The Clinical and Laboratory Standards Institute (CLSI) has published guidelines covering all preanalytical requirements for collection, transportation, preparation and storage of specimens for genetic testing.

KQ 1b: Within and Between Laboratory Precision and Reproducibility of Enzymatic Measurement of TPMT and Determination of TPMT Allelic Polymorphisms

Enzymatic assays measure the S-methylation of 6-MP by TPMT to form 6-methylmercaptapurine. Initially, 6-methylmercaptapurine was measured using a radiolabel method, which was later replaced by high performance liquid chromatography (HPLC). Alternatively, 6-thioguanine (6-TG) may be used as a substrate in HPLC-based methods, which measure 6-methyl-TG. All methods used to measure the TPMT activity are highly precise and accurate. The radiolabel method reported by eight studies had interassay and intra-assay variation coefficients from 0.51 to 8.4 percent and from 0.72 to 6.8 percent, respectively. The HPLC based methods produced inter-assay and intra-assay coefficients of variation ranging from 0.2 to nine percent and from zero to 9.5 percent respectively, in 16 studies. Among surveyed laboratories, enzymatic analysis repeatability ranged from three to 10 percent within runs, and from five to 20 percent between runs.

We found only three studies that investigated TPMT genotyping test performance. Matrix-assisted laser desorption/ionization, with a time-of-flight (MALDI-TOF) mass spectrometry multiplex assay and TaqMan® 5' nuclease assays were compared with denaturing HPLC, and 100 percent concordance was observed for 586 and 50 genotypes, respectively. The MALDI-TOF study also measured reproducibility to be in 100 percent agreement, when 10 percent of randomly selected samples of the study population were genotyped in duplicate. A novel microchip platform was compared with TaqMan® and with a conventional restriction fragment length polymorphism (RFLP) assay, resulting in 100 percent concordance.

KQ 1c: Diagnostic Sensitivity and Specificity of TPMT Allelic Polymorphism Measurement, Compared to the Measurement of TPMT Enzymatic Activity

A total of 16 studies, mostly of cross-sectional and prospective observational design, contributed to quantitative syntheses. Studies did not specifically examine diagnostic accuracy of genetic testing with the TPMT enzymatic activity test as the reference standard, so we designated the activity test to be the reference standard and genotyping to be the index test. The pooled sensitivity of the carrier genotype (i.e. homozygous plus heterozygous patients) to correctly identify all those patients with subnormal (intermediate, or low to absent) enzymatic activity was imprecise and ranged from 70.70 to 82.10 percent across the different subgroups of alleles tested (95 percent CI, lower bound range 37.90 to 54.00 percent; upper bound range 84.60 to 96.90 percent). The pooled sensitivity of a homozygous TPMT genotype to correctly identify patients with low to absent enzymatic activity was based on two studies with few homozygotes (87.10, 95 percent CI 44.30 to 98.30 percent). Meta-regression analysis did not identify any significant effect modifiers. Compared with the reference standard of TPMT enzymatic activity, the specificity of TPMT genotyping to correctly identify patients with normal/high enzymatic activities, or normal/high and intermediate enzymatic activities, was very high (greater than 90 percent) across all combinations of alleles tested.

There was insufficient data to determine the optimum combination of TPMT alleles for testing. Approximately 80 percent of the studies tested at least the TPMT *3A, *3B, and *3C or TPMT *2, *3A, and 3C variant alleles, irrespective of testing additional polymorphisms.

Among the surveyed laboratories, reported concordance between enzymatic analysis genotyping ranged from 60 percent to 100 percent.

KQ 2: Knowledge of TPMT Status and Change in Management

Evidence from one randomized controlled trial in 333 patients with chronic inflammatory conditions of whom only one patient was homozygous for a variant allele suggests that physician azathioprine prescribing practice may not entirely be guided by pharmacogenetic testing. Cautious prescribing is adopted by physicians regardless of pre treatment genotyping results. No significant differences could be observed between the prethiopurine tested and nontested groups in azathioprine starting doses or mean doses at the end of the study period, however, heterozygotes received lower doses compared with noncarriers in the group pretested before therapy.

KQ 3a and 3b: Knowledge of TPMT Status to Guide Therapy

Table 1. Rating the strength of evidence

Pretreatment genotyping to guide thiopurine treatment vs. thiopurine treatment without pretesting								
Outcome	N of studies	N of Subjects	Domains pertaining to strength of evidence				OR (95% CI)	Strength of evidence
			Risk of Bias	Consistency	Directness	Precision		
Mortality	1 RCT ¹	333	Medium	Unknown	Direct	Imprecise	0.33 (0.03 to 3.18)	Insufficient
Serious adverse events	1 RCT ¹	333	Medium	Unknown	Direct	Imprecise	0.48 (0.14 to 1.64)	Insufficient
Health-related quality of life	0	0	-	-	-	-	-	Insufficient
Myelotoxicity	0	0	-	-	-	-	-	Insufficient
Applicability of evidence	There is limited applicability of evidence for the outcomes of mortality and serious adverse events since there was just one homozygous carrier of TPMT variant allele in the entire sample of mostly IBD patients observed for just 4 months. Also, patients likelier to experience adverse events were excluded during the screening phase							

Abbreviations: RCT = randomized controlled trial

Evidence comparing efficacy of prior TPMT status determination with no pretesting from one fair quality randomized controlled trial and a poor quality retrospective cohort study demonstrated no significant differences in the outcomes of leukopenia, neutropenia and pancreatitis, while significantly higher odds were observed for hepatitis in the group randomized to prior TPMT genotyping, odds ratio 2.54 (95 percent CI 1.08 to 5.97). Other intermediate outcomes were not reported.

KQ 3c: Association Between TPMT Status and Thiopurine Toxicity

TPMT enzymatic activity. Among 15 studies, mostly cross-sectional in design, quantitative syntheses demonstrated a dose response relationship associating subnormal TPMT enzymatic activities with leukopenia and myelotoxicity. In comparison with normal enzymatic activity, greater odds of leukopenia were noted with low enzymatic activity (OR 80.00, 95 percent CI 11.5 to 559), than intermediate activity (OR 2.96, 95 percent CI 1.18 to 7.42). Greater odds of myelotoxicity were also noted with low activity when compared with intermediate (OR 10.20, 95 percent CI 2.23 to 46.60) and normal (OR 13.60, 95 percent CI 3.52 to 52.80) TPMT enzymatic activities.

Pooling of the few small studies with events for the outcomes of withdrawal due to adverse events, anemia, hepatitis or elevated hepatic transaminases and pancreatitis, revealed no significant associations.

No evidence was available for the outcomes of mortality, hospitalization, serious adverse events, and health related quality of life. The sparse data available for the outcomes of infection, neutropenia and thrombocytopenia did not permit a meaningful synthesis.

TPMT genotype. Thirty studies contributed to quantitative syntheses. A dose response relationship was suggested between TPMT genotypic status and leukopenia. In studies testing TPMT *2, *3A, *3B, and *3C, plus/minus additional genetic variants, homozygosity for a variant TPMT allele yielded the highest odds ratio for leukopenia (18.60, 95 percent CI 4.12 to 83.60) compared with noncarrier status, while heterozygous patients experienced lower, but still significantly increased odds (4.62, 95 percent CI 2.34 to 9.16) compared with noncarriers. However, with only 6 homozygous participants, direct comparison with heterozygous carriers did not yield statistically significant results.

For all other outcomes of mortality, hospitalization, serious adverse events (SAE), health related quality of life (HQOL), neutropenia, infection, withdrawal due to adverse events, myelotoxicity, anemia, thrombocytopenia, hepatitis or elevated hepatic transaminases, and pancreatitis, evidence was either absent, insufficient or lacked power to demonstrate significant differences between heterozygous and homozygous carriers in comparisons with noncarriers, and between themselves.

KQ 4: Costs of TPMT Testing, Costs of Care, and Costs of Treating Drug-Associated Complications

Eleven studies reported data on the costs of determining TPMT activity and/or genotyping for patients with chronic autoimmune disease being considered for thiopurine therapy. The studies were conducted in Canada, United States, New Zealand, Europe (Italy, Scotland, United Kingdom, Spain), and Korea. All data were converted into U.S. dollars (2009).

Eight studies reported 11 cost estimates for TPMT genotyping, which were obtained from public and private laboratories or hospitals. The cost of obtaining a test per patient ranged from \$29.43 to \$617.80, with the highest cost being reported by a private laboratory. Excluding the cost from the private laboratory, the average cost for the genotype test per patient was \$89.94.

Four studies reported five cost estimates for the TPMT enzymatic analysis, which were also from laboratories or hospitals. The cost of obtaining a test per patient ranged from \$46.36 to \$320.98 and the source for the highest cost was not reported. Excluding the highest costing item, the average cost for the TPMT phenotype test per patient was \$53.13.

Seven studies reported eight cost estimates for treating AZA related complications. The costs were obtained from hospitals and governmental agencies. The one-time cost of adverse events associated with AZA ranged from \$1,366.82 to \$7,110.02 USD (2009), with an average of \$4,019.29. One study reported two cost estimates for the average cost per identified TPMT-deficient individual, with an average of \$11,848.51.

Conclusions

There is currently insufficient evidence regarding effectiveness of determining TPMT status prior to thiopurine treatment in terms of improvement in clinical outcomes and incident myelotoxicity in comparison with routine monitoring of full blood counts and adverse events. It is also unclear whether pretesting guides appropriate prescribing. Indirect evidence confirmed previously known strong associations between lower levels of TPMT enzymatic activity and the presence of TPMT variant alleles with thiopurine related leukopenia. Sufficient preanalytical data were available to recommend preferred specimen collection, stability and storage conditions for determination of TPMT status. There was no clinically significant effect on TPMT activity of age, gender, various coadministered drugs, or most morbidities (with the exception of renal failure and dialysis). The available methods for determination of TPMT enzymatic activity showed good precision, with coefficients of variation generally below 10 percent. Based upon limited evidence, the reproducibility of TPMT allelic polymorphism determination is acceptable. However, the sensitivity of genetic testing to identify patients with low or intermediate TPMT enzymatic activity is imprecisely known. Thus, if knowledge of TPMT status is desired and there has been no recent transfusion of RBCs, with the current evidence enzymatic assay (phenotyping) rather than allelic polymorphism determination is preferred. Enzymatic assay will capture effects of other polymorphisms that are not detected by genotyping the common alleles; laboratories tend to use genotyping as a confirmatory test for low TPMT activity. The average cost of TPMT phenotyping was approximately half of the average cost of TPMT genotyping, but these costs may not be generalizable to all TPMT tests. More research has been conducted examining TPMT genotyping but the cost estimates are heterogeneous, likely due to different methodological choices.

Remaining Issues

There is insufficient evidence examining the effectiveness of TPMT pretreatment enzymatic or genetic testing, to minimize thiopurine related toxicity in patients with chronic autoimmune diseases. As a priority, well powered, good quality, randomized controlled studies need to be conducted, in diverse and representative patient populations, to compare the effectiveness of TPMT genotyping and phenotyping with one another, and with no TPMT testing. These studies should be large enough to include a sizable number of patients homozygous for the variant alleles and should be pragmatic in conduct, mimicking routine clinical practice. Outcomes would include both treatment efficacy and harms associated with thiopurine therapy. Another objective would be to establish the optimum initial dose adjustment for a given TPMT status. These studies should ensure that outcomes are truly assessed without prior knowledge of results of TPMT testing and administered drug dose, by employing appropriate blinding procedures. The recently concluded pragmatic TARGET study by Newman and associates was under-powered to detect differences in clinically important outcomes, largely because it faced recruitment

problems. In future such recruitment problems may be mitigated by educating the public and clinicians that the evidence base for pretreatment TPMT testing is lacking and that it is unclear whether pretreatment testing does more good (i.e. reduction in thiopurine related toxicity) than harm (i.e. reduction in thiopurine efficacy because of overzealous dose reductions based on prior testing).

Until such experimental high quality evidence becomes available, alternative evidence may be sought in prospectively designed observational studies that estimate health related quality of life, drug prescription patterns, and myelotoxicity related mortality as important outcomes associated with and with no pretreatment TPMT testing. With availability of empiric evidence from such studies, decision-analytic modeling that comprehensively consider alternative strategies such as regular blood cell count and liver enzyme testing, metabolite monitoring, and dose adjustments for concomitant medications that impact the TPMT enzymatic pathway can help guide practice until evidence becomes available from well powered pragmatic trials. Subsequent models might also need to consider new information as technologies develop and knowledge evolves.

TPMT genotyping should test for the most common TPMT polymorphisms in the population of interest. There is little direct evidence identifying the optimum set of alleles to be tested, and this may need to be established for specific populations if TPMT genotyping turns out to be effective in future studies.

TPMT activity analyses are reported on one of two bases: per milliliter of packed red blood cells; or per gram of hemoglobin. These are not readily or exactly comparable. Common reporting units are needed, as well as cutoffs for low/absent, intermediate, normal TPMT enzymatic activity, and high enzymatic activities.

Future studies should clearly report numbers of uninterpretable or equivocal test results.

Evidence Report

Chapter 1. Introduction

Background

Thiopurines make up a class of immunosuppressive and chemotherapeutic drugs that is used effectively in the treatment of chronic autoimmune inflammatory conditions, hematological malignancies, and prevention of organ transplant rejection. Azathioprine (AZA), 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) are the thiopurine drugs currently used in clinical practice. The clinical response to thiopurines varies according to the nature of disease, dose and patient metabolism of the drugs.

AZA and 6-MP are currently widely used as steroid sparing agents in chronic autoimmune inflammatory conditions, including pemphigoid, inflammatory bowel disease, and rheumatoid arthritis, among others. AZA and 6-MP are effective in inducing remission in 50 percent to 60 percent of inflammatory bowel disease patients, and permit steroid reduction or withdrawal in up to 65 percent of patients.² Clinical response rates using AZA to treat nonbullous inflammatory dermatoses can be as high as 75 percent.³ However, use of AZA or 6-MP in other chronic inflammatory disorders including lupus and rheumatoid arthritis has been variable, and they are often not the primary drugs of choice.

When used among organ transplant patients, although AZA has been associated with a 5-year renal graft survival ranging from 70 percent to 92 percent,^{4,5} use of AZA and 6-MP in transplantation has declined somewhat in favor of other immunosuppressive drugs.

Both 6-MP and 6-TG have been used effectively in treatment of childhood acute lymphoblastic leukemia, with remission rates (5-year relapse free survival) of approximately 80 percent using 6-MP.⁶

Patients with cancer or transplanted organs are clinically more complex, so this review focuses on thiopurine use in autoimmune conditions.

Biochemistry of Thiopurines

AZA and 6-MP are pro-drugs that have no intrinsic biological activity, and require extensive metabolism for activity (Figure 1). After oral administration of AZA or 6-MP, between 27 percent and 83 percent is biologically available. AZA is often used clinically, as it is more stable and soluble than 6-MP. AZA doses are higher because the molecular weight of 6-MP is 55 percent of that of AZA.

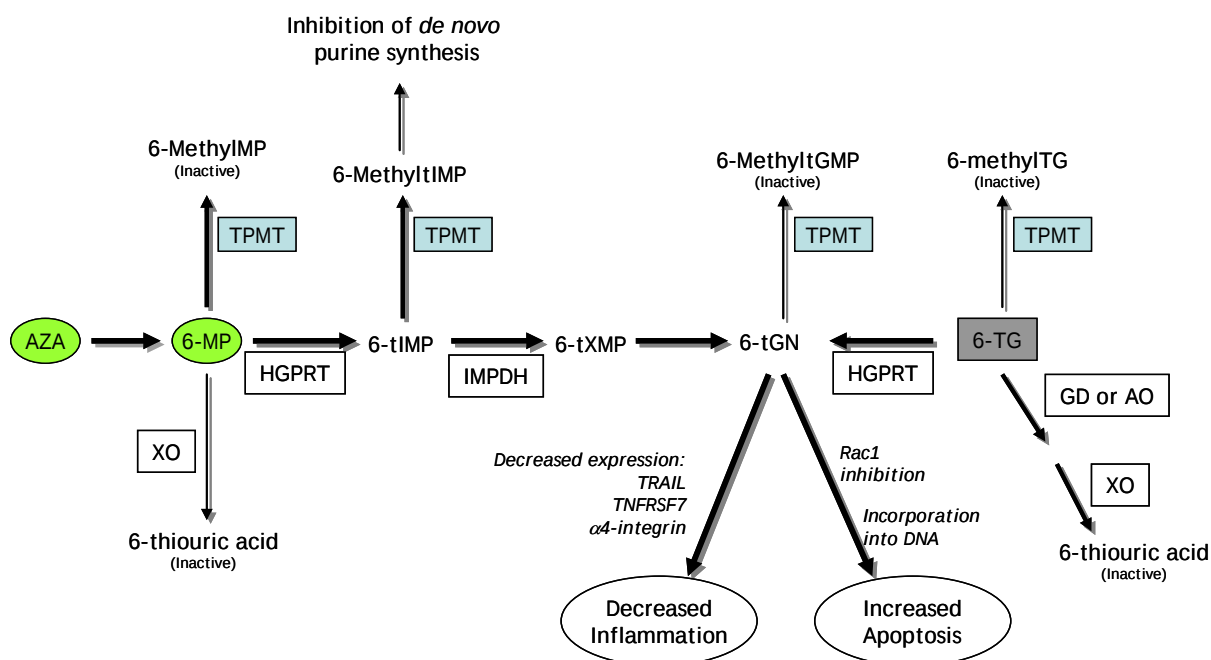
In the gut, approximately 90 percent of AZA is converted to 6-MP, a thiopurine analogue of the purine base hypoxanthine, by cleavage of the imidazolyl moiety which is thought to be catalyzed through the action of glutathione transferase.⁷ 6-MP is then enzymatically converted to its active metabolite, deoxy-6-thioguanosine 5' triphosphate (6-tGN), through successive enzymatic conversions by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and inosine monophosphate dehydrogenase (IMPDH). Inactivation of 6-MP (and hence AZA) occurs primarily through S-methylation by thiopurine S-methyltransferase (TPMT), and to a minor degree by catabolism, to thiouric acid by xanthine oxidase (XO).

6-TG is converted to its active metabolite (6-tGN) in a single step involving HGPRT, while inactivation occurs through two pathways. The major metabolic pathway involves guanine deaminase (GD) and aldehyde oxidase (AO) to form inactive 6-thiouric acid. Metabolism by

TPMT, to form inactive 6-methyl-TG, is a minor contributor to drug inactivation. TPMT also plays a minor role in directly methylating and inactivating 6-tGN.

Incorporation of 6-tGN into DNA triggers cell-cycle arrest and apoptosis through the mismatch repair pathway. Until recently, this was considered the primary mechanism of action.⁸ However, recent evidence has suggested other mechanisms of immunosuppression not directly related to 6-tGN incorporation into DNA. Metabolism by TPMT of 6-thiomercaptopurine (6-tIMP), an intermediate metabolite, to produce 6-methyl –tIMP has been shown to inhibit *de novo* purine synthesis in lymphocytes, which likely contributes to the immunosuppressive effects of thiopurines.⁹ Furthermore, accumulation of 6-tGN in lymphocytes has been demonstrated to decrease the expression of TRAIL, TNFRSF7, and α -4 integrin, effectively decreasing inflammation. Thiopurine drugs have also been shown to induce apoptosis in T-cells through modulation of Rac1 activation upon CD28 costimulation. Rac1 is a GTPase upstream of MEK, NF- κ B, and bcl-xL. Upon binding of 6-thio-GTP with Rac1, activation of its downstream mediators is blocked, inducing apoptosis.¹⁰

Figure 1. Metabolic pathways of thiopurine drugs



Abbreviations: 6-MP = 6-mercaptopurine; 6-tGN = 6-thioguanine nucleotides; 6-tIMP = 6-thiomercaptopurine; 6-TG = 6-thioguanine monophosphate; 6-tGN = deoxy-6-thioguanosine 5' triphosphate; 6-tXMP = 6-thiooxanthosine; AO = aldehyde oxidase; AZA = azathioprine; GD = guanine deaminase; HGPRT = hypoxanthine guanine phosphoribosyltransferase; IMPDH = inosine monophosphate dehydrogenase; TPMT = thiopurine S-methyltransferase; XO = xanthine oxidase

Thiopurine Toxicity

Thiopurine-based drugs have been associated with various toxic adverse events, including myelosuppression, hepatotoxicity, pancreatitis, and flu-like symptoms, among others. One of the most serious dose-dependent reactions is myelosuppression, which is believed to be caused by increased 6-tGN levels (the active metabolite), either due to overdosing or a low rate of

thiopurine metabolism. The most extensively characterized enzyme in the metabolism of thiopurines is TPMT.

TPMT polymorphisms. The TPMT gene is located on chromosome 6 at 6p22.3. It is approximately 27 kb in size and contains 9 exons.^{11,12} A nonfunctional TPMT pseudogene has also been identified on chromosome 18 at 18q21.1.¹³ TPMT is widely expressed in many tissues, but TPMT expression in lymphocytes, red blood cells and bone marrow is most relevant clinically for immunosuppression by thiopurine drugs. To date, at least 30 variant (or mutant) alleles of TPMT have been identified, the majority of which have been associated with lower TPMT enzymatic activity or protein expression (Table 2).¹⁴ Several studies have highlighted the importance of thiopurine drug metabolism by TPMT, as lower TPMT may place patients at higher risk of developing drug-related toxicity.^{15,16} The four most common alleles (TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C) seen in Caucasians, Asians, and Africans account for approximately 80 percent to 95 percent of individuals with lower TPMT activity.¹⁷⁻²² When comparing genotype to phenotype (enzymatic activity), homozygous mutant individuals have very low or absent enzymatic activity while those heterozygous for a mutant allele demonstrate intermediate enzymatic activity, between those of noncarrier and homozygous individuals. The frequency of the common alleles within each ethnic group varies, as does the overall number of individuals with lower TPMT activity. Heterozygous individuals with intermediate enzymatic activity comprise five percent to 15 percent of patients, while approximately 0.3 percent are homozygous, with very low or absent enzymatic activity.^{17,18,23}

Table 2. TPMT polymorphisms

Allele	Nucleotide	Amino acid substitution	Enzyme activity
TPMT*1	WT		Wild-type activity.
TPMT*2	238G→C	80Ala→Pro	Low ²⁴
TPMT*3A	460G→A	154Ala→Thr	Low ²⁴
	719A→G	240Tyr→Cys	
TPMT*3B	460G→A	154 Ala→Thr	In vitro assay: significant enzyme activity decrease ²⁴
TPMT*3C	719A→G	240Tyr→Cys	In vitro assay: enzyme activity decrease ²⁵
TPMT*3D	460G→A	154Ala→Thr	Intermediate ²⁴
	719A→G	240Tyr→Cys	
	292G→T	98Glu→X	
TPMT*4	_1G→A (intron 9)	Splicing defect	Low ²⁴
TPMT*5	146T→C	49Leu→Ser	In vitro assay: enzyme activity decrease ^{14,25}
TPMT*6	539A→T	180Tyr→Phe	Low ²⁴
TPMT*7	681T→G	227His→Gln	Intermediate ²⁴
TPMT*8	644G→A	215Arg→His	Intermediate ²⁴
TPMT*9	356A→C	119Lys→Thr	Intermediate/normal ²⁴
TPMT*10	430G→C	144Gly→Arg	In vitro assay: enzyme activity decrease ^{25,26}
TPMT*11	395G→A	132Cys→Tyr	Low ²⁴
TPMT*12	374C→T	125Ser→Leu	In vitro assay: enzyme activity decrease ²⁴
TPMT*13	83A→T	28Glu→Val	In vitro assay: enzyme activity decrease ^{25,26}
TPMT*14	1A→G	1Met→Val	Low ²⁴
TPMT*15	_1G→A (intron 7)	Splicing defect	Low ²⁴
TPMT*16	488G→A	163Arg→His	Intermediate ²⁴
TPMT*17	124C→G	42Gln→Glu	Intermediate ²⁴
TPMT*18	211G→A	71Gly→Arg	Intermediate ²⁴
TPMT*19	365A→C	122Lys→Thr	Normal ²⁴
TPMT*20	712A→G	238Lys→Glu	Intermediate ²⁴
TPMT*20 ^a	106G→A	36Gly→Ser	In vitro assay: significant enzyme activity decrease ²⁴
TPMT*21	205C→G	69Leu→Val	Intermediate ²⁴
TPMT*22	488G→C	163Arg→Pro	Intermediate ²⁴
TPMT*23	500C→G	167Ala→Gly	Low ²⁴
TPMT*24	537G→T	179Gln→His	Intermediate ²⁴
TPMT*25	634T→C	212Cys→Arg	Intermediate ²⁴
TPMT*26	117T→ C	208Phe→Leu	Intermediate ²⁷
TPMT*27	19T→ G	107Tyr→ Asp	Intermediate ²⁸

Note: ^a Originally called TPMT*20,²⁹ another paper by the same group refers to the same allele as TPMT*24.¹⁴ Abbreviation: TPMT = Thiopurine methyltransferase

TPMT analysis. Analysis of TPMT status can be accomplished through either analysis of the red blood cell TPMT enzymatic activity, or genotyping. Genetic analysis in routine clinical laboratories involves targeting specific TPMT mutations, usually at least three of the four common alleles. Depending on the mutant alleles targeted and the ethnic background of the patient, genotyping can identify up to 95 percent of affected individuals, but it will not identify those patients with rare mutations. Since the frequency of the rare mutations is exceedingly low, the probability of missing patients with rare mutations is also low. The enzymatic assay is currently considered to be the gold standard measurement, since it should identify all patients with reduced enzymatic activity, regardless of mutations. However, the enzymatic assay is technically more challenging to perform. In current clinical practice, both enzymatic testing and genetic analysis are being performed, depending on the laboratory.

Clinical utility and validity of TPMT analyses. Currently, there is no evidence that the presence of one or more mutant TPMT alleles causes disease or places one at increased risk for disease. However, the presence of a mutant allele has been suggested to increase the risk of thiopurine-related drug toxicity, particularly when using AZA or 6-MP (6-TG is not metabolized to as great an extent by TPMT). Therefore, a fraction of patients prescribed thiopurines are at greater risk of developing drug-related toxicity. Until recently, all patients were prescribed a standard starting dose of either AZA or 6-MP. The current starting dose for AZA ranges from 1.0 to 2.5 mg/kg/day and 0.75 to 1.25 mg/kg/day for 6-MP. Patients with either intermediate or low to absent TPMT activity may benefit from a decreased starting dose.

Various clinical guidelines suggest measuring TPMT enzymatic activity or screening for TPMT alleles associated with reduced enzymatic activity before starting patients on thiopurine drugs.^{30,31} However, measuring TPMT activity may not lead to reduced drug-related toxicity since regular monitoring is recommended. Complete blood counts, including platelet counts are recommended to be done weekly during the first month, twice monthly for the second and third months of treatment, then monthly or more frequently if dosage alterations or other therapy changes are necessary.³² As such, there is a need to review the current literature regarding the assessment of TPMT status prior to administration of thiopurine drugs, to determine if TPMT testing will reduce drug-related toxicity.

Scope, Topic Development, and the Key Questions

This review of the effectiveness of determining thiopurine methyl transferase (TPMT) enzymatic activity prior to initiation of thiopurine therapy in patients with chronic autoimmune diseases was nominated by the American Association for Clinical Chemistry (AACC), and commissioned by the Agency for Healthcare Research and Quality (AHRQ).

TPMT status can be assessed by direct determination of the TPMT enzymatic activity (phenotyping), or by genotyping TPMT gene coding for the enzyme for common single nucleotide polymorphisms (SNPs), also referred to as variant alleles, coding for the enzyme.

In theory, TPMT status determination before initiating thiopurine therapy may be undertaken in order to address two potential clinical scenarios:

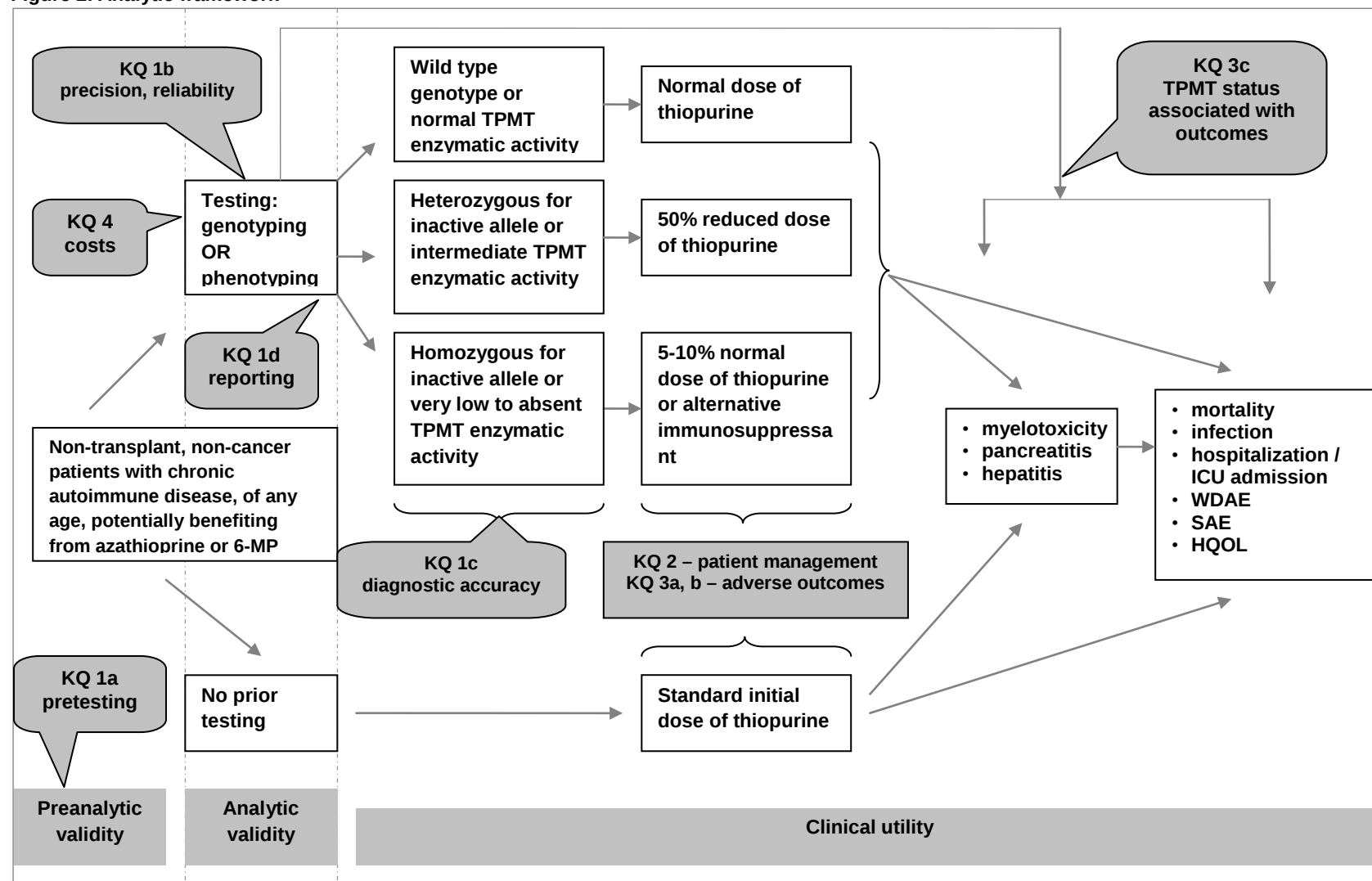
- minimize thiopurine toxicity in up to 15 percent of patients with lower TPMT enzymatic activity, by thiopurine dose reduction or switching to alternative treatment
- optimize clinical responsiveness in patients with abnormally elevated TPMT enzymatic activity, by dose escalation

In the first scenario, thiopurine dose reduction, in order to minimize drug toxicity associated with excessively elevated levels of thioguanine nucleotides, may negatively affect treatment efficacy (i.e. overzealous dose reduction to subtherapeutic thiopurine levels). Hence, an additional concern in tandem with the first scenario is what an optimally effective dose reduction should be, in light of pretreatment knowledge of TPMT status, in patients likely to experience increased thiopurine toxicity. Based on a scoping review of the literature, it was anticipated that this additional concern had not been adequately investigated in primary research. Therefore, the current systematic review of literature does not investigate the relative efficacy or the effectiveness of thiopurine dose adjusted treatment of chronic autoimmune diseases with pretreatment TPMT testing. As such, this review focuses on the equipoise of whether pretreatment determination of the TPMT status (using genotyping and/or phenotyping) mitigates harms associated with thiopurine therapy. In addition, the accuracy of TPMT status determination by genotyping is also investigated, in reference to the enzymatic activity assay, as well as the costs and potential savings associated with TPMT testing.

The second clinical scenario could not be investigated because abnormally high TPMT enzymatic activity has not been investigated in any detail in primary research, so its significance is not yet appreciated.

The analytic framework (Figure 2) depicts the causal pathways forming the basis of the key questions. Since study of myelosuppression in organ transplant and cancer patients poses several potential confounders (namely, concomitant myelosuppressive treatment and the short-term complications induced by the procedure or disease), the eligibility criteria were restricted to populations with chronic autoimmune diseases. As well, since the metabolism of the drug 6-thioguanine does not involve the TPMT enzyme, we focused on the two thiopurine drugs, AZA and 6-MP.

Figure 2. Analytic framework



Abbreviations: KQ = key question; TPMT = thiopurine methyltransferase

Key Questions Addressed in This Report

The following key questions were generated by the University of Ottawa Evidence Based Practice Centre in consultation with American Association for Clinical Chemistry and the Agency for Health Research Quality. Outcomes are considered only in the context of drug toxicity/adverse events, and not of efficacy.

KQ1. In terms of the analytical performance characteristics of enzymatic measurement of TPMT activity and determination of TPMT allelic polymorphisms:

- a) What are the preanalytical requirements for enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms? (e.g. specimen types and collection procedures, lab transportation, interference of coadministered drugs, patient preparation and identification etc.)
- b) What are the within and between laboratory precision and reproducibility of the available methods of enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms (proficiency testing)?
- c) What is the diagnostic sensitivity and specificity of TPMT allelic polymorphism measurement compared to the measurement of TPMT enzymatic activity in correctly identifying chronic autoimmune disease patients eligible for thiopurine therapy with low or absent TPMT enzymatic activity? How do effect modifiers (e.g. underlying disease prevalence and severity, different activity thresholds, Hardy-Weinberg equilibrium, number and types of alleles tested) explain any observed heterogeneity in sensitivity and specificity?
- d) Are there any postanalytical requirements specific to measurement of TPMT enzymatic activity or TPMT allelic polymorphism measurement? (E.g. timely reporting of data, reference intervals, immediate or reporting within a time-frame, highlighting of extreme results)

KQ2. Does the measurement of TPMT enzymatic activity or determination of TPMT allelic polymorphisms change the management of patients with chronic autoimmune disease when compared with no determination of TPMT status?

KQ3. In chronic autoimmune disease patients prescribed thiopurine-based drugs (AZA or 6-MP), does the assessment of TPMT status to guide therapy, when compared with no pretreatment assessment, lead to:

- a) reduction in rates of mortality, infection, hospitalization, withdrawal due to adverse events (WDAE), serious adverse events (SAE) and improvement in health-related quality of life?
- b) reduction in rates of myelotoxicity, liver toxicity, and pancreatitis?
- c) In the absence or inconclusiveness of evidence answering key question 3a and/or 3b above, is there an association between TPMT status (as determined by TPMT enzymatic activity and/or TPMT allelic determination) and/or the following amongst chronic autoimmune disease patients treated with thiopurines?
 - i. the clinical outcomes of mortality, infections, hospitalization, WDAE, SAE and health-related quality of life?
 - ii. surrogate outcomes of myelotoxicity, liver toxicity, and pancreatitis?

KQ4. What are the costs of determining TPMT enzyme activity and/or genotyping for patients with chronic autoimmune disease being considered for thiopurine-based therapy (e.g., costs of testing, costs of care, and costs of treating drug-associated complications)?

Chapter 2. Methods

Literature Search Strategies and Peer Review of Electronic Search Strategies

Electronic search strategies were developed and tested through an iterative process by an experienced medical information specialist (BS) in consultation with the team. The search strategy was peer reviewed by RD according to the PRESS guideline.³³ The following databases were searched: Ovid MEDLINE® 1950 to May Week 3 2010; The Cochrane Library® (CLIB 2009 3) including CENTRAL, CDSR, DARE, HTA, and NHSEED; BIOSIS® May 6 2009; EMBASE® 1980 to 2010 Week 21; Genetics Abstracts: May 7 2009; and Ovid Healthstar 1966 to April 2010. EconLit™ was searched May 7 2009 for the economics section (question 4). Strategies utilized a combination of controlled vocabulary and keywords. No language or date restrictions were imposed on any of the searches and animal studies were excluded. Additional references were identified through scanning reference lists of relevant articles, and by expert nomination. Grey (unpublished, unindexed or difficult to locate) literature were sought through searching the websites of relevant specialty societies and organizations. The detailed search strategies are available in Appendix A.

Study Selection

We employed three levels of screening of retrieved records. Study selection was based on predefined eligibility criteria of interventions (testing and treatment), patient populations, outcome measures, and study design (Table). Studies that included patients reporting to a clinic then tested their TPMT genotype or phenotype to correlate with AEs in which adverse events were noted from patients charts were considered cross-sectional in design. The electronic literature search and expert-nominated records were uploaded to the software program EPPI-Reviewer 3.0,³⁴ along with screening questions developed by the review team. Titles and abstracts were screened by one reviewer for potential relevance, and exclusions at this level were verified by a second reviewer. In case of disagreement or uncertainty about relevance, the record was passed through to the next level for full-text review. Two reviewers reviewed full text reports independently, applying a priori eligibility criteria. Discrepancies were resolved through discussion and consensus or by third party adjudication if consensus could not be reached. Reviewers were not masked to the reports' authors, institution or journal. For key questions 1a and 1b, a third level of screening was undertaken by content experts (RB and EL) to confirm records marked as relevant at a relatively liberal level II screening. Records that were unavailable as abstract or full text reports, or were commentaries, letters, editorials and reviews were excluded. Only articles in the English language were eligible.

Table 3. Study selection criteria

Criterion	Key question	Included	Excluded	Eligible study design
Patient population	All key questions	Patients with chronic autoimmune diseases, of any age	Cancer, organ transplant	-
Drug treatment(s)	All key questions	Azathioprine (AZA), and 6-mercaptopurine (6-MP)	6-thioguanine, excluded because it is not metabolized by the TPMT enzyme	-
Test(s)	All key questions	1. TPMT enzymatic activity as determined by HPLC, radioassay or mass spectrometry 2. Genotyping using PCR, direct sequencing or RFLP	Mass quantitation was excluded because it is rarely used	-
Outcomes	1a	Specimen types and collection procedures, lab transportation, interference of coadministered drugs, patient preparation and identification, enzyme stability, and factors affecting preanalytic enzymatic variability (e.g. gender, age, comorbid conditions etc.)	-	Any
	1b	Inter-, and intra-assay coefficients of variations and/or test concordance	-	Any
	1c	Testing sensitivity and specificity	-	Any
	2	Patients requiring thiopurine dose reduction, patients switching to nonthiopurine treatment, and number of specific monitoring tests per person time at risk, or per patient per unit treatment time	Differences in efficacy (i.e. clinical response to treatment)	Randomized controlled trials, controlled clinical trials, cohort, and case-control studies reporting numerical data in which administration of thiopurine treatment followed thiopurine status testing and was adjusted or replaced (by another disease modifying treatment) in at least one group
	3a	Mortality, infection, hospitalization (including ICU admissions if available), withdrawal due to adverse events (WDAE), serious adverse events (SAE), Health-related quality of life measures (HQOL)		
	3b	Myelotoxicity (two or more of leukopenia, thrombocytopenia, and anemia), leukopenia, neutropenia, thrombocytopenia, and anemia, hepatotoxicity (raised ALT/AST) and pancreatitis		
	3c	Mortality, hospitalization (including ICU admissions if available), withdrawal due to adverse events (WDAE), serious adverse events (SAE), Health-related quality of life measures (HQOL), myelotoxicity (two or more		
				Any study design in which thiopurine treatment was not guided by prior knowledge of TPMT status

Table 3. Study selection criteria (continued)

Criterion	Key question	Included	Excluded	Eligible study design
		of leukopenia, thrombocytopenia, and anemia), leukopenia, neutropenia, thrombocytopenia, anemia, hepatotoxicity (raised ALT/AST) and pancreatitis		
	4	Costs of testing, costs of care, costs of treating drug-associated complications	Economic analyses (e.g., cost analysis, cost-benefit analysis, cost-effectiveness analysis, cost-utility analysis)	Studies that provide costing data
Publication status	All key questions		NonEnglish publication, editorial, review, commentary, letter, news report or case report. Also records unavailable as abstracts or full text reports	

Abbreviations: 6-MP = 6-mercaptopurine; AZA = azathioprine; HQOL = Health-related quality of life; ICU = intensive care unit; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; WDAE = withdrawal due to adverse events; SAE = serious adverse events; TPMT = thiopurine methyltransferase

Data Extraction

Sample data extraction forms are presented in Appendix B. Where applicable for a key question, we extracted the following data from studies: first author's name, year of publication, country, study design (prospective or retrospective), sample size, eligibility criteria, population characteristics, type of genetic testing (e.g. alleles tested, method of testing and source of DNA) and enzymatic assay, testing thresholds, percentage of nondiagnostic results, deviation from Hardy-Weinberg equilibrium (HWE), reported sensitivity and specificity, drug treatment and dose, definitions of outcomes, number of patients with and without events as dichotomous outcomes data for a given genotypic or phenotypic enzymatic status, adjusted and crude effect estimates with standard error or confidence intervals, validation of health profiles, coefficient of variations, preanalytic variables for TPMT activity, costing items (e.g., costs of adverse events, cost of testing, costs of care, currency, discount rate, source of cost data) and determinants of preanalytic validity as reported in papers.

For the diagnostic accuracy question, numerical and meta-analyzable data were extracted in two 2×2 tables as shown in Table . Patients tested negative for any of the SNPs were considered noncarriers (wild type homozygous), while carriers were either heterozygous for the variant allele or homozygous for it. For data clarification and missing data we wrote to study authors. *Heterozygous* carrier state implied presence of one variant allele on one of the two paired chromosomes; *homozygous* state implied presence of one of the same variant alleles on each one of the two paired chromosomes, or presence of two different mutant alleles on each of the two paired chromosomes (the latter is also called a compound heterozygous state). In other words, compound heterozygosity was extracted and interpreted as homozygous carrier data. We did not differentiate between normal and high TPMT activities, and most studies used the terms interchangeably. When investigators reported raw data, we considered TPMT activity of less than 5.0 Units per milliliter of packed red blood cells (U/mL RBCs) as low, between 5.0 and 13.5 U/mL RBCs as intermediate, and greater than 13.5 U/mL RBCs as normal activity. Extracted data were verified and corrections made where necessary. For clarity of presentation, we standardized and presented TPMT activity data according to two standard units, U/mL RBCs or U/gram of hemoglobin (g Hb). Note that 1 Unit is equivalent to 1 nanomole of product per hour (nmol/h), and 1 nmol/h/g Hb is equivalent to 1 pmol/h/mg Hb. Unless otherwise stated, the product was 6-MMP.

When there were multiple reports of the same study we referenced the most relevant record as the primary identifying study, and also extracted additional data as available from companion report(s).

Table 4. Two by two tables for analysis of diagnostic accuracy

2×2 Table A		Phenotyping (enzymatic activity assay) Reference standard	
		Normal or high activity	Intermediate or low activity
Genotyping Index test	Heterozygous or homozygous carriers		
	Noncarriers		
2×2 Table B		Phenotyping (enzymatic activity assay) Reference standard	
		Intermediate, normal, or high activity	Low activity
Genotyping Index test	Homozygous carriers		
	Noncarriers or heterozygous carriers only		

Assessment of Risk of Bias

For key questions 1c (diagnostic accuracy); 2, 3a, and 3b (effectiveness of testing versus no testing;) and 3c (association studies), we assessed risk of bias of studies using standard questionnaires.

For diagnostic studies we used a modified QUADAS tool³⁵ in which we replaced the item number 4 about the time period between the two tests with an item questioning whether Hardy-Weinberg equilibrium (HWE) was tested or not.

For randomized controlled trials and cohort studies we assessed the following:

- similarity of groups at baseline in terms of baseline characteristics and prognostic factors
- extent to which outcomes were described
- blinding of subjects and providers
- blinded assessment of the outcome
- intention-to-treat analysis
- differential loss to follow-up between the compared groups or overall high loss to follow-up,
- Potential for financial conflict of interest

For trials, two additional elements were considered:

- methods used for randomization and
- allocation concealment
- Treatment adherence

For cohort studies, yet another set of elements was considered:

- sample size
- methods for selecting participants (inception cohort, methods to avoid selection bias);
- methods for measuring exposure variables

- methods to control confounding (e.g. design features of matching or restriction to particular subgroups; and analytic methods of stratification or regression modeling with propensity scores or covariates)
- appropriateness of methods of measuring TPMT status

For key question 3c, risk of bias items included assessment of comparability of groups, blinded assessment of outcome and categorization of TPMT genotyping or phenotyping testing results, sample selection and survival bias, adequacy of description of TPMT testing methodology and its reliability, reporting of ambiguous and uninterpretable results of TPMT testing, compliance with thiopurine treatment, sources of TPMT genotyping or phenotyping for all patients, loss to followup, clear description of outcomes definitions, adequacy of sample size, methods to control for confounding, potential for financial conflict of interest, and finally assessment of HWE and gene-gene interaction where applicable.

Each study was given an overall risk of bias assessment of Good (low risk of bias), Fair or Poor (high risk of bias) (Table). Because of a lack of standards for assessment of risk of bias or quality of preanalytic, analytic, postanalytic, and costing data studies, risk of bias was not assessed for key questions 1a, 1b, 1d and question 4. To rate the strength of the body of evidence, we planned to qualify the corpus of evidence into low, medium and high risk for an outcome of interest. Since only one study contributed to the outcomes preplanned for rating the strength of evidence, individual risk of bias of this study determined the overall risk of bias of the evidence for those outcomes in form of a single study. In other words, fair risk of bias of the individual study for the outcomes determined the medium risk of bias of the available evidence. Details of items contributing to risk of bias assessment for each study are reported in Appendix C (Evidence tables).

Table 5. Categorization of overall risk of bias of individual studies

<u>Good (low risk of bias).</u> These studies have the least bias and results are considered valid. A study that adheres mostly to the commonly held concepts of high quality including the following: a formal randomized controlled study; clear description of the population, setting, interventions, and comparison groups; appropriate measurement of outcomes; appropriate statistical and analytic methods and reporting; no reporting errors; low dropout rate; and clear reporting of dropouts
<u>Fair.</u> These studies are susceptible to some bias, but it is not sufficient to invalidate the results. They do not meet all the criteria required for a rating of good quality because they have some deficiencies, but no flaw is likely to cause major bias. The study may be missing information, making it difficult to assess limitations and potential problems
<u>Poor (high risk of bias).</u> These studies have significant flaws that imply biases of various types that may invalidate the results. They have serious errors in design, analysis, or reporting; large amounts of missing information; or discrepancies in reporting.

Evidence Synthesis and Analysis

For key questions 1a, 1b, and 4, evidence was synthesized qualitatively.

Data synthesis was not possible for key questions 1d due to lack of evidence, and for question 2, 3a and 3b sparse evidence precluded evidence synthesis.

For key question 4, costing data were converted to United States Dollar 2009 values using purchasing power parities³⁶ and were inflated to reflect 2009 values using the consumer price index for US medical care for all urban consumers (series identification # CUUR0000SAM).³⁷

For the main quantitative syntheses, we did not consider underlying autoimmune disease, method of genotyping or phenotyping (i.e. enzymatic activity testing), population demographics, and the different observational study designs, as contributors to any important clinical or methodological diversity. In this light, the underlying assumption was that differences in outcomes of thiopurine toxicity are explained by differences in TPMT enzymatic activities across similar doses of the drugs. We used DerSimonian and Laird's random-effects model for pooling individual study estimates, weighting them by individual study variance and the estimated between-study heterogeneity. We pooled whenever there were two studies in a given analysis for an outcome. Pooled estimates of diagnostic sensitivity and specificity percentages, and odds ratios and their 95 percent confidence intervals were calculated using CMA software (version 2.2.046).

Despite the underlying assumption, we planned to explore clinical and methodological diversity in a random effects univariate meta-regression based upon preidentified covariates of study design, overall risk of study bias, TPMT testing methodology, specific age subgroups, and underlying autoimmune disease(s) subgroups. Data on variables such as racial groups were too sparse to be considered as covariates. HWE was not considered as a covariate because it has been shown that including studies that appear to violate the HWE in the meta-analysis does not bias the summary estimates.³⁸ Enzymatic activity cutoffs used in the studies differed trivially between them. This variability is not unexpected because laboratories employ in-house reference intervals based on slight population and assay differences. As such, the TPMT enzymatic activity cutoff was also not considered to be an important effect modifier. For key questions other than the diagnostic question 1c, an additional covariate considered was blinded assessment of outcomes and TPMT status (by genotyping or phenotyping). Meta-regression was done in SAS using the NLMIXED procedure whenever six or more studies were involved in the pooled result of a binary covariate and 12 or more if the covariate had three distinct levels. When possible, we tested for statistical heterogeneity using Cochran's Q and reported it when found to be substantial (p value for chi-squared test of heterogeneity less than 0.10, and I^2 greater than or equal to 50 percent).

Pooling of Evidence of Diagnostic Accuracy (Key Question 1c)

We considered the TPMT enzymatic activity assay as the reference for the index test of genotyping of the different SNPs. Since the index test reported presence or absence of variant allele as a dichotomous outcome, implicit or explicit cut-off threshold effects were ruled out. We, therefore, pooled for the outcomes of test sensitivity and specificity instead of summary receiver-operating characteristics.

The role of testing for statistical heterogeneity in effect estimates from studies of diagnostic accuracy is less well understood. Since the estimates of sensitivity and specificity are inter-related, the significance of heterogeneity in one and not the other is unclear. We, therefore, did not formally test for the extent of statistical heterogeneity; however, we did carry out univariate meta-regression based upon preidentified covariates stated above when possible.

Since studies identified homozygous and heterozygous carrier states by testing for different TPMT variant alleles, we pooled only the studies testing for the same set of SNPs.

When possible, two meta-analyses were considered for each set of variant alleles investigated by at least two studies. In *meta-analysis 1*, genotypes were dichotomized into noncarriers and carriers (or homozygotes and/or heterozygotes) and phenotypes were dichotomized into normal

(or high) and subnormal (or intermediate and/or low and/or absent) enzymatic activities. In *meta-analysis 2*, we dichotomized genotypes into noncarriers and/or heterozygotes and homozygotes, and phenotype into normal and/or high and/or intermediate and low/absent activities (Table). For studies with 100 percent sensitivity or specificity, 0.5 was added to either both TP (true positive) and FN (false negative) or TN (true negative) and FP (false positive), in order to estimate pooled proportions.

Table 6. Meta-analyses 1 and 2, for Key Question 1c

Meta-analysis 1					
	Normal/high activity	Intermediate / low / absent activity		Sensitivity 1	Specificity 1
Homozygotes and/or heterozygotes	A1	B1		B1/(B1+D1)	C1/(C1+A1)
Noncarrier (or Wild types)	C1	D1			
Meta-analysis 2					
	Normal/high/intermediate activity	Low/absent activity		Sensitivity 2	Specificity 2
Homozygotes	A2	B2		B2/(B2+D2)	C2/(C2+A2)
Noncarrier (or Wild types) and/or heterozygotes	C2	D2			

Pooling of Evidence of Association of Thiopurine Toxicity With TPMT Genotypic or Phenotypic Status (Key Question 3c)

Outcomes data were number of patients with and without events. With respect to genotype, we considered a codominant model in which TPMT enzymatic activities are highest when both TPMT alleles are Wild type (i.e. noncarrier state without TPMT polymorphisms), followed by somewhat decreased enzymatic activity when the one of the two alleles is mutant or variant (i.e. heterozygous carrier of mutation, or heterozygote) and lowest to absent TPMT enzymatic activities when both alleles are variants (i.e. homozygous carrier of mutation, or homozygote). The possible comparisons were heterozygotes versus noncarriers, homozygotes versus noncarriers, and homozygotes versus heterozygotes. For the association with TPMT enzymatic activity, the comparisons were intermediate enzymatic activity versus normal/high, low to absent activity versus normal/high and low/absent activity versus intermediate activity.

For estimating association with genotype, the primary analysis included all studies as long as they tested for the most common TPMT alleles, irrespective of testing additional polymorphisms. Alleles we considered most common were TPMT*2, *3A, *3B, *3C (Table). Secondary analyses were allele specific and restricted to only those subgroups of studies that tested specific alleles. Additionally, we assessed deviations of the genotype frequencies from those predicted by the Hardy-Weinberg law (chi-square test). A p-value less than 0.05 indicated possible genotyping error (other factors, such as inbreeding could also be at play). The Hardy-Weinberg law states that if there are 2 alleles at a particular locus, named A and a, with frequency p and q, respectively, then after 1 generation of random mating the genotype frequencies of the AA, Aa, and aa groups in the population will be p^2 , $2pq$, and q^2 , respectively. Given that there are only 2 alleles possible, A or a, then $p + q = 1$, and $p^2 + 2pq + q^2 = 1$.

Rating the Strength of Evidence and Assessing Applicability

For the prespecified outcomes of mortality (critical), serious adverse events (critical), myelotoxicity (important), and health-related quality of life (important) in Key Questions 3a, and 3b, two methodologists rated the strength of the body of evidence across the domains of risk of bias, consistency, directness and precision as per published guidance.³⁹ Disagreements were resolved by consensus. Applicability was assessed according to the domains of patient population, intervention and dose, comparator, outcome and study duration. We examined eligibility criteria, participant demographics, and distribution of genotype to assess generalisability of population. To assess applicability of testing we examined the type of TPMT tests employed and their validity and reproducibility. We also ascertained dose and type of thiopurine. We also examined whether non-pretreated group of patients received routine blood count monitoring or not, and the study duration.

Survey of Laboratories Conducting TPMT Analyses

To supplement the limited published literature to answer key questions 1a, 1b, 1c, and 1d, further data regarding the preanalytical and postanalytical requirements and performance characteristics of TPMT laboratory analyses was collected. With advice from the Technical Expert Panel, the review team decided to survey laboratories that provide TPMT analytical services.

Our target population included all laboratories in which medium of language was English and provide TPMT analytical services. Potential participating laboratories were identified by the Technical Expert Panel, which also queried two listservs: Clinical Chemistry General Topics (American Association for Clinical Chemistry) and CSCC Listserv (Canadian Society of Clinical Chemists). Based on these expert recommendations, two organizations and seven laboratories were contacted to determine their willingness either to complete a questionnaire or to disseminate the questionnaire to other relevant laboratories. Contact was initiated using an emailed letter explaining the intent behind the request, as well as background information on the questionnaire. The letter and questionnaire are included in Appendix B.

The review team developed a draft questionnaire, which was assessed by the Technical Expert Panel for face validity and comprehensiveness and the logical flow of questions and responses were ensured for both paper and electronic versions. The questionnaire was also revised after some initial responses revealed ambiguity. The final questionnaire included 11 questions with multiple subquestions relating to TPMT analytical methods (e.g., sample type and handling), preanalytical requirements (e.g., specimen stability), quality control procedures, reporting of results and cost of TPMT services. The questionnaire took approximately 15-20 minutes to complete. The full questionnaire is included in Appendix B.

The survey was administered electronically via Survey Monkey™ to representatives of the six laboratory representatives who had agreed to participate, with up to three weeks provided for completion. Reminder emails were sent one week after initial contact as well as three days before responses were due. Responses were captured in a Survey Monkey™ database and were subsequently downloaded to Microsoft Excel to conduct the analysis. We conducted a descriptive analysis, and summarized results using frequencies and ranges as appropriate.

Ethics approval was obtained from the Ottawa Hospital Research Ethics Board before initial contact with any potential participating laboratory.

Chapter 3. Results

Screening and Inclusion of Records for Key Questions

The PRISMA diagram in Figure 3 depicts the flow of retrieved records through the phases of screening and inclusion. Overall, 1783 records were identified by systematic searches of databases and seven^{1,40-45} were nominated by the Technical Expert Panel and reviewers. A third level of content expert screening was employed to ensure that laboratory studies identified for key questions 1a, 1b and 1d did not include irrelevant records, as a result of relatively liberal screening at the two prior levels.

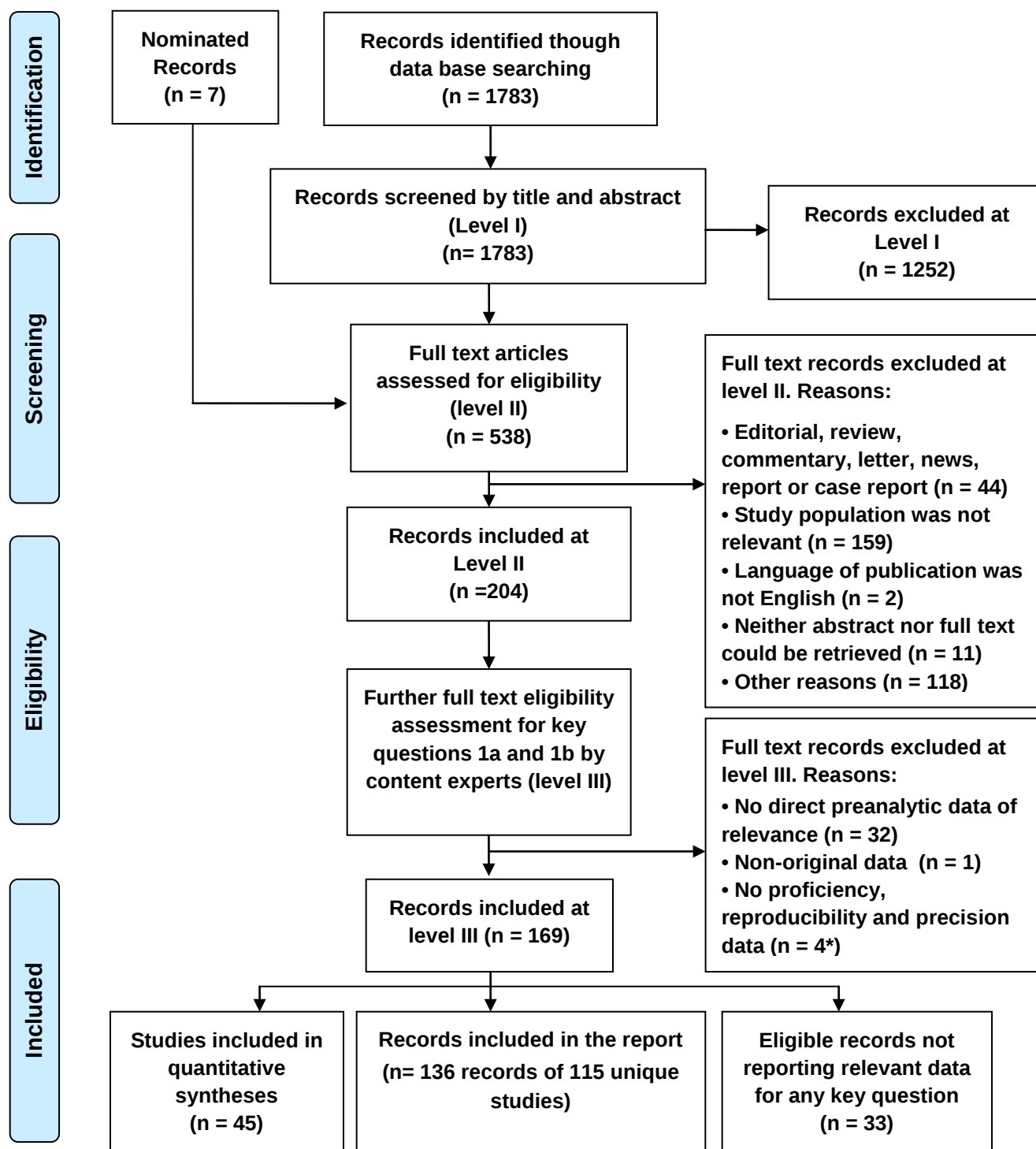
Studies that were reported in more than one included record are listed in Table . We identified one of these records as the primary report and others as companion papers, however, where applicable, data from both were used in evidence syntheses.

For 33 of 169 included records, extractable data were not available (Table 8).

We contacted authors of 24 records requesting additional data or seeking data clarification,^{1,46-68} and additional data received were incorporated in evidence syntheses.

One hundred and thirty six records associated with 115 unique studies addressing the key questions were included in this systematic review. Additional individual study data and quality assessment are presented in Appendix C. Forty-five studies contributed to meta-analyses and the remaining were included in qualitative syntheses of evidence. No record was identified that answered Key Questions 1d (Table).

Figure 3. PRISMA diagram of record identification, screening and inclusion



* Two records had two primary reasons for exclusion at level III

Table 7. Primary records with companion reports passing level III screen

Primary record	Companion record(s)
Palmieri, 2007 ⁴⁷	Palmieri, 2006 ⁶⁹
Winter, 2007 ⁷⁰	Winter, 2007 ⁷¹
Gisbert, 2006 ⁷²	Gisbert, 2007 ⁷³
Lindqvist, 2006 ⁴⁹	Hindorf, 2006 ⁷⁴ , Hindorf, 2005 ⁷⁵
Heneghan, 2006 ⁷⁶	Heneghan, 2003 ⁷⁷
Hindorf, 2006 ⁵⁰	Hindorf, 2006 ⁷⁸
Czaja, 2006 ⁷⁹	Czaja, 2004 ⁸⁰
Gearry, 2004 ⁸¹	Gearry, 2003 ⁸²
Winter, 2004 ⁸³	Winter, 2004 ⁸⁴
Derijks, 2004 ⁵⁴	Derijks, 2003 ⁸⁵
Marinaki, 2004 ⁸⁶	Ansari, 2003 ⁸⁷
Campbell, 2002 ⁵⁸	Campbell, 2002 ⁸⁸
Black, 1998 ⁸⁹	Black, 1998 ⁹⁰
Stolk, 1998 ⁹¹	Stolk, 1996 ⁹²
Snow, 1995 ⁹³	Snow, 1994 ⁹⁴
Kerstens, 1995 ⁹⁵	Kerstens, 1992 ⁹⁶
Walsh, 2008 ⁹⁷	Walsh, 2008 ⁹⁸
Schmeling, 2007 ⁹⁹	Schmeling, 2007 ¹⁰⁰
Ansari, 2004 ⁶²	Ansari, 2004 ¹⁰¹
Colletti, 2009 ⁴⁵	Colletti, 2006 ⁶⁰
Xin, 2005 ¹⁰²	Xin, 2005 ¹⁰³

One companion record was considered primary paper for key question 1b⁷⁴

Table 8. Distribution of records by key questions

Key question	Total number of studies	Full text records	Abstracts	Eligible studies not reporting relevant data	Studies for which (some or all) data were received from authors
1a	35 ^{102,104-137}	33 ^{102,104-135}	2 ^{136,137}	0	0
1b	36 ^{74,106-108,111-116,118,121,124,128,131,135,136,138-156}	33 ^{74,106-108,111-116,118,121,124,128,131,135,138-154}	3 ^{136,155,156}	3 ^{139,141,149}	0
1c	24 ^{1,46,49-53,56,59,70,93,99,157-168}	20 ^{1,46,49-53,56,59,70,93,157-163,167,168}	4 ^{99,164-166}	5 ^{99,164-166,168}	5 ^{51-53,56,59}
1d	0	NA	NA	NA	NA
2	1 ¹	1 ¹	0	0	0
3a	2 ^{1,45}	2 ^{1,45}	0	1 ⁴⁵	1 ¹
3b	3 ^{1,45,169}	3 ^{1,45,169}	0	1 ⁴⁵	0
3c TPMT allelic determination	49 ^{1,46-54,57-59,61,63,64,67,70,76,81,86,89,93,99,157,162,165,170-191}	34 ^{1,46-54,57-59,70,76,81,86,89,93,157,162,170-180,190,191}	15 ^{61,63,64,67,99,165,181-189}	15 ^{49,58,63,64,67,76,162,165,178,183-185,187-189}	5 ^{46-48,52,54,57}
3c TPMT enzymatic activity	36 ^{46,49-53,55,58,59,62,65,68,70,72,79,91,93,95,97,119,157,161,162,165,192-203}	26 ^{46,49-53,55,58,59,70,72,79,91,93,95,119,157,161,162,192-197,203}	10 ^{62,65,68,97,165,198-202}	20 ^{46,49,55,58,59,62,65,68,95,97,119,162,165,194,198-203}	4 ⁵⁰⁻⁵³
4	11 ^{40,83,204-212}	11 ^{40,83,204-212}	0	0	0
Overall across key questions					
Companion records (N=21)	60,69,71,73,75,77,78,80,82,84,85,87,88,90,92,94,96,98,100,101,103				
Studies contributing to the evidence base (N=115)	1, 46-54, 56, 57, 59, 61, 70, 72, 74, 79, 81, 83, 86, 89, 91, 93, 99, 102, 104-138, 140, 142-148, 150-163, 167, 169-177, 179-182, 186, 190-193, 195-197, 204-212 One companion record was considered unique paper for key question 1b ⁷⁴				
Included records that did not report relevant data N=33	45,55,58,62-65,67,68,76,95,97,139,141,149,164-166,168,178,183-185,187-189,194,198-203				

Abbreviations: NA = not applicable; TPMT = thiopurine methyltransferase

Key Question 1a: In terms of the analytical performance characteristics of enzymatic measurement of TPMT activity and determination of TPMT allelic polymorphisms, what are the preanalytical requirements for enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms? (e.g. specimen types and collection procedures, lab transportation, interference of coadministered drugs, patient preparation and identification etc.)

A total of 35 studies reported data relevant to answer this key question, all in relation to enzymatic analysis of TPMT activity (Table). No evidence was available regarding preanalytical performance characteristics for determination of TPMT allelic polymorphisms. Genotyping is most commonly performed by using the restriction fragment length polymorphism (RFLP) technique, polymerase chain reaction (PCR), or direct sequencing.

Stability. Thirteen studies assessing the stability of TPMT enzyme activity are summarized in Table 9.^{08,115,116,118,121,124,127-129,131,134,136,137} Two studies were conducted in the USA^{134,136}, and eleven in Europe.^{108,115,116,118,121,124,127-129,131,137} TPMT stability was assessed at room temperature, 4°C; -20°C, -21°C, -23°C, -25°C, -30°C, -70°C, -80°C and -85°C. Time periods from a few hours to 16 months were studied.

TPMT was found to be stable at room temperature for a maximum of seven days in control blood samples, while in a case of acute lymphocytic leukemia, patient blood TPMT was stable for three days.¹²¹ At -20°C, TPMT was stable for up to three months. Four studies of storage at -80°C showed that TPMT was stable from a minimum of a few days to 25 days,^{116,129,131} but TPMT activity decreased by 15 percent after 16 months of storage.¹¹⁶ Repeated freeze-thaw cycles of the red blood cell (RBC) lysate were reported to result in a 16.2 percent decrease in activity.¹¹⁵ However, the decrease was not statistically significant after three cycles (initial values: 9.2, 11.7, and 16.5 U /mL RBCs; final values: 8.1, 9.8, and 14.3 U/mL RBCs). TPMT activity was stable in six blood samples shipped via regular mail, and received two to seven days post sampling (coefficient of variance, 5.6 percent).¹²¹

Table 9. Stability of TPMT enzymatic activity

Storage temperature	Sample	Storage time, anticoagulant study reference	Stability
Room temperature	Whole blood	24 h, heparin ¹³⁴ 36 h, EDTA ¹⁰⁸ 3 days, heparin ^{121*} 4 days, heparin ¹²⁷ 5 days, heparin ¹¹⁸ 6 days, EDTA ¹²⁴ 7 days, heparin ^{121*}	Stable
	Whole blood	72 h, EDTA ¹³⁷	25% +/- 6% decrease in 24 hours
4°C	Whole blood	24 h, heparin ¹³⁴ 4 days, heparin ¹²⁷ 6 days, EDTA ^{124*} 8 days, unspecified anticoagulant ¹³⁶	Stable
-20°C to -30°C	RBC lysate	7 days ¹¹⁶ 21 days ¹¹⁵ 3 month ¹⁰⁸ Several month ¹²⁴	Stable
	RBC lysate	3 month ¹²⁸	7% decrease
-70°C to -80°C	RBC lysate	Few days ^{129,131} 25 days ¹¹⁶	Stable
	RBC lysate	16 month ¹²⁸	15% decrease

Notes: * Control blood was stable for 7 days. Leukemia patient blood was stable for 3 days, and the median activity showed a small but statistically significant decrease after 6 days.

Abbreviations: EDTA = ethylene diamine tetra-acetic acid; RBC = red blood cell; TPMT = thiopurine methyltransferase.

Gender. Eighteen studies evaluated gender-related differences in TPMT activity (Table 10).^{106,107,110-113,116,117,122,124,125,127-129,131,133-135} Seventeen studies reported the TPMT activity in RBCs and one in renal tissue.¹³³ In 16 studies, no significant differences associated with gender were found in TPMT activity.^{106,107,110-113,116,117,122,124,125,127,128,131,134,135} One study reported TPMT values to be higher for males in Caucasian and mixed-race groups (30 U/g Hb in females versus 38 U/g Hb in males, and 33 U/g Hb in females versus 39 U/g Hb in males, respectively, for Caucasian and mixed race groups).¹²⁹ TPMT values in renal tissue were 10 percent higher in males than in females.¹³³ Fifteen studies reported TPMT values, whereas three studies stated only the outcomes of comparisons.^{107,122,127}

Table 10. Gender related differences in TPMT enzymatic activity

Study	Male	Female	Significance
Hindorf 2004 ¹¹¹	12.9 (range, 0.2–24.6) U/mL RBCs (n=607)	12.7 (range, 0.4–25.4) U/mL RBCs (n=544)	p = 0.08
Weinshilboum 1978 ¹³⁴	10.0 ± 2.3 U/mL RBCs (n = 37)	10.4 ± 2.5 U/mL RBCs (n = 36)	
Tinel 1991 ¹³¹	14.6 ± 6.7 U/mL RBCs (n=119)	16.8 ± 7.7 U/mL RBCs (n=184)	
Jacqz-Aigrain 1994 ¹²⁸	19.6 ± 4.9 U/mL RBC (n=134)	18.9 ± 4.9 U/mL RBC (n=166)	
Kroplin 1998 ¹²⁴	38.8 nmol 6-MTG/h/g Hb (n=117)	36.9 nmol 6-MTG/h/g Hb (n=82)	
Keizer-Garritsen 2003 ¹¹⁶	15,8 ± 6,4 pmol/h/107 RBCs (n=59)	15,1 ± 4,8 pmol/h/107 RBCs (n=44)	
Ganiere-Monteil 2004 ¹¹³	Adults: 19.55 ± 4.25 U/mL RBCs (n= 229) Children: 18.34 ± 4.21 U/mL RBCs (n=97)	Adults: 18.61 ± 3.59 U/mL RBCs. (n=75) Children: 18.87 ± 3.98 U/mL RBCs (n=50)	Adults: p=0.057. Children: p=0.310
Ford 2004 ¹¹²	Mean and median TPMT activities: 34 and 34 nmol 6-MTG/h/g Hb (n=469)	Mean and median TPMT activities: 33 and 32 nmol 6-MTG/h/g Hb (n=531)	No significant difference
Zhang 2007 ¹⁰⁶	Wild type Healthy: (n=155) 17.05 ± 3.12 U/ml RBCs Heterozygous Healthy: (n=4) 7.50 ± 1.58 U/ml RBCs	Wild type Healthy: (n=86) 15.90 ± 2.87 U/ml RBCs. Heterozygous Healthy: (n=3) 8.17 ± 1.30 U/ml RBCs	p=0.01
Zhang 2006 ¹³⁵	12,36 U/mL RBCs (n=5)	13,16 U/mL RBCs (n=14)	
Chocair 1993 ¹²⁹	Caucasians: (n = 21) 38 U/g Hb Black: (n = 24) 38 U/g Hb Mixed-race: (n = 19) 39 U/g Hb Japanese: (n = 11) 37 U/g Hb	Caucasians: (n = 12) 30 U/g Hb Black: (n = 15) 36.5 U/g Hb Mixed-race: (n = 11) 33 U/g Hb Japanese: (n = 21) 38 U/g Hb	
Micheli 1997 ¹²⁵	Adults (20-59 y): (n=7) 21 ± 5 U/g Hb Children (1-14y): (n = 6) 23 ± 5.8 U/g Hb	Adults (20-59 y): (n=7) 15 ± 8 U/g Hb Children (1-14y): (n = 2) 18.21 U/g Hb	
Menor 2002 ¹¹⁷	19.7 - 6.2 U/mL RBCs (n=1671)	19.5 - 6.1 U/mL RBCs (n=1873)	
Brouwer 2005 ¹¹⁰	ALL patients: Median TPMT: 12.4 (range 1.7–30.7) pmol/hr /10 ⁷ RBCs (n=116)	ALL patients: Median TPMT: 12.8 (range 5.8–30.4) pmol/hr /10 ⁷ RBCs (n=57)	No significant difference for ALL patients (p = 0.841)
Oselin 2006 ¹⁰⁷	n=52	n=47	No significant difference TPMT activity:

Study	Male	Female	Significance
			21.5 to 129.6 ng/h/mL RBCs
Lennard 1994 ¹²⁷	In children, boys versus girls TPMT activity for the entire group (n=100) not significantly different (p > 0.25, Mann-Whitney). High TPMT activity group (n=100) boys versus girls not significantly different (p=0.8)		
Alves 2001 ¹²²	(n=76)	(n=67)	No statistically significant association (p = 0.796, ANOVA).
Lee 1982 ¹³³	Renal cell carcinoma patients 232 ± 9 U/g tissue or 4.24 ± 0.15 U/mg protein (n=37)	Renal cell carcinoma patients 210 ± 21 U/g of tissue or 3.93 ± 0.4 U/mg of protein (n=14)	No significant differences. Imbalance in sample numbers likely due to the 2:1 male:female incidence of renal cell carcinoma.

Abbreviations: 6-MTG = 6-methylthioguanine ALL = acute lymphoblastic leukemia; ANOVA = analysis of covariance; g = gram; mL RBCs = milliliter of red blood cells; p = probability; pmol = picomole; TPMT = thiopurine methyltransferase; U = Unit = nanomole of product per hour

Age. Ten studies investigated variation of TPMT activity with age (Table 11)^{104,110,111,113,116,123,125,131,133,135} In eight studies TPMT activity was analyzed in RBCs, while one study examined each of renal tissue¹³³ and lymphocytes.¹²³ Only a single study reported a statistically significant difference in TPMT activities between adults and children (12.0 U/mL RBCs in children and teenagers, versus 12.9 U/mL RBCs in adults; p less than 0.001).¹¹¹

Table 11. Effect of age on TPMT enzymatic activity

Study	Children (<20 years)	Adults (>20 years)	Significance
Hindorf 2004 ¹¹¹	12.0 (range 0.6–25.4) U/mL RBCs (n = 192, <15 y)	12.9 (range 0.2 – 24.6) U/mL RBCs (n = 959, median age 33.4 y)	p < 0.001
Tinel 1991 ¹³¹		14.5 ± 6.7 U/mL RBCs (n = 175, 20-40 y; 16.7 ± 7.6 U/mL RBCs (n = 128) ages from 40 to 60 years.	No significant difference
Ganiere-Monteil 2004 ¹¹³	18.49 ± 4.13 U/mL RBCs. range: 8.25–30.0 U/mL RBCs (n = 165)	19.34 ± 4.09 U/mL RBCs range: 0.43-30.38 U/mL RBCs (n = 304)	p = 0.310 ; no significant difference
Zhang 2006 ¹³⁵		14.38 U/mL RBCs (22 - 38 y, n = 8) 11.90 U/mL RBCs for age 40-59, n = 11. 12.95 (SD, 3.07) U/mL RBCs entire sample	No significant difference

Micheli 1997 ¹²⁵	22 ± 5 U/mL RBCs (1-14 y, n = 8)	18 ± 7 U/mL RBC (20-59 y, n=14)	No significant difference
Brouwer 2005 ¹¹⁰	2-31 U/mL RBCs Patients with ALL, at diagnosis (5.9 ± 4.1 y, n = 173)		TPMT activity not correlated with age.
Keizer- Garritsen 2003 ¹¹⁶	TPMT data various ages. Children, young adults (n=103)		No significant correlation between TPMT activity and age among children and young adults.
Gisbert 2007 ¹⁰⁴		40 ± 16 y (n = 14,545)	No significant correlation between TPMT activity and age
Lee 1982 ¹³³		renal TPMT [average 225 ± 9 U/g of tissue (n = 51)	No significant correlation of patient age with TPMT activity with patient age. Correlation coefficients = 0.04 for all samples.
Coulthard 1998 ¹²³	0.24 nU/mg lymphocytes Range: 0.1 and 0.76 nU/mg protein 11 months to 15.5 y (n = 35)	0.16 nU/mg lymphocytes Range: 0.04 - 0.86 nU/mg protein 16 - 77 y (n = 37)	No significant correlation between TPMT activity and age

Abbreviations: ALL = acute lymphoblastic leukemia; g = gram; mg = milligram; mL RBCs = milliliter of red blood cells; nU = nanoUnit; p = probability; pmol = picomole; TPMT = thiopurine methyltransferase; U = Unit = nanomole per hour

Drugs. Ten studies evaluated the influence of drugs on TPMT activity in blood (Table 12).^{102,104,105,110,114,117,119,120,128,131} Four studies were conducted in vitro.^{102,110,114,128} The following drugs were studied: 5-aminosalicylate^{102,104}, sulfasalazine^{102,114,122}, mesalazine^{105,122}, azathioprine^{105,117}, mesalamine⁵⁸, ac-5-aminosalicylate¹⁰², syringic acid¹²⁸, prednisone¹²⁸, prednisolone¹²⁸, 6-methylprednisolone¹²⁸, cyclophosphamide¹²⁸, methotrexate^{110,128}, trimethoprim-sulphamethoxazole¹²⁸, SKF 525-A¹³¹, 3,4-dimethoxy-5-hydroxybenzoic acid¹³¹, trimethoprim¹¹⁰, vincristine¹¹⁰, dexamethasone¹¹⁰, and L-asparaginase.¹¹⁰ Two studies reported significant inhibition. 3,4-dimethoxy-5-hydroxybenzoic acid decreased TPMT activity by 97 percent in vitro.¹³¹ One in vitro study showed concentration-dependent inhibition of TPMT from 11 to 55 percent, in the presence of 80 to 640 mol/L sulfasalazine.¹¹⁴ One study reported 147 to 148 percent stimulation of TPMT activity in vitro by methotrexate and trimethoprim.¹¹⁰ Interestingly, another study of methotrexate in vitro found no effect.¹¹⁶ The rest of the studies reported no significant inhibition by any of the drugs studied.

Table 12. Effects of drugs on TPMT enzymatic activity

Study	Drug ID	Effect on TPMT activity
Tinel 1991 ¹³¹	SKF 525-A	None
	3,4-dimethoxy-5-hydroxybenzoic acid	Decreased by 97%

Study	Drug ID	Effect on TPMT activity
Dewit 2002 ¹²⁰	Acetylated metabolite of 5-aminosalicylic acid, with either sulfasalazine or mesalazine.	No significant change after aminosalicylate withdrawal. TPMT activity before withdrawal of aminosalicylate: whole group: 12.29 U/mL RBCs (range 8.25±16.85); sulfasalazine subgroup: 12.14 U/mL RBCs; mesalazine subgroup 12.43 U/mL RBCs. TPMT activity after aminosalicylate withdrawal: whole group: 11.41 U/mL RBCs (7.3±14.5) (p=0.245, not significant); sulfasalazine subgroup: 11.43 U/mL RBCs; mesalazine subgroup: 11.39 U/mL RBCs.
Dilger 2007 ¹⁰⁵	Azathioprine versus mesalazine	No significant differences between patients on azathioprine compared with those on mesalazine, at baseline or at any further visit.
Gisbert 2007 ¹⁰⁴	5-aminosalicylates	No differences between patients on azathioprine or 5-aminosalicylates versus controls. Azathioprine: no treatment 20.7 U/mL RBCs versus treatment 21.2 U/mL RBCs 5-aminosalicylates: no treatment 20.9 U/mL RBCs versus treatment 21.2 U/mL RBCs
Dubinsky 2002 ¹¹⁹	Mesalamine	No differences between patients on mesalamine medications versus controls (median 32.4 (range 14.7-49.2) ELISA units vs. 31.8 (15.3-49.1 ELISA units)
Menor 2002 ¹¹⁷	azathioprine	No significant difference between patients on azathioprine versus no treatment 19.9 ± 6.0 U/mL RBCs versus 19.7±5.8 U/mL RBCs
Xin 2005 ¹⁰²	sulfasalazine, 5-aminosalicylate, Ac-5-aminosalicylate	No significant effects in vitro. IC50s: Sulfasalazine: 9.4 (±3.1) µM 5-aminosalicylate: 236 (±55) µM Ac-5-aminosalicylate: 73 (±20) µM
Jacqz-Aigrain 1994 ¹²⁸	syringic acid, prednisone, prednisolone, 6-methylprednisolone, cyclophosphamide, methotrexate, trimethoprim sulphamethoxazole	No significant effect in vitro. Residual TPMT activity was always more than 70% of control activity.
Shipkova 2004 ¹¹⁴	sulfasalazine	Significant, concentration-dependent inhibition 11% for 80 µM to 45% for 640 µM sulfasalazine.
Brouwer, 2005 ¹¹⁰	methotrexate, trimethoprim, vincristine, dexamethasone, L-asparaginase	TPMT activity significantly increased in vitro. 2.4 µM trimethoprim: 148% 0.01 µM methotrexate: 147%. Vincristine, dexamethasone and L-asparaginase had nonsignificant inhibitory effects on TPMT activity.

Abbreviations: IC50 = concentration of inhibitor at which enzyme activity is 50 percent of uninhibited activity; mL RBCs = milliliter of red blood cells; p = probability; TPMT = thiopurine methyltransferase; U = Unit = nanomole per hour; µM = micromoles per liter.

Race. Two studies of TPMT activity differences among different races are summarized in Table 13.^{109,129} No significant differences among studied races (Caucasians, blacks, Japanese, mixed races) were found.^{109,129}

Table 13. Variation of TPMT activity with race

Study	Population	TPMT activity (range) (U/mL RBCs)
Heckmann 2005¹⁰⁹	Black (n=50) 8.75	8.75 (6.5–10.1)
	Mixed race (n=50)	10.15 (8.7–11.3)
	Caucasian (n=15)	13.40 (10.9–15.1)
Chocair 1993¹²⁹	Black (n=39)	37 (13-84)
	Mixed race (n=30)	36.5 (14-65)
	Japanese (n=32)	37.5 (29-48) **
	Caucasian (n=33)	32 (15-54) **

Note: ** (p<0.04)

Abbreviations: n = number in group; RBCs = red blood cells; TPMT = thiopurine methyltransferase; U = Unit = nanomole per hour.

Hematocrit. The effect of hematocrit on TPMT activity was measured in three studies.^{117,126,104} One study proposed a decrease of TPMT activity by seven percent (range 1.2 percent to 12.0 percent) when comparing the high and low hematocrit levels among 12 participants.¹²⁶ They studied TPMT activity between erythrocyte fractions of 0.1 and 0.5 and reported the slopes to be significantly different from zero (p ranging from 0.02 to 0.0001). A single study reported young RBCs to have TPMT activity 8.8 units higher than old RBCs (Wilcoxon median difference 8.8 units (95 percent CI 7.2 to 10.8, p=0.006)).¹²¹ Among 10 participants, TPMT activities in the 60 percent, 63 percent, 66 percent and 69 percent gradients were analysed and the TPMT activities in each gradients differed (p less than 0.001). Another study described no hematocrit dependant difference in TPMT activity.¹⁰⁴

Morbidities. Two studies^{104,106} evaluated the effect of morbidities on TPMT activity (Table 14). Inflammatory bowel disease (ulcerative colitis, Crohn disease, and indeterminate colitis), autoimmune hepatitis, multiple sclerosis, myasthenia gravis, pemphigus and chronic renal failure were studied. Statistically significant differences (p less than 0.001) in TPMT activity were observed among some disease groups, such as inflammatory bowel disease, autoimmune hepatitis, multiple sclerosis, myasthenia gravis, and pemphigus.¹⁰⁴ Patients with chronic renal failure had almost double the TPMT activity compared with the healthy control before hemodialysis (33.88 ± 12.33 U/mL RBCs versus 16.03 ± 4.16 U/mL RBCs).¹⁰⁶ Posthemodialysis TPMT activity levels were comparable to patients without renal failure.

Table 14. TPMT activity in various disease groups

Study	Population	TPMT activity (U/mL RBCs)
Gisbert 2007¹⁰⁴	All study participants	20.1 ± 6 (Range 0-46.4)
	Inflammatory bowel disease (n=7046)	20.4 ± 6
	Autoimmune hepatitis (n=359)	21.5 ± 6
	Multiple sclerosis (n=814)	18.4 ± 6
	Myasthenia gravis (n=344)	20.9 ± 6
	Pemphigus (n=133)	21.1 ± 6

Zhang 2007¹⁰⁶	Healthy control (n=241)	16.03 ± 4.16
	Chronic renal failure (n=30): before hemodialysis	33.88 ± 12.33
	Chronic renal failure (n=30): after hemodialysis	17.89 ± 5.24

Abbreviations: n = number in group; RBCs = red blood cells; TPMT = thiopurine methyltransferase; U = Unit = nanomole per hour.

Key points. TPMT is a stable enzyme that apparently can be stored for up to seven days at room temperature, and up to three months at -30°C. However, further research is required to confirm this.

Age and gender do not appear to affect TPMT activity. Furthermore, although the current data does not demonstrate racial differences between TPMT activities, further research is required to confirm this.

A number of drugs were studied for their effect on TPMT activity, including 5-aminosalicylate, sulfasalazine, mesalazine, azathioprine, mesalamine, ac-5-aminosalicylate, syringic acid, prednisone, prednisolone, 6-methylprednisolone, cyclophosphamide, methotrexate, trimethoprim-sulphamethoxazole, SKF 525-A, 3,4-dimethoxy-5-hydroxybenzoic acid, trimethoprim, vincristine, dexamethasone, L-asparaginase. Studies showing potentially clinically significant effects were conducted in vitro, and therefore their in vivo influence on TPMT activity remains unknown.

In patients with renal failure, TPMT activity is elevated prior to hemodialysis, and returns to normal levels following hemodialysis.

No studies were identified that addressed preanalytic variables for TPMT genotyping.

Key Question 1b: In terms of the analytical performance characteristics of enzymatic measurement of TPMT activity and determination of TPMT allelic polymorphisms, what are the within and between laboratory precision and reproducibility of the available methods of enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms (proficiency testing)?

Thirty-three studies reported information relevant to question 1b enzymatic measurement.^{74,106-108,111-116,118,121,124,128,131,135,136,138,140,142-148,150-156} Detailed information is available in Appendix C. Two studies were conducted in North-America,^{136,155} three studies in China,^{106,135,154} and the remaining 28 in Europe.

TPMT enzymatic activity is usually determined by measuring the formation of 6-methyl mercaptopurine (6-MMP) from 6-mercaptopurine (6-MP), with S-adenosyl-L-methionine (SAM) as the methyl donor. This was originally described by Weinshilboum et al, who used radiolabelled SAM.¹³⁴ More recently, 6-MMP has also been measured using high performance liquid chromatography (HPLC). TPMT is prevalent in many tissue types, but in clinical practice it is normally measured in red blood cells (RBCs).

Nine studies used radiolabelled SAM for TPMT assays.^{74,111,138,140,154,156} The reported inter-assay coefficient of variation (CV) ranged from 0.51 to 8.4 percent. The intra-assay CV ranged from 0.72 to 6.8 percent. One study reported regression values as $y = 0.72x + 0.43 = 0.75$, where x represents the value for first estimate of TPMT activity and y the value for the second estimate.¹³¹ This result indicates high reproducibility. One study reported a 95 percent confidence interval as 13.94 to 14.88 U/mL RBCs for a high TPMT enzyme sample and 8.07 to 8.78 U/mL RBCs for an intermediate TPMT sample.¹⁴⁸ One study¹⁴⁴ used thin layer chromatography and quantitative scanning to measure TPMT activity, with 6-MP as a substrate, and radiolabelled SAM as cosubstrate. The day to day variance was 8.5 ± 1.7 percent.

Seventeen studies used an HPLC assay to determine 6-MMP.^{106,108,113-116,118,121,135,143,145,146,150-153,155} The inter-assay CV ranged from 0.2 to nine percent and the intra-assay CV ranged from zero to 9.5 percent. One study reported the assay to be accurate, and highly reproducible when the samples were kept at -80°C for up to 25 days.¹¹⁶ Another study reported 4 percent precision and 96 to 103 percent accuracy.¹⁰⁷ However, the authors did not clearly describe how the variability was measured. Five studies used an HPLC method to measure 6-methylthioguanine (6-MTG) formed using 6-thioguanine (6-TG) as a substrate,^{112,124,136,142,147} and reported CVs from two to five percent for intra-assay, and from four to 10 percent for inter-assay performance.

Two studies measured the between laboratory precision and reproducibility.^{113,126} Two labs in France, Laboratoire de Pharmacologie Pediatrique et Pharmacogenetique (Hospital Robert Debre, France) and Laboratoire de Pharmacologie (Hotel-Dieu, France) studied HPLC precision with 6-MMP in erythrocyte lysate (25, 75, 125 ng/mL). The intra-day and inter-days CV values were 1.7 to 4.3 percent, and 0.8 to six percent respectively, for six samples. Inter-assay CV values were 5.8 percent for 20 samples, and 5.2 percent for five samples of quality control blood. Klemetsdal et al (Department of Pharmacology, Institute of Medical Biology, University of Tromso, Norway) compared RBC TPMT activity in five samples with those measured in Dr. Weinshilboum's laboratory at the Mayo Clinic (Rochester, USA), finding CV values of 1.8 percent to 2.6 percent for 20 analyses.¹²⁶

Overall, no obvious trend, depending on time or study characteristics, was observed between studies reporting lower versus those reporting higher accuracy and reproducibility.

No study was found that specifically investigated the accuracy and precision of TPMT allelic polymorphism determination. However, three studies compared different genotyping methods to one other.^{105,139,141} One group developed a novel multiplex assay using matrix-assisted laser desorption/ionization, with a time-of-flight mass spectrometer (MALDI-TOF mass spectrometry) based on Sequenom iPLEX technology.¹³⁹ All genotypes for 586 samples were 100 percent concordant with the results from a previous denaturing HPLC genotyping assay. The new method was accurate, with 100 percent agreement for duplicate analyses. The other study used TaqMan® 5' nuclease assay for genotyping.¹⁰⁵ Fifty samples genotyped by denaturing HPLC direct sequencing were in 100 percent concordance with TaqMan®.²¹³ In the third study, a microchip based method that includes PCR, RFLP and capillary electrophoresis in a single platform was developed and tested. 100 percent concordance was reported for 80 patients, comparing the microchip method with both the conventional RFLP assay and the commercial TaqMan® assay.¹⁴¹

Key points. The various methods used to determine TPMT enzymatic activity are based on a method developed by Weinshilboum et al.¹³⁴ While the enzymatic reaction has remained relatively unchanged, with a few minor adjustments, the method of product detection has evolved from radiolabel detection to HPLC.

Both detection techniques produce reliable results, with intra- and inter-assay CVs of less than 10 percent.

Choice of TPMT substrate, 6-MP or 6-TG, does not appear to affect the precision of the assay significantly; however, 6-MP appears to be more widely used.

Three studies have compared MALDI-TOF, TaqMan® and microchip assays with denaturing HPLC and RFLP, and demonstrated 100 percent concordance in genotypes.

Key Question 1c: What is the diagnostic sensitivity and specificity of TPMT allelic polymorphism measurement compared to the measurement of TPMT enzymatic activity in correctly identifying chronic autoimmune disease patients eligible for thiopurine therapy with low or absent TPMT enzymatic activity? How do effect modifiers (e.g. underlying disease prevalence and severity, different activity thresholds, Hardy-Weinberg equilibrium, number and types of alleles tested) explain any observed heterogeneity in sensitivity and specificity?

Nineteen studies reported relevant data on testing of variable numbers and types of variant thiopurine methyl transferase alleles (Table 8). For certain combinations of alleles tested, only single studies provided data, and for those combinations, evidence was considered insufficient for any meaningful syntheses.^{159,160,163} One early study undertaken in 1993 did not report the specific variant alleles that were tested.⁹³ In a sensitivity analysis we assumed the common TPMT *3A, *3B, *3C alleles were tested in this study, in keeping with the early date of publication when fewer alleles were being identified and tested.

Characteristics of included studies and risk of bias. Study characteristics are summarized in Table 15.

Over 50 percent of included studies were cross-sectional, and 21 percent were of prospective observational design. Studies did not specifically examine diagnostic accuracy of genetic testing with the TPMT enzymatic activity test as the reference standard, so we designated the activity test to be the reference standard and genotyping to be the index test. Over 75 percent of studies were conducted in patients with inflammatory bowel disease (IBD). Most studies used standard TPMT activity cutoffs.

Risk of bias is summarized in Figure 4 with studies grouped according to items of the QUADAS risk of bias scale.³⁵ Only one study clearly reported that genotyping and enzymatic activity measurements were not influenced by prior knowledge of the other test result.¹⁶² Sixty-three percent of studies were considered to be of fair quality and the rest were rated poor.

Diagnostic sensitivity and specificity of TPMT genotyping with respect to the enzymatic activity assay as the reference standard are presented below. Diagnostic groups are organized according to the specific set of TPMT variant alleles tested. Insufficient evidence was available for the following sets of single nucleotide polymorphisms (SNPs):

TPMT *2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8¹⁶⁰

TPMT *2, *3A, *3B, *3C, *3D¹⁶³

TPMT *3A, *3B, *3C, *3D¹⁵⁹

Table 15. Characteristics of studies of diagnostic accuracy of TPMT genetic testing compared with TPMT enzymatic activity

Characteristic		Number of studies	References
Study Design			
	Cross-Sectional	11	1,50,53,56,59,70,93,160-162,167
	NonRandomized Intervention Study	1	46
	Prospective Observational	4	49,51,158,159

Table 15. Characteristics of studies of diagnostic accuracy of TPMT genetic testing compared with TPMT enzymatic activity (continued)

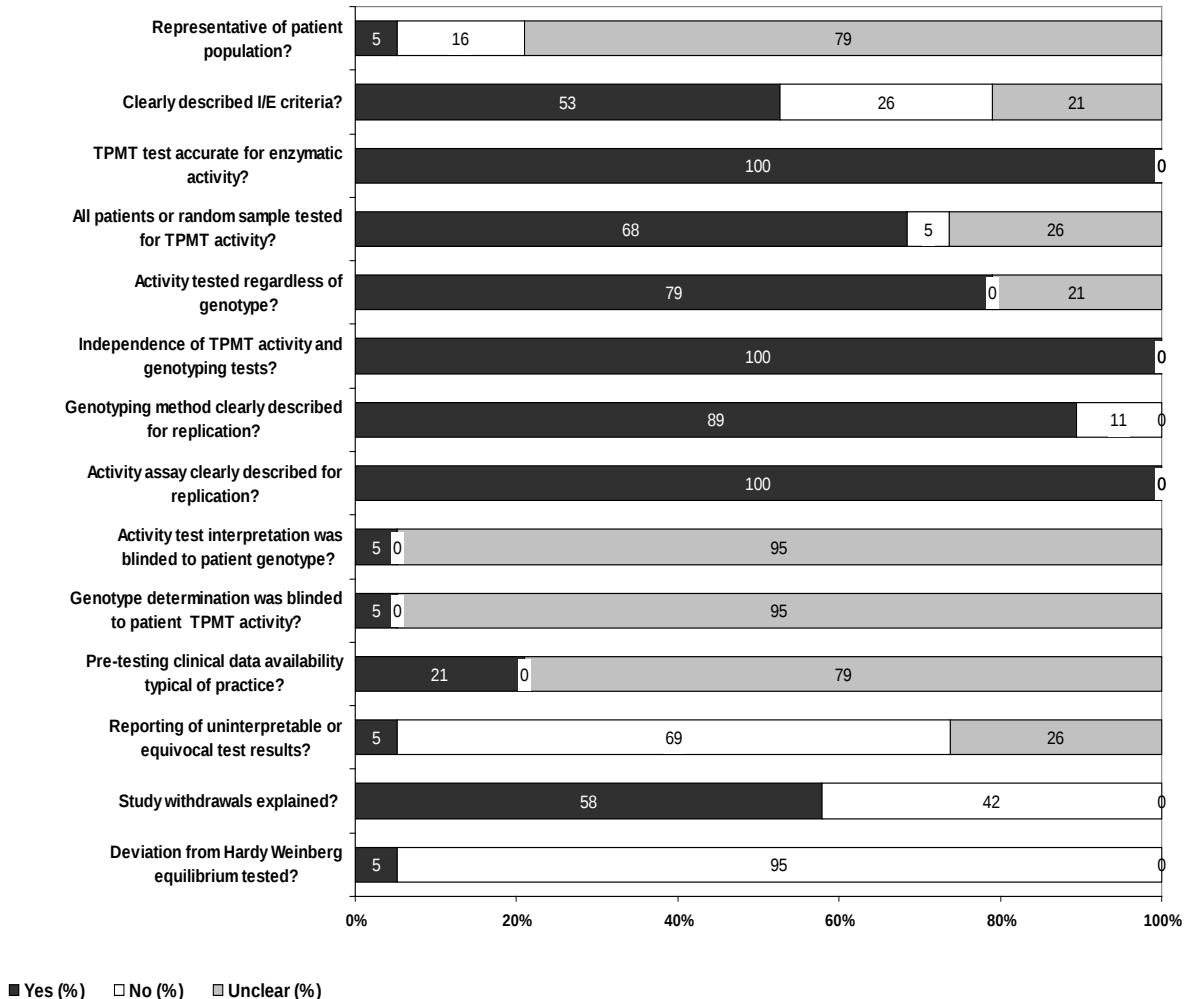
Characteristic		Number of studies	References
	Randomized Controlled Trial	1	52
	Retrospective Review of Records	2	157,163
Chronic Autoimmune Disease			
	AntiNeutrophil Cytoplasmic Antibody Associated Vasculitis	1	157
	Autoimmune Dermatologic Conditions	1	93
	Autoimmune Hepatitis	2	162,167
	Inflammatory Bowel Disease	14	1,46,49-52,56,59,70,158-161,163
	Systemic Lupus Erythematosus	1	53
Allelic Variants Tested			
	TPMT*2, *3A, *3B, *3C	7	1,51-53,59,70,157
	TPMT*2, *3A, *3B, *3C, *3D	1	163
	TPMT*2, *3A, *3C	3	56,158,161
	TPMT*3A, *3B, *3C	2	46,162
	TPMT*3A, *3B, *3C, *3D	1	159
	TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8	1	160
	TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, 10, *14, *15	3	49,167,214
	Not Reported	1	93
TPMT Assay Type			
	High Performance Liquid Chromatography	7	1,51,53,59,157,163,167
	Mass Spectrometry	2	46,70
	Radioassay	10	49,50,52,56,93,158-162
Genotyping Method			
	Denatured High Performance Liquid Chromatography	1	163
	Polymerase Chain Reaction	12	1,51-53,56,59,70,157-159,161,162
	Pyrosequencing	4	49,50,160,167
	Not Reported	2	46,93
Age Group			
	Adults	11	1,46,49,50,52,93,158,159,161,163,167
	Mixed	4	51,59,160,162
	Not Reported	4	53,56,70,157
Setting			
	Outpatient Specialty Clinics	9	1,46,49,51,56,59,161-163
	Inpatients, and Outpatient Specialty Clinic	1	158
	Not Reported	8	50,52,70,93,157,159,160,167,214
Region			
	Asia	1	53
	Europe	14	1,46,49-52,56,59,70,159,160,162,163,167
	North America	1	93

Table 15. Characteristics of studies of diagnostic accuracy of TPMT genetic testing compared with TPMT enzymatic activity (continued)

Characteristic		Number of studies	References
	Oceania	1	158
	Not Reported	2	157,161
Risk of Bias			
	Fair	12	1,46,49,50,52,56,70,157,159,161,163,167
	Poor	7	51,53,59,93,158,160,162

Abbreviations: TPMT = thiopurine methyltransferase

Figure 4. Risk of bias of studies of diagnostic accuracy of TPMT genetic testing compared with TPMT enzymatic activity



As described above in the “Methods” section, when possible, two meta-analyses were considered for each set of variant alleles. In meta-analysis 1, genotypes were dichotomized into noncarriers (or wild types) and carriers (or homozygotes and/or heterozygotes) and phenotypes were dichotomized into normal (or high) and subnormal (or intermediate and/or low and/or absent) enzymatic activities. In meta-analysis 2, we dichotomized genotypes into noncarriers and/or heterozygotes and homozygotes, and phenotype into normal and/or high and/or intermediate and low/absent activities (Table 16).

Table 16. Meta-analyses 1 and 2, for sensitivity and specificity of variant allelic determination to diagnose TPMT enzymatic activity

Meta-analysis 1					
	Normal/high activity	Intermediate/low/ absent activity		Sensitivity 1	Specificity 1
Homozygotes and/or heterozygotes	A1	B1		B1/(B1+D1)	C1/(C1+A1)
Noncarrier (or Wild types)	C1	D1			
Meta-analysis 2					
	Normal/high/intermediate activity	Low/absent activity		Sensitivity 2	Specificity 2
Homozygotes	A2	B2		B2/(B2+D2)	C2/(C2+A2)
Noncarrier (or Wild types) and/or heterozygotes	C2	D2			

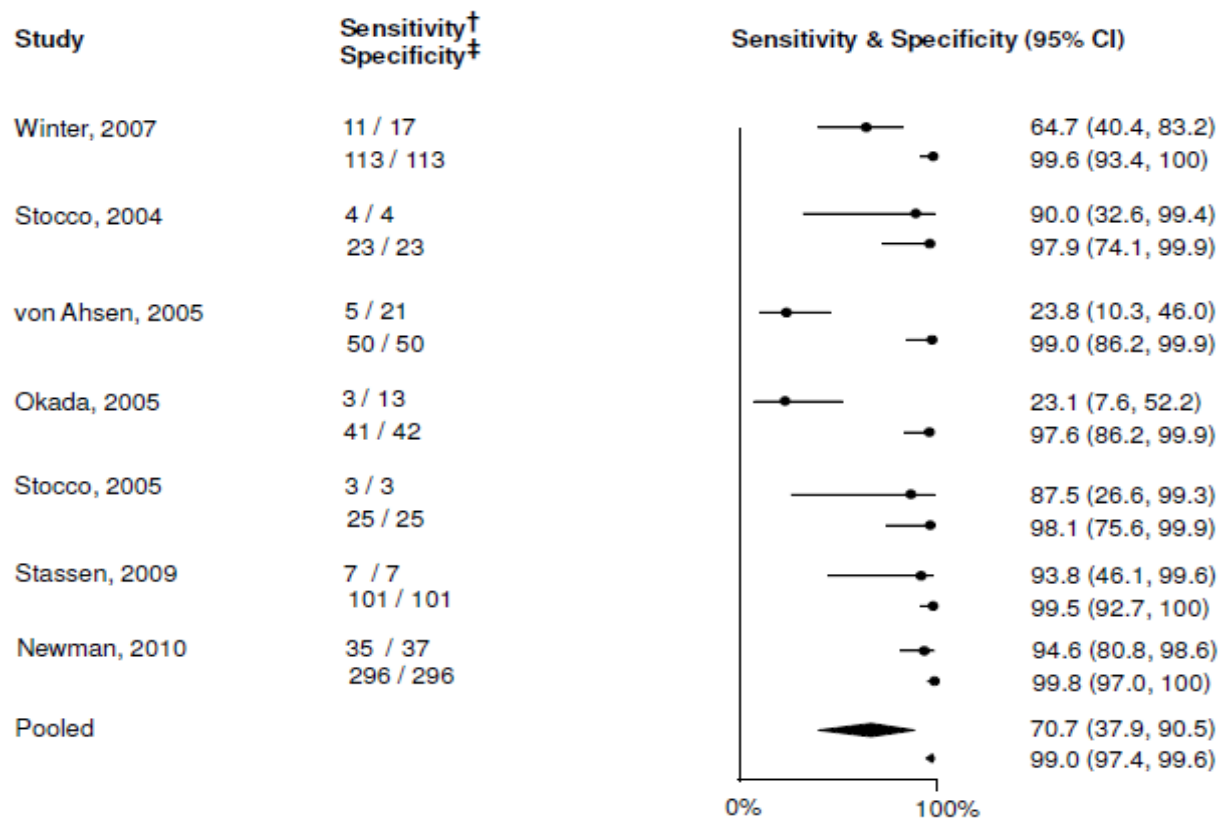
TPMT *2, *3A, *3B, and *3C. These variant alleles were genotyped in seven studies involving 752 participants.^{1,51-53,59,70,157} In meta-analysis 1, the pooled sensitivity of the carrier genotypes (i.e. homozygosity and heterozygosity) to correctly identify all those patients with subnormal enzymatic activity as determined by activity assays was 70.70 percent (95 percent CI 37.90 percent to 90.50 percent). Risk of bias was not found to be significantly associated with variation in effect estimates. No explanation was identified for this heterogeneity. The pooled specificity of the noncarrier or wild type genotype to correctly identify all of those with normal/high enzymatic activity was 99.90 percent (95 percent CI 97.40 percent to 99.60 percent) (Figure 5).

In meta-analysis 2, since only one patient was homozygous for a TPMT allele, only the pooled specificity of noncarrier and heterozygous carrier genotype to correctly identify all those who do not have low or absent enzymatic activities could be calculated. This approached 100 percent (Figure 6).

In further sensitivity analyses, we assumed that studies that specifically tested the alleles TPMT *2, *3A, *3C (see below) also implicitly tested for *3B, because of shared *3B SNP 460G→A, which is one of the two TPMT *3A alleles. We therefore widened meta-analysis 1 and 2 to included the three studies that reported specifically testing for TPMT *2, *3A, *3C as additional sensitivity analyses.^{56,158,161} Still, only one of a total of 945 patients in nine studies was homozygous for a TPMT variant allele. In the sensitivity meta-analysis 1, the pooled sensitivity of the carrier genotype (i.e. homozygosity and heterozygosity) to correctly identify all those patients with subnormal enzymatic activity as determined by activity assays remained unchanged at 74.00 percent (95 percent CI 48.90 percent to 89.40 percent). Risk of bias was again not found to be significantly associated with variation in effect estimates. The pooled specificity of the noncarrier or wild type genotype to correctly identify all of those with normal/high enzymatic activity was 98.60 percent (95 percent CI 97.10 percent to 99.30 percent) (Figure 7).

In the sensitivity meta-analysis 2, since only one patient was homozygous for a TPMT allele, only the pooled specificity of noncarrier and heterozygous carrier genotype to correctly identify all those who do not have low or absent enzymatic activities could be calculated. This approached 100 percent (Figure 8)

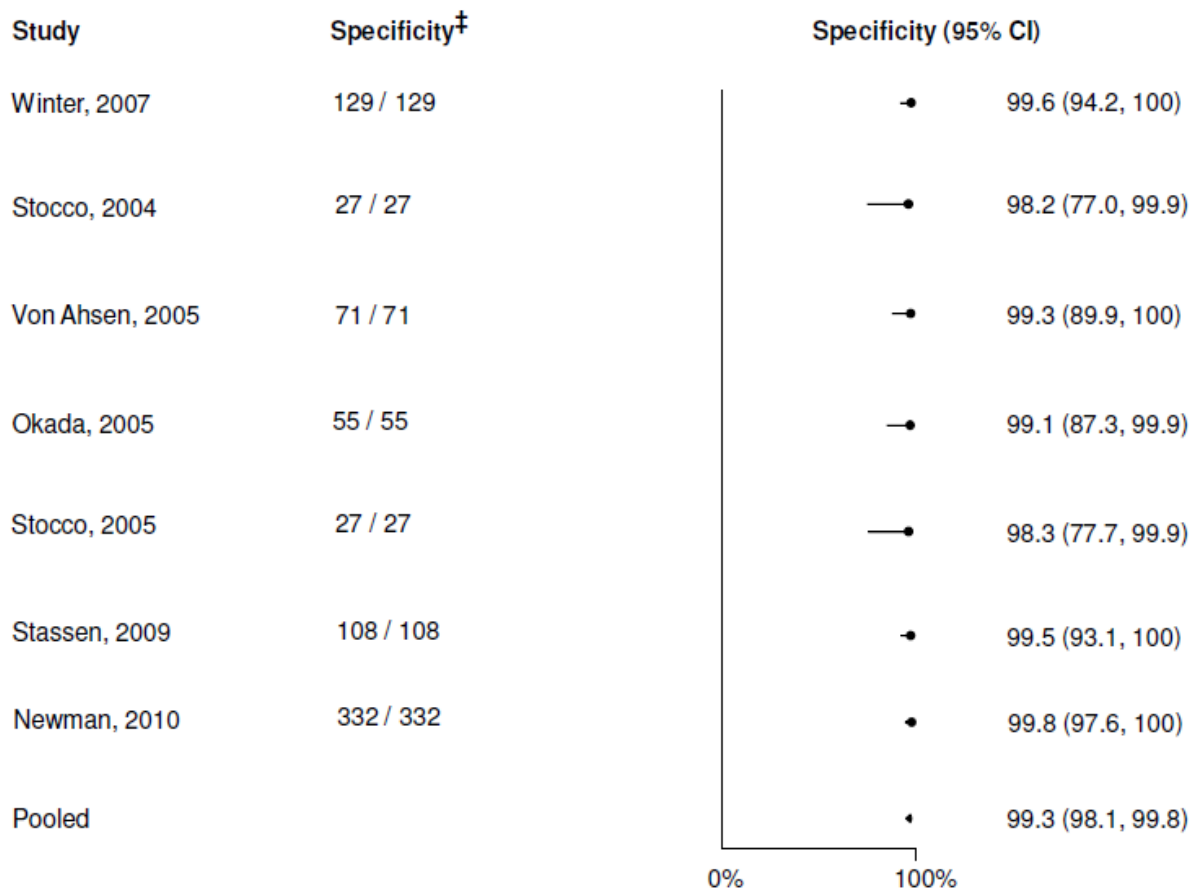
Figure 5. Meta-analysis 1 of sensitivity and specificity of genotyping TPMT *2, *3A, *3B and *3C, to diagnose TPMT activity



[†] (Homozygotes or heterozygotes with intermediate or low to absent enzymatic activity)
(all with intermediate or low enzymatic activity)

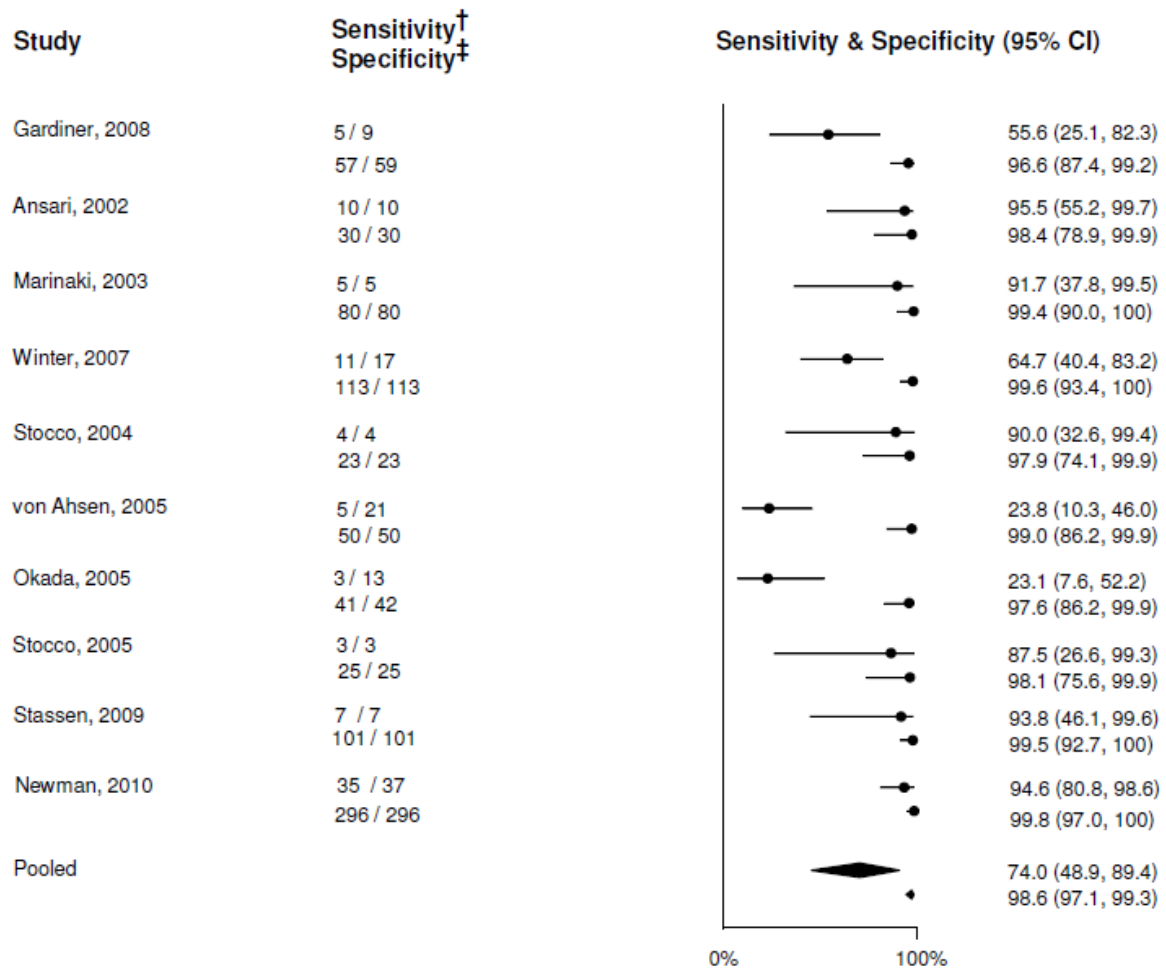
[‡] (Noncarriers or wild type genotype with normal/high enzymatic activity)
(all with normal or high enzymatic activity)

Figure 6. Meta-analysis 2 of specificity of genotyping TPMT *2, *3A, *3B and *3C, to diagnose TPMT activity



[‡] (Noncarriers (wild type) and/or heterozygotes with normal/intermediate/high enzymatic activity)
(all with normal, intermediate or high enzymatic activity)

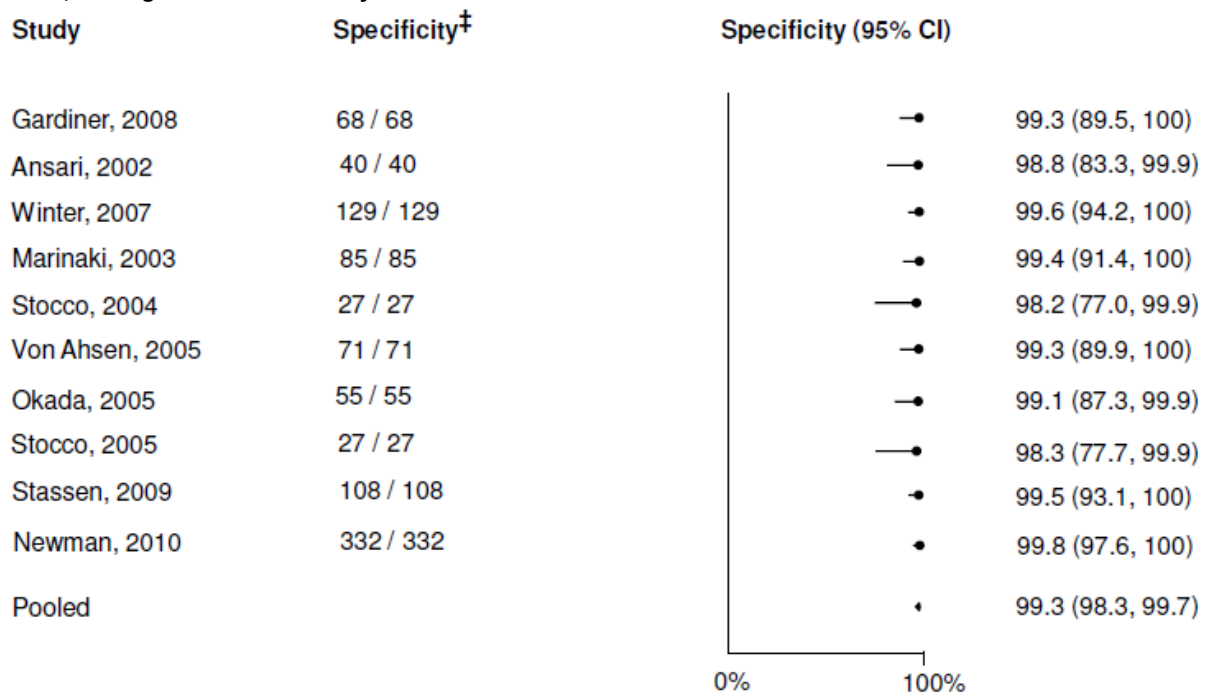
Figure 7. Additional meta-analysis 1 of sensitivity and specificity of genotyping TPMT *2, *3A, *3B and *3C, or TPMT *2, *3A and *3C, to diagnose TPMT activity



[†] (Homozygotes or heterozygotes with intermediate or low to absent enzymatic activity)
(all with intermediate or low enzymatic activity)

[‡] (Noncarriers or wild type genotype with normal/high enzymatic activity)
(all with normal or high enzymatic activity)

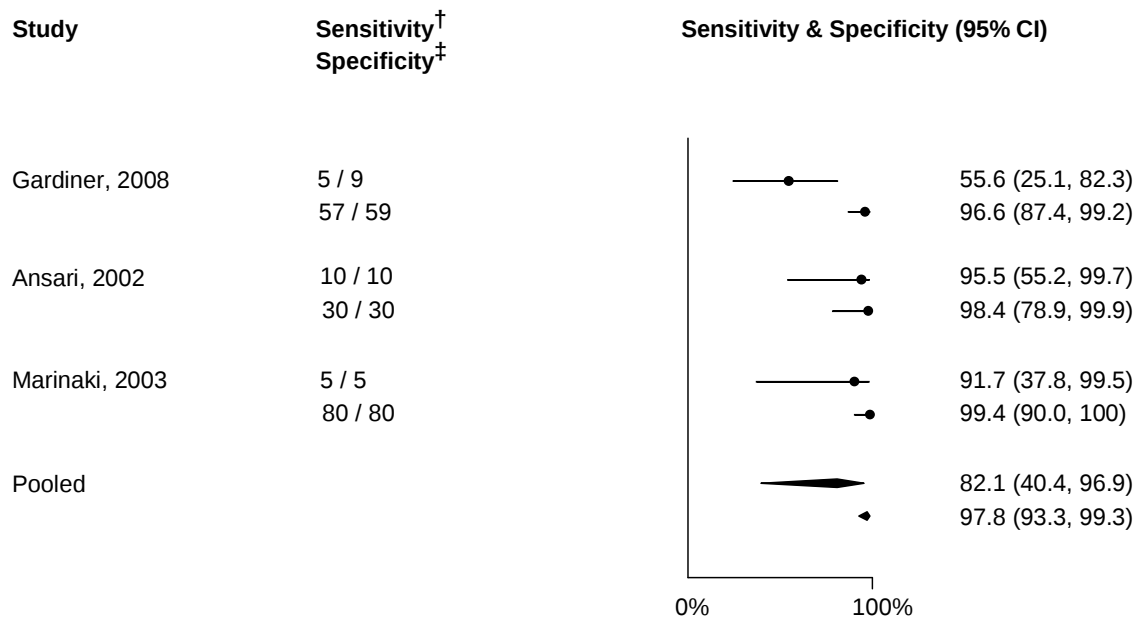
Figure 8. Additional meta-analysis 2 of specificity of genotyping TPMT *2, *3A, *3B and *3C, or TPMT *2, *3A and *3C, to diagnose TPMT activity



[‡] (Noncarriers (wild type) and/or heterozygotes with normal/intermediate/high enzymatic activity)
(all with normal, intermediate or high enzymatic activity)

TPMT *2, *3A, and *3C. These variant alleles were genotyped in three studies including 386 patients.^{56,158,161} In meta-analysis 1, the pooled sensitivity of the carrier genotype (i.e. homozygosity and heterozygosity) to correctly identify all those patients with subnormal enzymatic activity was 82.10 percent (95 percent CI 40.40 percent to 96.90 percent). The pooled specificity of noncarrier or wild type genotypes to correctly identify all those with normal/high enzymatic activity was 97.80 percent (95 percent CI 93.30 percent to 99.30 percent).(Figure 9). This group of studies did not include any homozygous participant; hence only the specificities of noncarrier and heterozygous carrier genotypes to correctly identify all those who do not have low/absent enzymatic activity could be pooled for meta-analysis 2. The pooled estimate approached 100 percent (Figure 10).

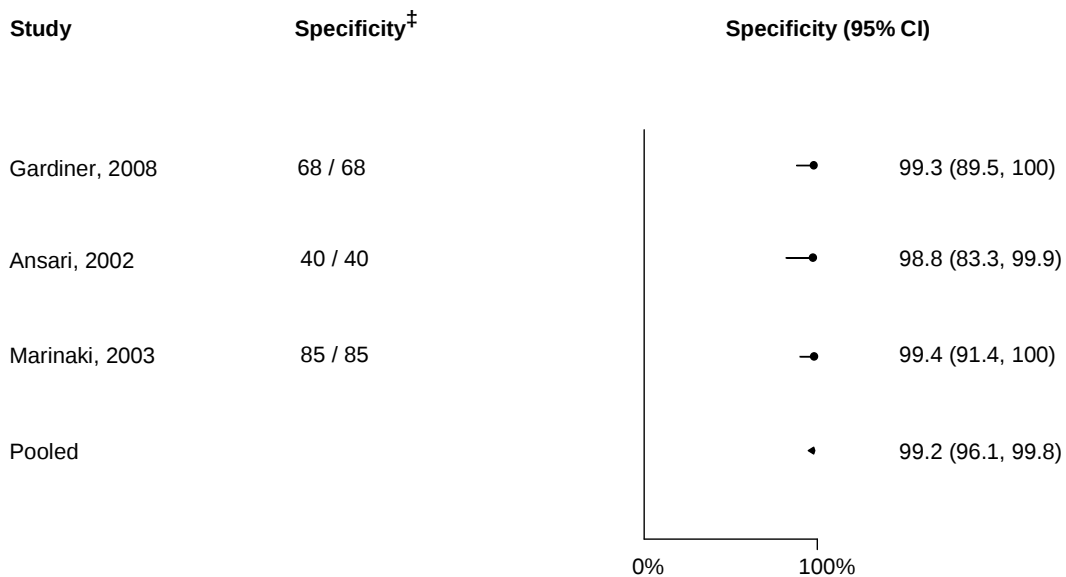
Figure 9. Meta-analysis 1 of sensitivity and specificity of genotyping TPMT *2, *3A, and *3C, to diagnose TPMT activity



[†] (Homozygotes or heterozygotes with intermediate or low to absent enzymatic activity)
(all with intermediate or low enzymatic activity)

[‡] (Noncarriers or wild type genotype with normal/high enzymatic activity)
(all with normal or high enzymatic activity)

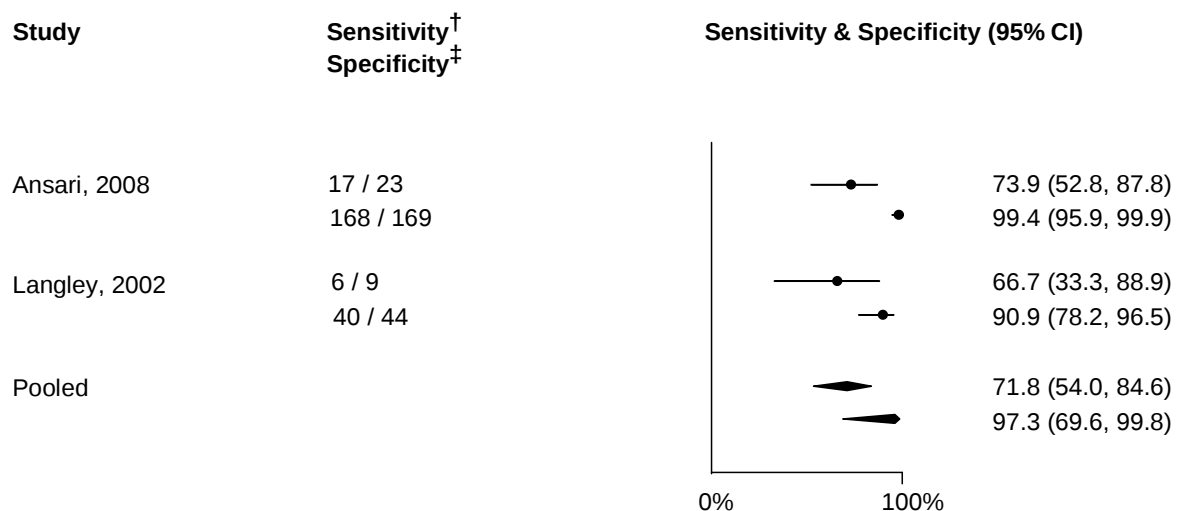
Figure 10. Meta-analysis 2 of specificity of genotyping TPMT *2, *3A, and *3C, to diagnose TPMT activity



[‡] (Noncarriers (wild type) and/or heterozygotes with normal/intermediate/high enzymatic activity)
(all with normal, intermediate or high enzymatic activity)

TPMT *3A, *3B, and *3C. These variant alleles were genotyped in two studies that included a total of 245 participants.^{46,162} In meta-analysis 1, the pooled sensitivity of the carrier genotype (i.e. homozygosity and heterozygosity) to correctly identify all those patients with subnormal enzymatic activity was 71.80 percent (95 percent CI 54.00 percent to 84.60 percent). The pooled specificity of noncarrier or wild type genotypes to correctly identify all those with normal/high enzymatic activity was 97.30 percent (95 percent CI 69.60 percent to 99.80 percent) (Figure 11). In a sensitivity analysis, when we included an early study by Snow et al.,⁹³ a study that did not report variant alleles it tested, under the assumption that it tested TPMT *3A, *3B, *3C, the results remained essentially unchanged for pooled estimates of both sensitivity and specificity (N = 26). Because no homozygous carriers were included, meta-analysis 2 was only possible for specificity of noncarrier and heterozygous carrier genotypes to correctly identify all those who do not have low/absent enzymatic activity. The pooled specificity approached 100 percent.

Figure 11. Meta-analysis 1 of sensitivity and specificity of genotyping TPMT *3A, *3B and *3C, to diagnose TPMT activity



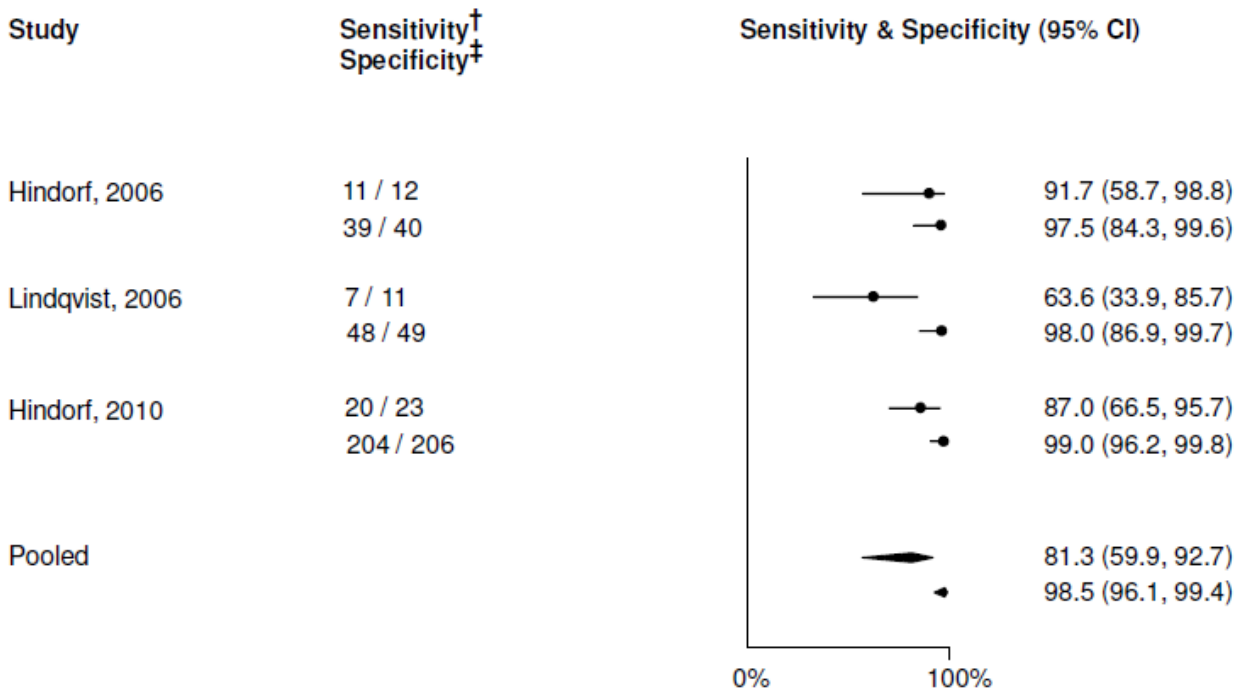
[†] (Homozygotes or heterozygotes with intermediate or low to absent enzymatic activity)
(all with intermediate or low enzymatic activity)

[‡] (Noncarriers or wild type genotype with normal/high enzymatic activity)
(all with normal or high enzymatic activity)

TPMT *2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, and *15. These variant alleles were genotyped in three studies that included a total of 341 patients of whom seven were homozygous for an SNP.^{49,50,167} In meta-analysis 1, the pooled sensitivity of the carrier genotype (i.e. homozygosity and heterozygosity) to correctly identify all those patients with subnormal enzymatic activity was 81.30 percent (95 percent CI 59.90 percent to 92.70 percent). The pooled specificity of noncarrier or wild type genotypes to correctly identify all those with normal/high enzymatic activity was 8.50 percent (95 percent CI 96.10 percent to 99.40 percent) (Figure 12). In meta-analysis 2, the sensitivity of the homozygous genotype to correctly identify all patients with low/absent enzymatic activity was 87.10 percent (95 percent CI 44.30 percent to 98.30 percent), and the specificity of noncarrier and heterozygous carrier genotype to correctly identify all those who do not have low/absent enzymatic activity approached 100 percent (Figure 13).

No other combinations of variant TPMT alleles were tested.

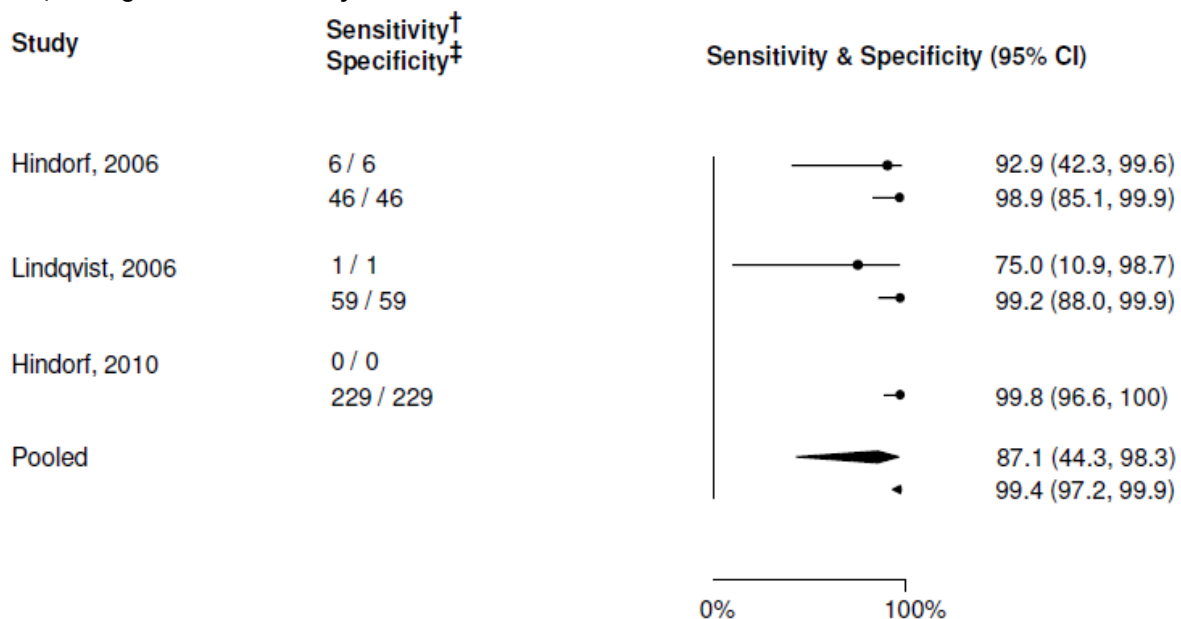
Figure 12. Meta-analysis 1 of sensitivity and specificity of genotyping TPMT *2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, and *15, to diagnose TPMT activity



[†] (Homozygotes or heterozygotes with intermediate or low to absent enzymatic activity)
(all with intermediate or low enzymatic activity)

[‡] (Noncarriers or wild type genotype with normal/high enzymatic activity)
(all with normal or high enzymatic activity)

Figure 13. Meta-analysis 2 of specificity of genotyping TPMT *2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, and *15, to diagnose TPMT activity



[†] (Homozygous with low/absent enzymatic activity)
(all with low/absent enzymatic activity)

[‡] (Noncarriers (wild type) and/or heterozygotes with normal/intermediate/high enzymatic activity)
(all with normal, intermediate or high enzymatic activity)

Key points. A total of 16 studies, mostly of cross-sectional and prospective observational design, contributed to quantitative syntheses.^{1,46,49-53,56,59,70,93,157,158,161,162,167} Studies did not specifically examine diagnostic accuracy of genetic testing with the TPMT enzymatic activity test as the reference standard, so we designated the activity test to be the reference standard and genotyping to be the index test. Overall, homozygosity for variant allele(s) was quite low in the study samples. Different combinations of TPMT variant alleles were analyzed in various studies. Thirty-seven percent of the studies were rated as of poor quality.

Across all the combinations of alleles tested, an imprecise pooled sensitivity of the carrier genotype (i.e. homozygous plus heterozygous patients) to correctly identify all those patients with subnormal (intermediate, or low to absent) enzymatic activity, as determined by TPMT assays, was in the range of 70.70 to 82.10 percent (95 percent CI, lower bound range 37.90 to 54.00 percent; upper bound range 84.60 to 96.90 percent).

The pooled sensitivity of a homozygous TPMT genotype to correctly identify patients with low to absent enzymatic activity was based on two studies with two percent of 341 patients identified as homozygous for variant allele. The pooled sensitivity was 87.10 percent (95 percent CI 44.30 to 98.30 percent).

Meta-regression analysis did not identify any significant effect modifiers.

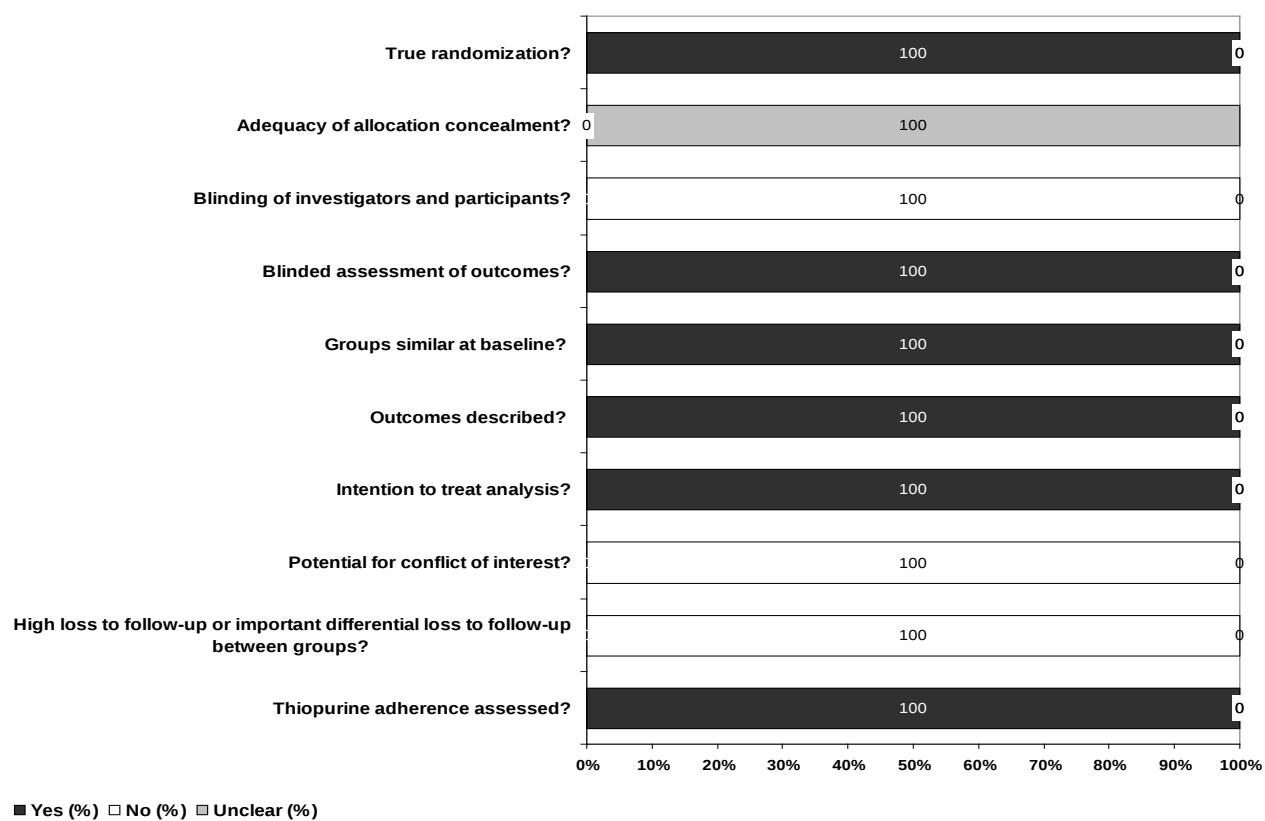
Compared with the reference standard of TPMT enzymatic activity, the specificity of TPMT genotyping to correctly identify patients with normal/high enzymatic activities, or normal/high and intermediate enzymatic activities, is very high (greater than 90 percent) across all combinations of alleles tested.

There is insufficient data to determine the optimum combination of TPMT alleles for testing. Most studies tested at least the TPMT *3A, *3B, and *3C variant alleles, irrespective of testing additional polymorphisms.

Key Question 2: Does the measurement of TPMT enzymatic activity or determination of TPMT allelic polymorphisms change the management of patients with chronic autoimmune disease when compared with no determination of TPMT status?

The evidence to answer this question came from one randomized controlled trial of fair quality in 333 patients with inflammatory conditions, mostly inflammatory bowel disease (Figure 14 and Appendix C, Tables C7-9).¹ Patients were randomized into either a group that had prior TPMT genotyping or one without pretesting. Where applicable, therapy was advised to be guided by genotyping results, however, all treatment decisions were at the discretion of treating physicians. Over a 4 month period, no significant differences between starting doses administered with or without prior knowledge of TPMT genotype were observed in both the noncarriers and heterozygous carrier patients. However, in the genotyped treatment arm, heterozygotes received significantly lower doses of TPMT when compared with noncarriers. Most patients in both groups were given starting doses lower than 2mg/kg/day including those with predisclosed noncarrier genotype. About seven percent of those in whom noncarrier genotype was predisclosed received AZA doses $\geq$ 2mg/kg/day as compared to 8.4 percent of those in whom noncarrier genotype was found out after the fact. Furthermore, there was no significant difference between the two groups in terms of mean AZA prescribed dose at the end of the study period. There is limited applicability of this evidence because there was just one homozygous carrier in the whole sample of mostly IBD patients.

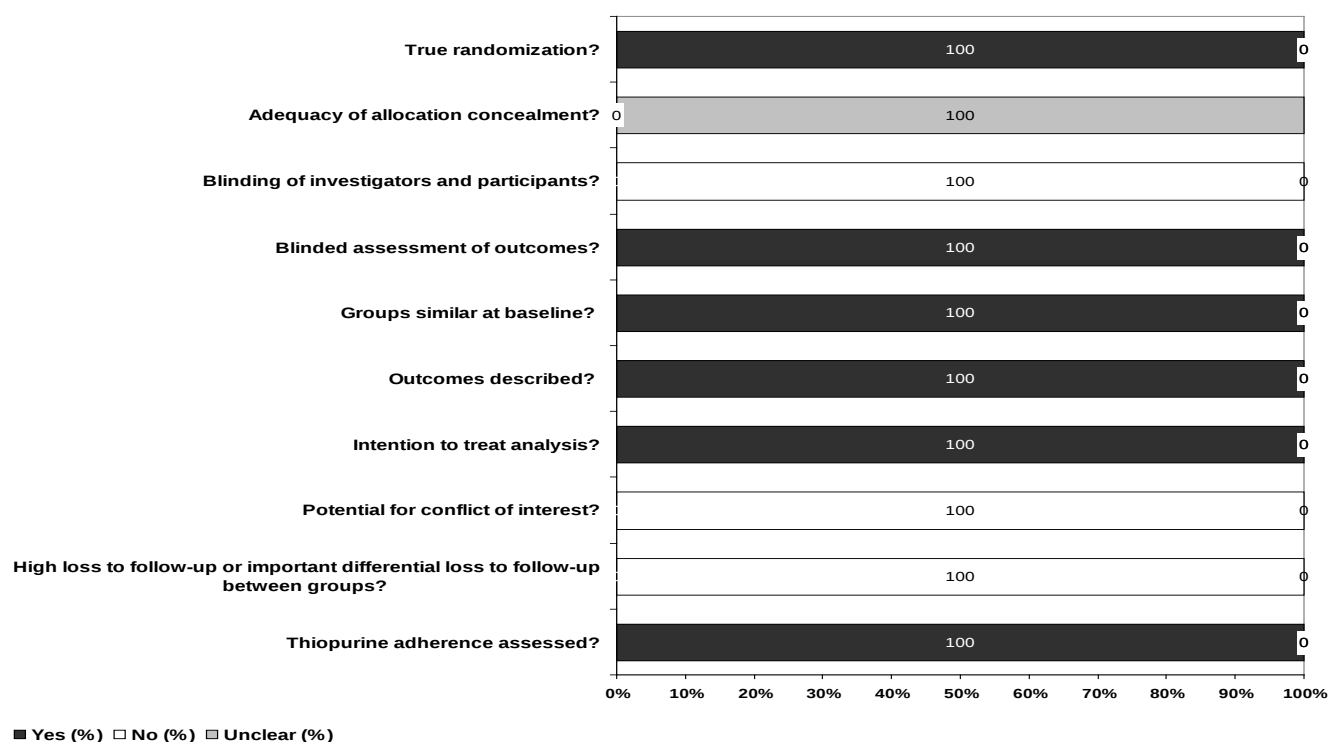
Figure 14: Risk of bias of the randomized controlled trial answering KQ 2



Key Question 3a: In chronic autoimmune disease patients prescribed thiopurine-based drugs (AZA or 6-MP), does the assessment of TPMT status to guide therapy, when compared with no pretreatment assessment, lead to reduction in rates of mortality, infection, hospitalization, withdrawal due to adverse events (WDAE), serious adverse events (SAE) and improvement in health-related quality of life?

The evidence to answer this question came from one randomized controlled trial of fair quality in 333 patients with inflammatory conditions, mostly inflammatory bowel disease (Figure 15 and Appendix C, Tables C 10-12).¹ Patients were randomized into either a group that had prior TPMT genotyping or another without pretesting. Where applicable, therapy was advised to be guided by genotyping results; however, all treatment decisions were at the discretion of treating physicians. Over a 4 month period, no significant differences were seen in the outcomes of mortality [1/167 versus 3/166; odds ratio 0.33 (95 percent CI, 0.03 to 3.18 percent)]; SAE [4/167 versus 8/166; odd ratio 0.48 (95 percent CI 0.14 to 1.64 percent)]; and WDAE (0/167 versus 0/166). Other outcomes were not reported including the important outcome of health-related quality of life.

Figure 15: Risk of bias of the randomized controlled trial answering KQ 3a



Strength of evidence answering key question 3a. The evidence was rated following published guidance³⁹ as follows:

Table 17. Rating the strength of evidence-key question 3a

Outcome	N of studies	N of Subjects	Domains pertaining to strength of evidence				OR (95% CI)	Strength of evidence
			Risk of Bias	Consistency	Directness	Precision		
Mortality	1 ¹	333	Medium	Unknown	Direct	Imprecise	0.33 (0.03, 3.18)	Insufficient
Serious adverse events	1 ¹	333	Medium	Unknown	Direct	Imprecise	0.48 (0.14, 1.64)	Insufficient
Health-related quality of life	0	0	-	-	-	-	-	Insufficient

There is limited applicability of this evidence as there was just one homozygous carrier of TPMT variant allele in the entire sample of mostly IBD patients observed for just 4 months. Also, patients likelier to experience adverse events were excluded.

Key Question 3b: In chronic autoimmune disease patients prescribed thiopurine-based drugs (AZA or 6-MP), does the assessment of TPMT status to guide therapy, when compared with no pretreatment assessment, lead to reduction in rates of myelotoxicity, liver toxicity, and pancreatitis?

The evidence to answer this key question was available from one randomized controlled trial and one retrospective cohort study (Appendix C, Tables C13-15).^{1,169} No significantly reduced event rates were noted for intermediate outcomes in the pretested versus the nonpretested groups.

One trial of fair quality in 333 patients with inflammatory conditions, mostly inflammatory bowel disease, in which patients were randomized into either a group that had prior TPMT genotyping or another without pretesting (Figure 16).¹ Where applicable, therapy was advised to be guided by genotyping results, however, all treatment decisions were at the discretion of treating physicians. Over a 4 month period, No significant differences were seen in the outcomes of neutropenia [2/167 versus 1/166; odd ratio 2.00 (95 percent CI 0.18 to 22.27 percent)]; and pancreatitis [1/167 versus 4/166; odd ratio 0.24 (95 percent CI 0.03 to 2.21 percent)]; while significantly higher odds were observed for hepatitis in the group that underwent prior TPMT genotyping [19/167 versus 8/166; odds ratio 2.54 (95 percent CI 1.08 to 5.97 percent)]. Other intermediate outcomes were not reported. There is limited applicability of this evidence as there was just one homozygous carrier in the whole sample of patients observed for just 4 months. Also, patients likelier to experience adverse events were excluded.

Banerjee et al.'s was a retrospective cohort study in a pediatric population with IBD, treated with at least 4 months of stable dose treatment with azathioprine (AZA) or 6-mercaptopurine (6-MP).¹⁶⁹ Ninety percent of patients received AZA. The study group was comprised of 64 patients who received initial AZA dosing based on TPMT enzymatic activity as measured by the high performance liquid chromatography (HPLC) method, with subsequent dose titrations based on 6-thioguanine nucleotide and 6-methylmercaptopurine levels. A historical control of 37 patients had been started on AZA 1.5 mg/kg/day and subsequent doses were adjusted based on clinical response and drug toxicity. Fourteen percent of patients had intermediate TPMT enzymatic activity and none had low/zero activity. On average, the study group received 1.7 mg/kg/day of AZA treatment and the control 1.2 mg/kg/day. A statistically significant difference was not observed for the outcome of leukopenia (study versus control group; 4.7 percent versus 0 percent) and hepatotoxicity (study versus control group; 9.4 percent versus 16.2 percent). The overall risk of bias assessment rated this evidence as poor, based on several limitation (Figure 17).

Figure 16: Risk of bias of the single randomized controlled trial answering KQ 3b

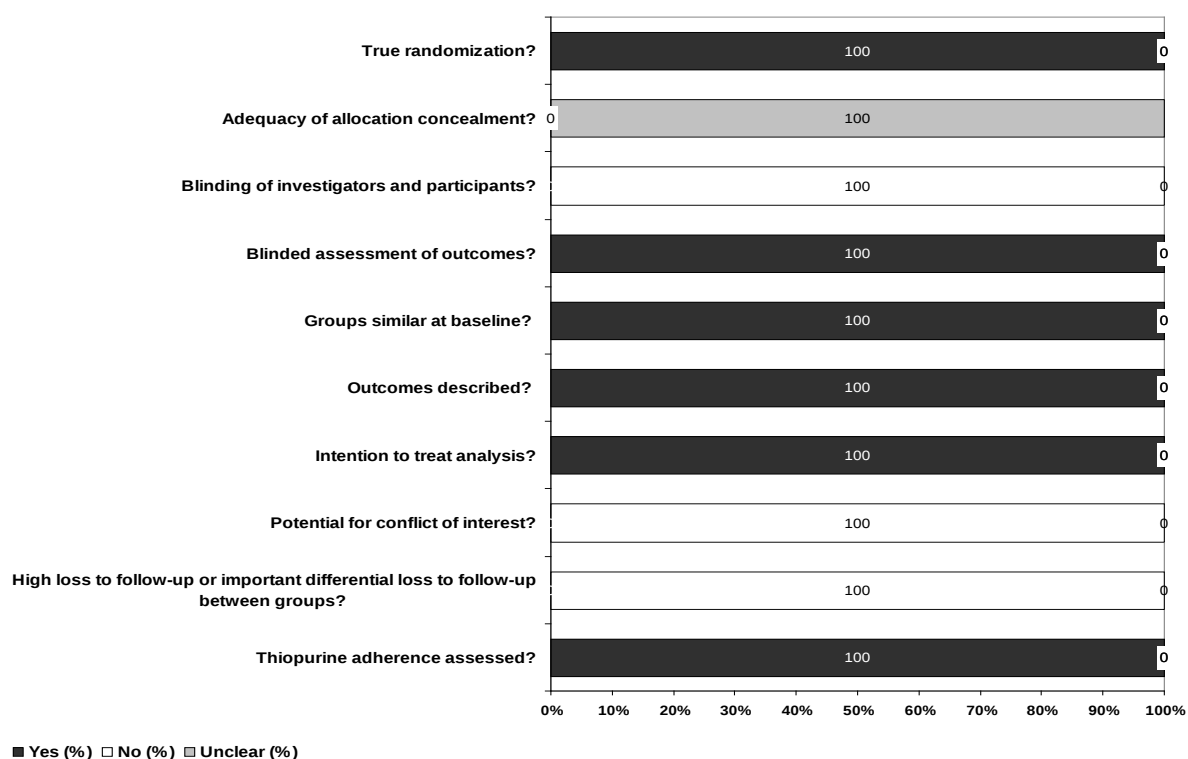
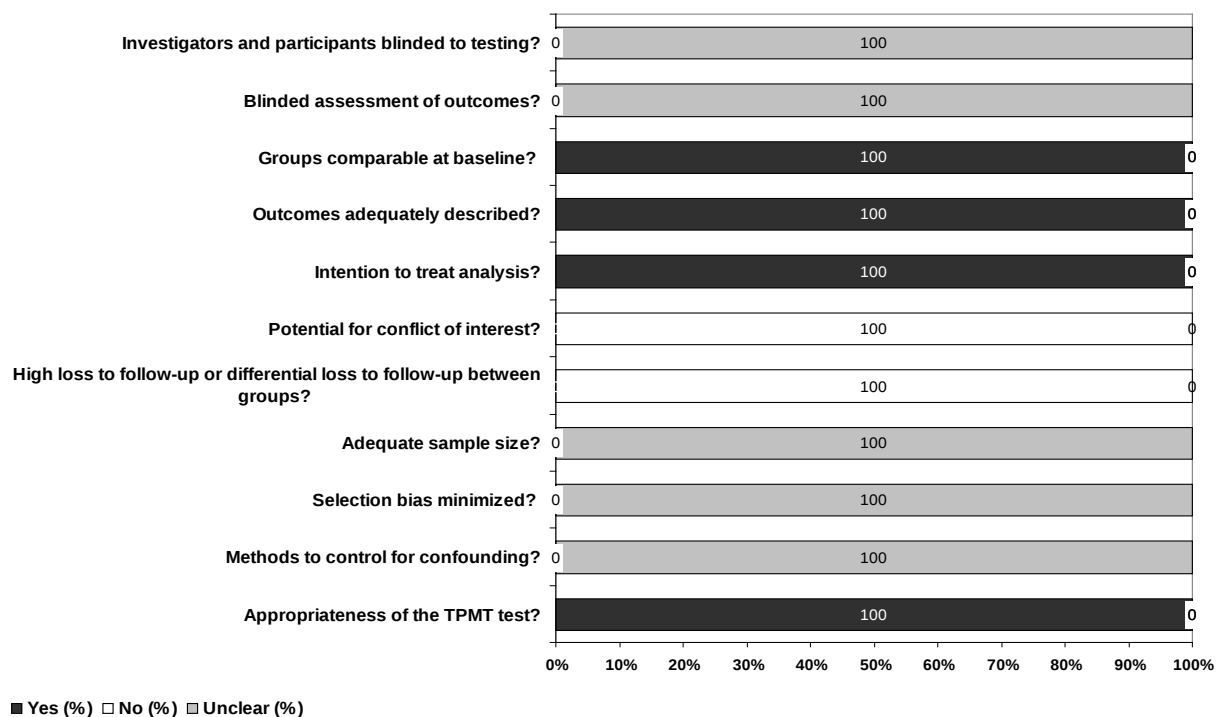


Figure 17: Risk of bias of the single retrospective cohort study answering KQ 3b



Strength of evidence answering key question 3b. The evidence was rated following published guidance³⁹ as follows:

Table 18. Rating the strength of evidence-key question 3b

Outcome	N of studies	N of Subjects	Domains pertaining to strength of evidence				OR (95% CI)	Strength of evidence
			Risk of Bias	Consistency	Directness	Precision		
Myelotoxicity	0	0	-	-	-	-	-	Insufficient

Key Question 3c: In the absence or inconclusiveness of evidence answering key question 3a and/or 3b above, is there an association between TPMT status (as determined by TPMT enzymatic activity and/or TPMT allelic determination) and/or the following amongst chronic autoimmune disease patients treated with thiopurines:

- i) the clinical outcomes of mortality, infections, hospitalization, withdrawal due to adverse events (WDAE), serious adverse events (SAE) and health-related quality of life?**
- ii) surrogate outcomes of myelotoxicity, liver toxicity, and pancreatitis?**

TPMT Enzymatic Activity Determination

Sixteen of the eligible studies reported relevant data (Table 19). No evidence was available for the outcomes of mortality, hospitalization, SAE, and HQOL. The sparse data available for the outcomes of infection, neutropenia and thrombocytopenia, did not permit a meaningful analysis.

Characteristics of included studies and quality are summarized in Table 19. Approximately 50 percent of studies were in populations with IBD. In 12 studies reporting gender distribution, female representation was on average 50 percent.^{50-52,70,72,79,91,93,161,193,196,197}

Racial distribution was very poorly reported. In 80 percent of included studies, thiopurine treatment was restricted to AZA, and when reported dosages were generally in the range 1.5-

2.5mg/kg/day.^{50,52,53,70,72,91,93,157,161,192,193,195,197} The adverse event observation period on thiopurine treatment ranged between six and 48 months (average 20 months) while four studies did not report this information.^{53,93,193,195}

Over sixty percent of studies were cross-sectional in design, and most studies were rated as fair quality. Most importantly, biased enzymatic activity determination based on prior knowledge of outcomes or outcomes assessment based on prior knowledge of enzymatic activity results could not be clearly ruled out in any one of the studies addressing this question. No study was rated as good quality, 25 percent were rated as poor and the rest were judged as fair.

Figure 18 depicts the distribution of studies by individual quality scoring items. Detailed results of all 16 studies are available in Appendix C.

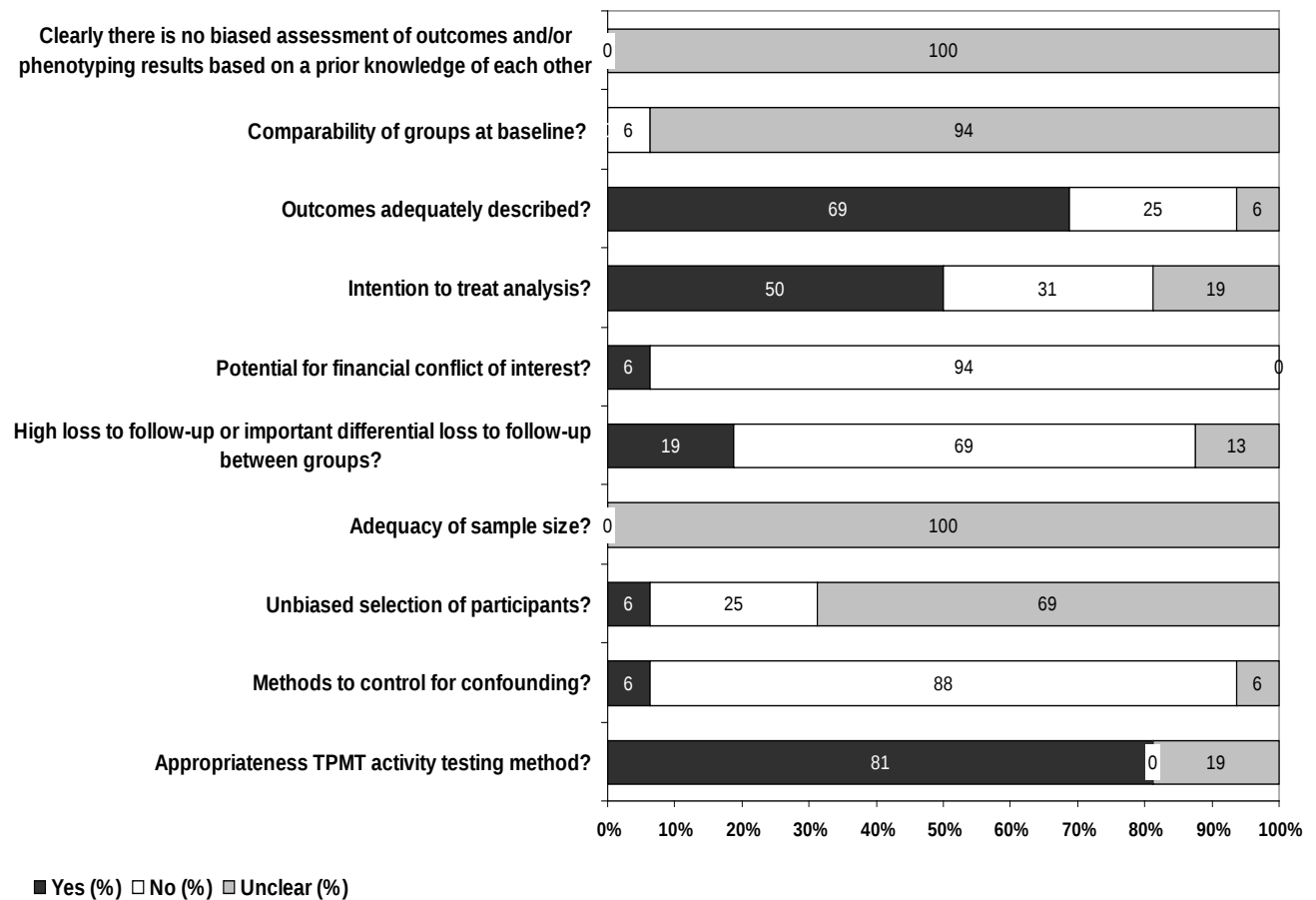
Table 19. Summary characteristics of TPMT enzymatic activity and thiopurine toxicity association studies

Characteristic		Number of studies	References
Study Design			
	Case Control	1	¹⁹⁷
	Chart Review	1	¹⁵⁷
	Cohort	1	¹⁹⁵
	Cross-Sectional	10	^{50,51,53,70,79,93,161,192,193,196}
	NonRandomized Intervention Study	1	⁹¹
	Prospective Observational	1	⁷²
	Randomized Controlled Trial	1	⁵²

Characteristic		Number of studies	References
Chronic Autoimmune Disease			
	AntiNeutrophil Cytoplasmic Antibody Associated Vasculitis	1	157
	Autoimmune Dermatologic Conditions	1	93
	Autoimmune Disorders	2	195,197
	Autoimmune Hepatitis	1	79
	Inflammatory Bowel Disease	8	50-52,70,72,161,192,196
	Pemphigus Vulgaris	1	193
	Rheumatoid Arthritis	1	91
	Systemic Lupus Erythematosus	1	215
	Not Reported		
Thiopurine Treatment	6-MP, AZA	1	196
	AZA	14	50,50,52,53,70,72,91,93,157,161,192,193,195,197
	Mixed Thiopurines	1	51
Assay Type	High Performance Liquid Chromatography	4	51,53,157,193
	Mass Spectrometry	1	70
	Radioassay	9	50,52,72,79,91,93,161,195,197
	Not Reported	2	192,196
Outcomes Assessed	Anemia	2	193,197
	Any Infection	1	91
	Hepatitis	9	50-52,70,72,91,93,193,196
	Leukopenia	8	53,70,93,157,193,195-197
	Myelotoxicity	7	50-52,72,91,195,197
	Neutropenia	1	161
	Pancreatitis	6	50-52,70,72,193
	Thrombocytopenia	1	197
	Withdrawal due to Adverse Events	4	52,79,192,193
Age Group	Adults	8	50,52,72,79,91,93,195,216
	Mixed	3	51,53,196
	Not Reported	5	70,157,161,192,193
Setting	Outpatient Specialty Clinics	5	51,70,91,161,193
	Not Reported	11	50,52,53,72,79,93,157,192,195-197
Region	Asia	1	53
	Europe	9	50-52,70,72,79,91,192,195
	Europe and North America	1	217
	Middle East and North Africa	1	193
	North America	2	93,196
	Not Reported	2	157,161
Risk of Bias	Fair	12	50-53,70,72,79,91,157,161,193,195
	Poor	4	93,192,196,197

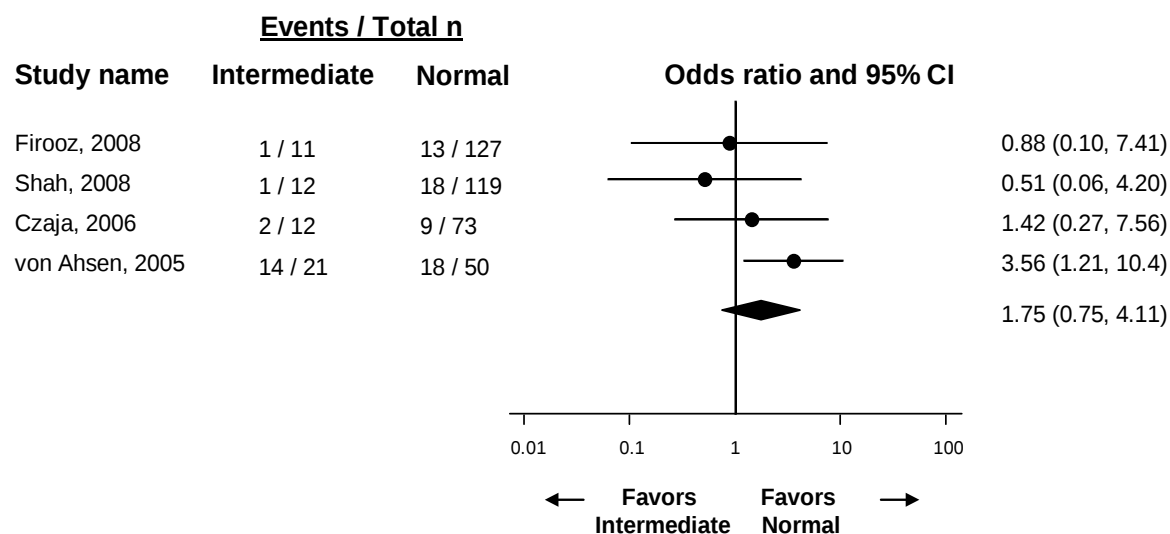
Abbreviations: 6-MP = 6-mercaptopurine; AZA = azathioprine; TPMT = thiopurine methyltransferase

Figure 18. Risk of bias of TPMT enzymatic activity, and thiopurine toxicity association studies



Withdrawal due to adverse events. Insufficient evidence addressed withdrawal due to adverse events in patients with low/absent TPMT activity. Compared with normal TPMT enzymatic activity, intermediate activity was not significantly associated with withdrawals (OR 1.75, 95 percent CI 0.75 to 4.11) (Figure 19).^{52,79,192,193}

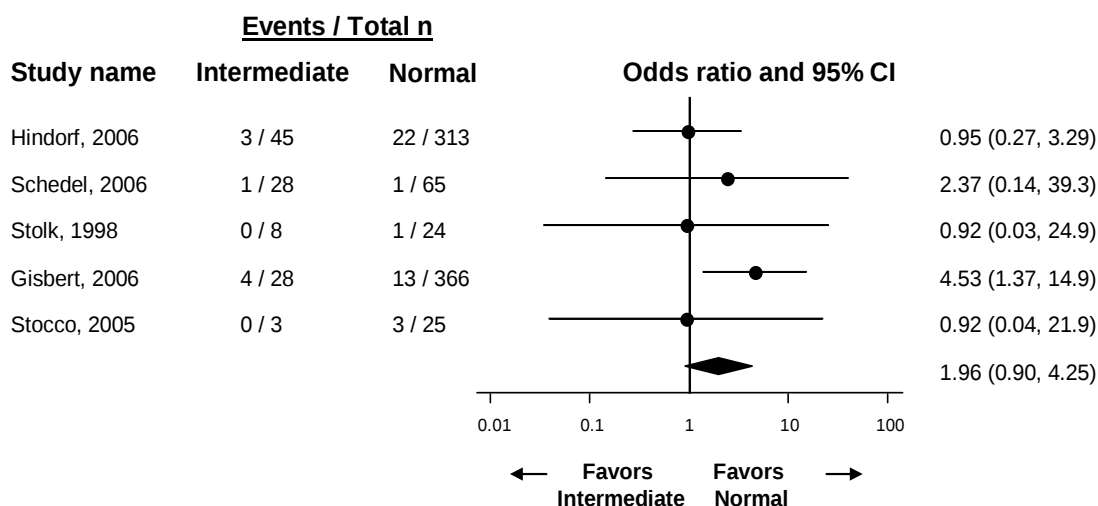
Figure 19. Odds ratio of withdrawal due to adverse events during thiopurine treatment of chronic autoimmune disease; intermediate versus normal enzymatic activities



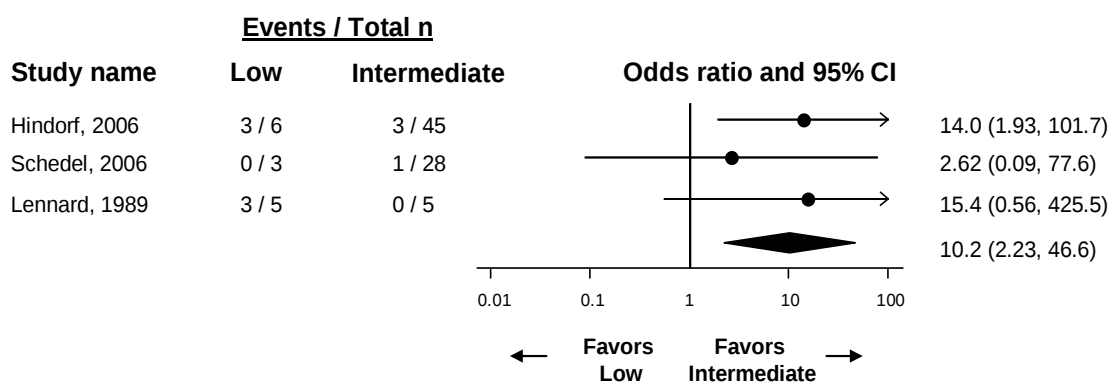
Myelotoxicity. Myelotoxicity was defined variably, in some studies including at least two myeloid cell lines (e.g. leukopenia and thrombocytopenia)^{50-52,91,195} or was not clearly defined.^{72,197} A nonsignificant odds ratio was noted for the outcome of myelotoxicity when intermediate enzymatic activity was compared with normal TPMT activity,^{50,51,72,91,195} but significant odds favoring higher activities were found when low enzymatic activity was compared with normal (OR 13.6, 95 percent CI 3.52 to 52.80) and intermediate (OR 10.2, 95 percent CI 2.23 to 46.60) activities (Figure 20).^{50,50,197} The overall event rate of myelotoxicity was 5 percent among 996 patients.

Figure 20. Odds ratios of myelotoxicity during thiopurine treatment of chronic autoimmune disease; intermediate/normal, low/intermediate and low/normal comparisons

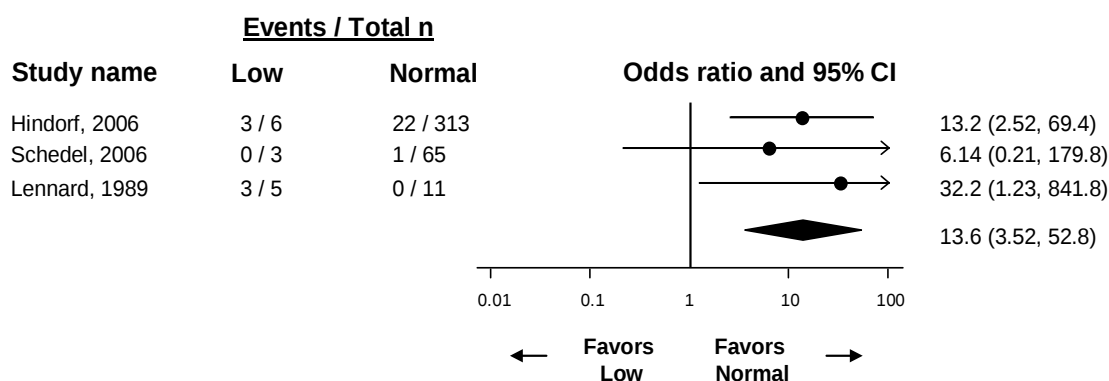
Intermediate vs. Normal



Low vs. Intermediate



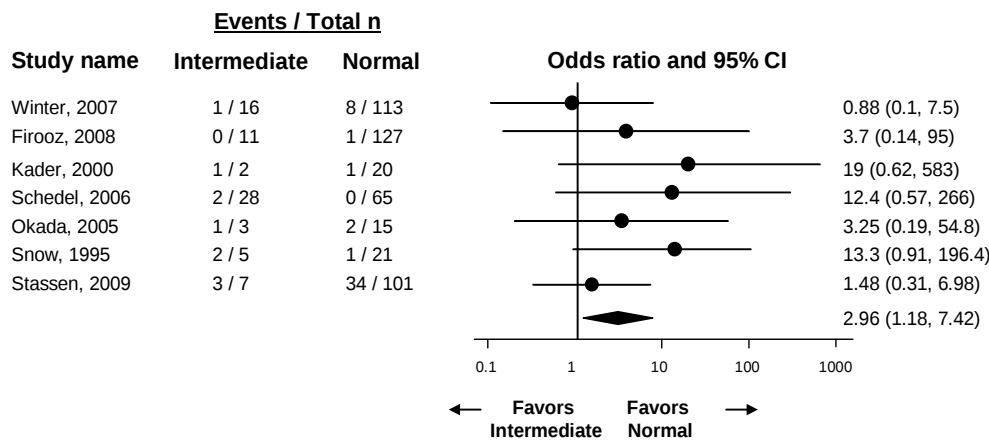
Low vs. Normal



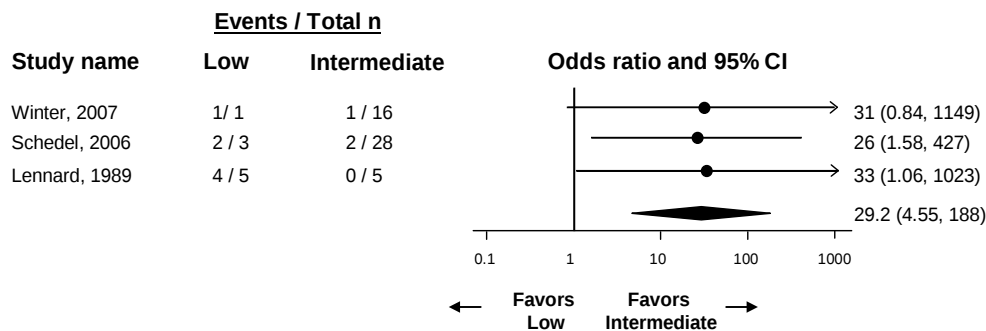
Leukopenia. Leukopenia was experienced by 12 percent of participants, and a significant dose response relationship was observed. When low and intermediate activities were compared with normal activities and with each other, a significantly higher number of patients with lower enzymatic activities experienced leukopenia. In a total of 538 patients in seven studies, significant odds ratios of intermediate versus normal were 2.96 (95 percent CI 1.18 to 7.42);^{53,70,93,157,193,195,196} low versus intermediate of 29.2 (95 percent CI 4.55 to 188) in three studies with 247 participants;^{70,195,197} and a low versus normal activity odds ratio of 80.00 (95 percent CI 11.5 to 559) in three studies with 247 participants (Figure 21).^{70,195,197}

Figure 21. Odds ratios of leukopenia during thiopurine treatment of chronic autoimmune disease; intermediate/normal, low/intermediate and low/normal comparisons

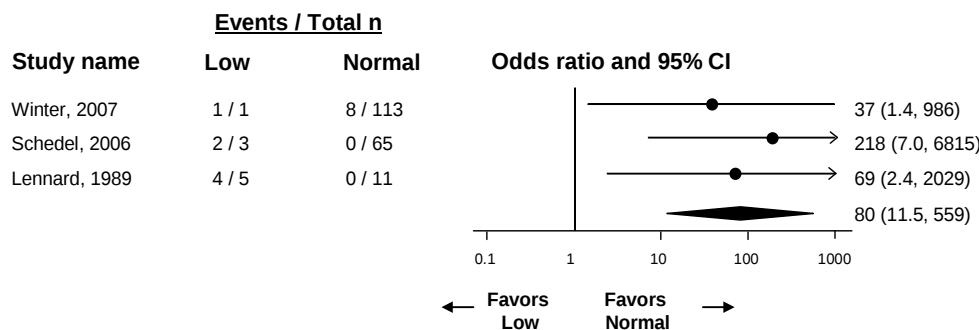
Intermediate vs. Normal



Low vs. Intermediate

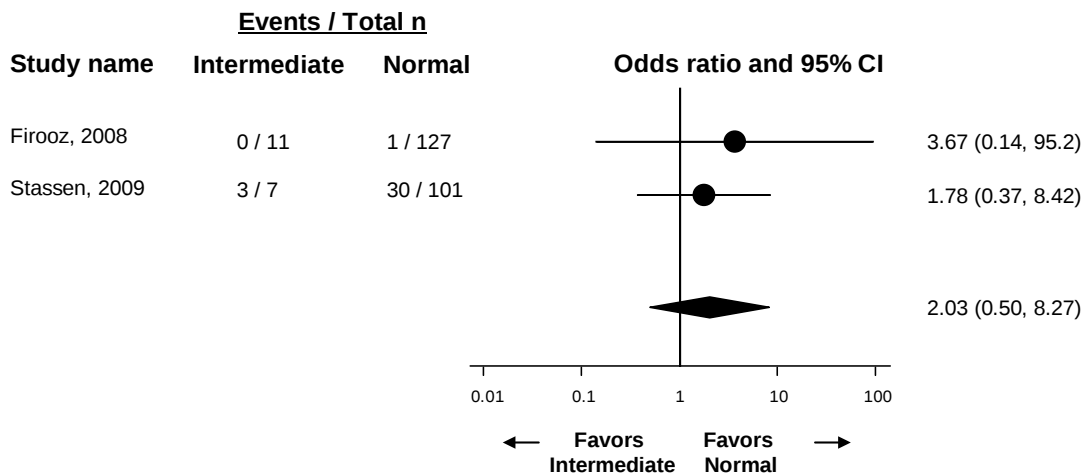


Low vs. Normal



Anemia. Two studies in 246 patients found no significant difference between patients with intermediate enzymatic activity and normal activity, in development of anemia during thiopurine treatment (Figure 22).^{157,193} Insufficient evidence was available to compare low enzymatic activity with higher activity.

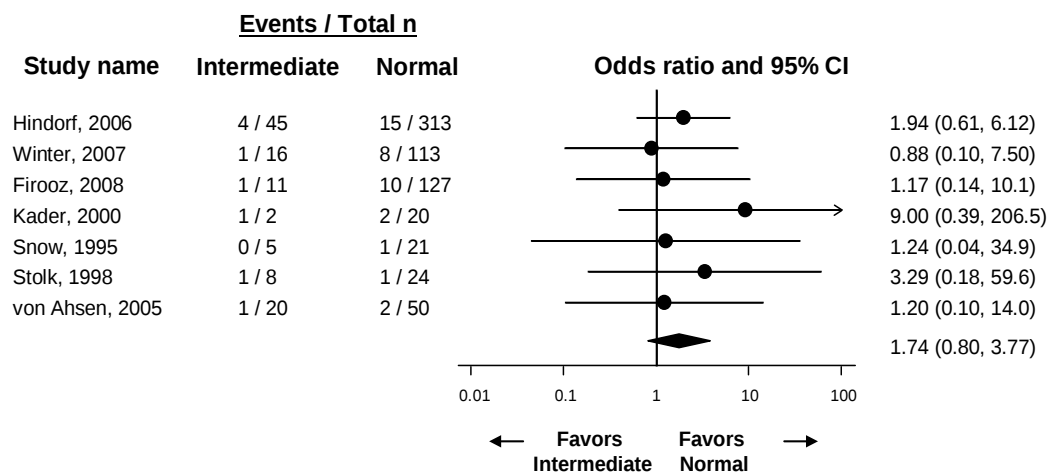
Figure 22. Odds ratio of anemia during thiopurine treatment of chronic autoimmune disease; intermediate versus normal TPMT enzymatic activities



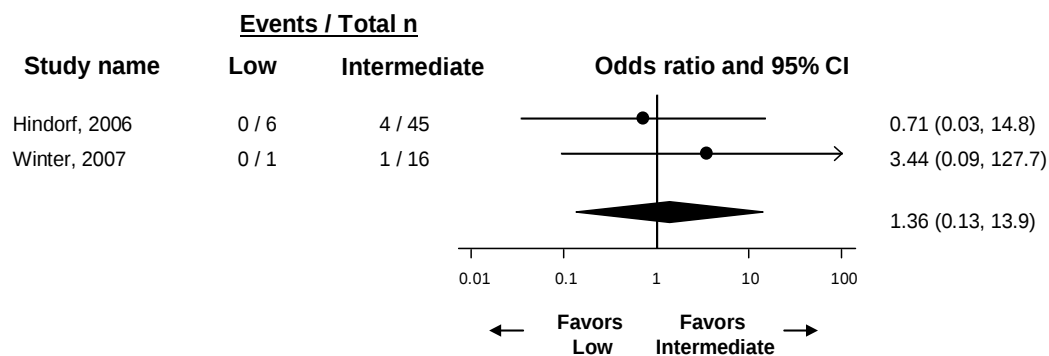
Hepatitis or elevated hepatic transaminases. No significant association between enzymatic activities and odds of developing hepatotoxicity was evident in seven studies. (Figure 23).

Figure 23. Odds ratios of hepatitis or elevated hepatic transaminases during thiopurine treatment of chronic autoimmune disease: intermediate/normal, low/intermediate and low/normal comparisons

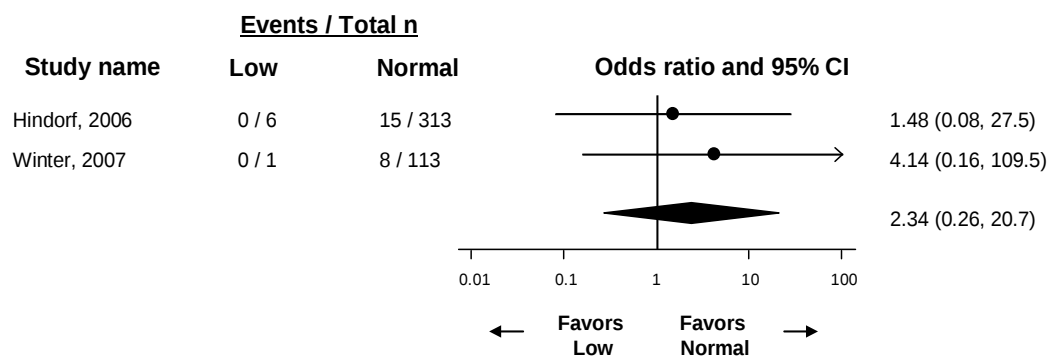
Intermediate vs. Normal



Low vs. Intermediate



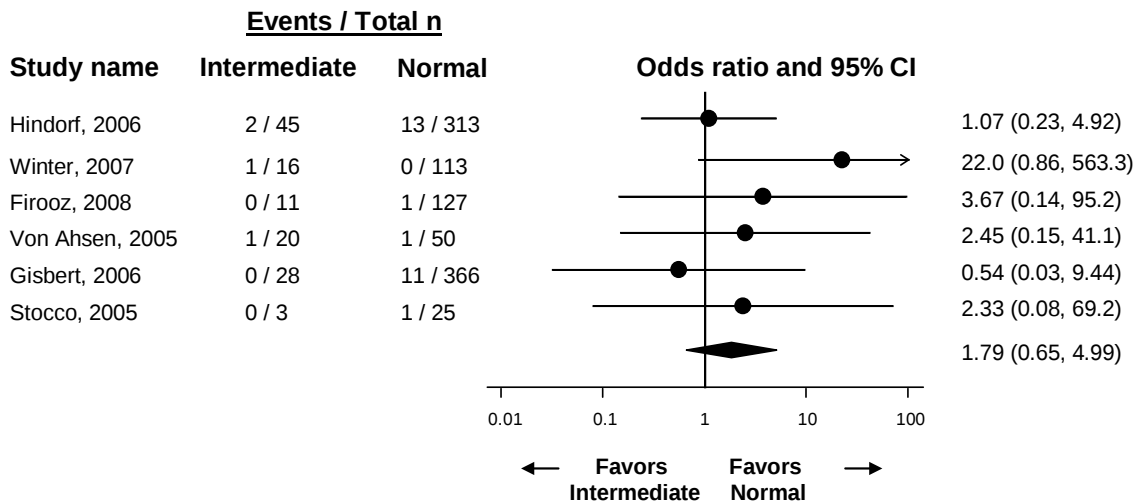
Low vs. Normal



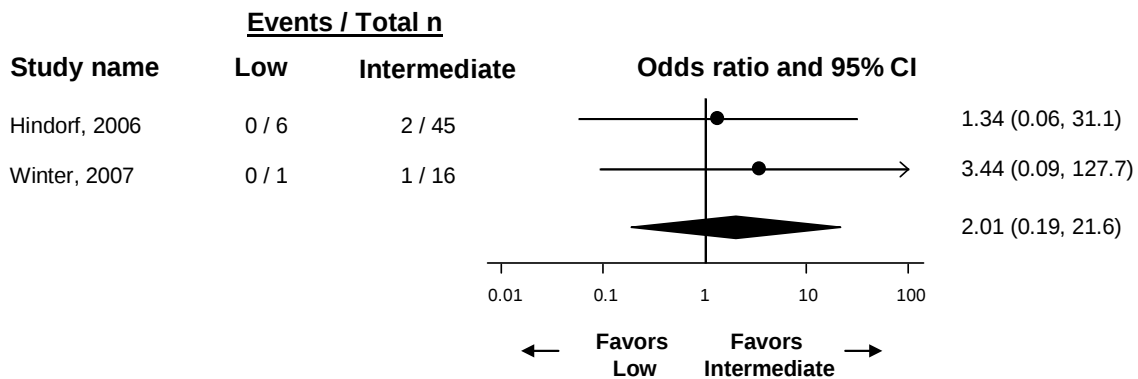
Pancreatitis. Insufficient evidence was available to compare low enzymatic activity with normal activity. Odds of pancreatitis were nonsignificant when intermediate activity was compared with normal activity (OR 1.79, 95 percent CI 0.65 to 4.99) and low with intermediate activity (OR 2.01, 95 percent CI 0.19 to 21.60) (Figure 24).

Figure 24. Odds ratios of pancreatitis during thiopurine treatment of chronic autoimmune disease: intermediate/normal, and low/intermediate comparisons

Intermediate vs. Normal



Low vs. Intermediate



Key points. Fifteen studies contributed to quantitative syntheses regarding association of thiopurine treatment toxicity with TPMT enzymatic activity.^{50-53,70,72,79,91,93,157,192,193,195-197}

A dose response relationship was demonstrated for the association of subnormal TPMT enzymatic activities and the outcome of leukopenia. The greatest odds of the outcome were noted when the enzymatic activity was low followed by when it was intermediate in comparison with normal enzymatic activity.

Greater odds for the outcome of myelotoxicity were also noted with low activity when compared with intermediate and normal TPMT enzymatic activities.

No evidence was available for the outcomes of mortality, hospitalization, SAE, and HQOL. Sparse data were available for the outcome of infection, neutropenia and thrombocytopenia, not permitting a meaningful analysis.

TPMT Allelic Determination

Genotyping was reported in thirty-four of the eligible studies (Table). Data reported for individual allelic variants were too sparse to permit a meaningful allele specific evidence synthesis. Insufficient evidence of association was available for the outcomes of mortality, hospitalization, serious adverse events (SAE), health related quality of life (HQOL) and neutropenia. Five studies reported outcomes data associated with compound heterozygosity, so these participants were evaluated as homozygous carriers.^{57,159,160,172,176}

Characteristics of included studies and risk of bias are summarized in Table 20. The majority (68 percent) of studies were in IBD patients. Overall, females were approximately equally represented, although this data could not be ascertained in nine studies^{53,57,61,81,157,170,176,179,182} and in five studies females were overrepresented.^{89,99,171,174,190} Racial distribution was very poorly reported.

In 17 studies, thiopurine treatment was restricted to AZA mostly in the doses varying from 1-2 mg/kg/day of AZA^{1,46,48,52,59,70,86,89,93,170-175,179,190} while eight studies did not report AZA dose.^{50,53,61,99,157,180,182,191} One study employed 6-MP in a dose of 50mg/kg/day,⁵⁴ while the rest used mixed thiopurines. Adverse event observation periods on thiopurine treatment varied but when reported were mostly greater than 2 months in duration. Nine studies did not report the period of on-thiopurine-treatment observation for adverse events.^{53,57,61,81,93,99,171,177,182} Most studies were cross-sectional in design, in which patients' past thiopurine treatment and related adverse events were correlated with study genotyping results. These were generally rated as fair risk of bias. Most importantly, biased genotyping based on prior knowledge of outcomes or outcomes assessment based on prior knowledge of genotyping results could be ruled out only in five studies.^{1,46,48,89,177}

Figure 25 depicts the distribution of studies by individual quality scoring items. Except for Ansari et al.'s study for which data on the homozygous carrier state was not reported, we tested studies for Hardy-Weinberg equilibrium (HWE) but did not consider it an item for sensitivity analyses.⁴⁶ Six studies were not found in equilibrium,^{47,50,86,172,173,190}. Detailed results of all 34 studies are available in Appendix C. Below we first present the pooled results from studies that tested for the most common of TPMT variant alleles (TPMT*2, *3A, *3B, *3C), whether or not testing additional polymorphisms. Subsequently we report results from subgroups of studies testing specific sets of variant alleles.

Table 20. Summary characteristics of TPMT allelic determination and thiopurine toxicity association studies

Characteristic		Number of studies	Studies
Study Design			
	Case Control	4	81,86,181,182
	Chart Review	1	157
	Cross-Sectional	18	48,50,51,53,57,59,70,93,99,170-176,190,191
	NonRandomized Intervention Study	2	46,54
	Prospective Observational	7	1,47,61,89,177,180,186
	Randomized Controlled Trial	1	52
	Unclear whether prospective or retrospective design	1	179
Chronic Autoimmune Disease			
	AntiNeutrophil Cytoplasmic Antibody Associated Vasculitis	1	157
	Autoimmune Dermatologic Conditions	2	93,170
	Autoimmune Hepatitis	1	171
	Inflammatory Bowel Disease	23	1,46-48,50-52,54,59,61,70,81,86,172,173,175-177,180-182,186,191
	Rheumatoid Arthritis	2	57,179
	Rheumatic Diseases	2	89,190
	Systemic Lupus Erythematosus	4	53,99,174,179
Thiopurine Treatment			
	6-MP	1	54
	6-MP, AZA	5	47,176,177,181,186
	AZA	25	1,46,48,50,52,53,59,61,70,86,89,93,99,157,170-175,179,180,182,190,191
	Mixed Thiopurines	2	51,81
	Not Reported	1	57
Allelic Variants Tested			
	TPMT*1, *2, *3A, *3B, *3C	4	173,179-181
	TPMT*1, *2, *3A, *3C	1	81
	TPMT*2, *3A	1	89

Table 20. Summary characteristics of TPMT allelic determination and thiopurine toxicity association studies (continued)

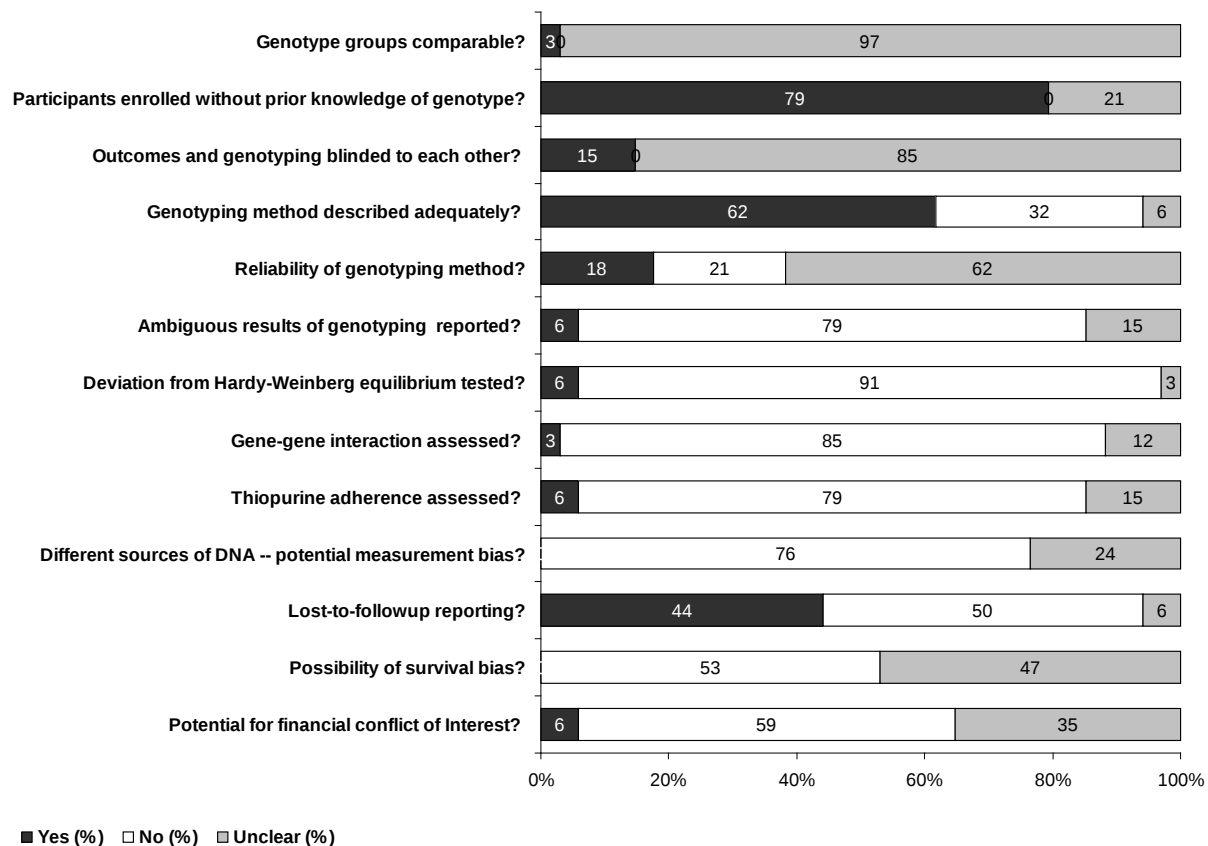
Characteristic		Number of studies	Studies
	TPMT*2, *3A, *3B, *3C	17	1,48,51-54,59,70,99,157,170-172,176,186,190,191
	TPMT*2, *3A, *3B, *3C, *3D	1	182
	TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, *15	1	50
	TPMT*2, *3A, *3B, *3C, *3D, *6	1	174
	TPMT*2, *3A, *3B, *3C, *7, *8	1	57
	TPMT*2, *3A, *3C	1	86
	TPMT*3A, *3B, *3C	4	46,47,175,177
	Not Reported	2	61,93
Genotyping Method			
	High Performance Liquid Chromatography	1	181
	Polymerase Chain Reaction	28	1,47,48,51-54,57,59,61,70,81,86,89,157,170-177,179,180,186,190,191
	Pyrosequencing	1	50
	Not Reported	4	46,93,99,182
Outcomes Reported			
	Mortality	1	1
	SAE	1	1
	Anemia	2	157,170
	Any Infection	3	59,170,190
	Hepatitis	20	1,46-48,50-52,54,59,61,70,81,86,89,93,99,172-175
	Leukopenia	25	46-48,53,54,61,70,81,89,93,99,157,170,172-177,179-181,186,190,191
	Myelotoxicity	6	50-52,59,171,180
	Neutropenia	3	Insufficient evidence with sparse events ^{1,86,170}
	Pancreatitis	14	1,46-48,50-52,54,59,70,81,86,172,182
	Thrombocytopenia	4	48,61,157,190
	Withdrawal due to Adverse Events	5	1,46,57,170,190
Age Group			
	Adults	15	1,46,47,50,52,54,81,93,171,173,175,180,186,190,191
	Children	3	99,172,177
	Mixed	5	48,51,53,86,181
	Not Reported	11	57,59,61,70,89,157,170,174,176,179,182
Setting			
	Inpatient	1	170
	Outpatient Specialty Clinics	15	1,48,51,54,57,59,70,81,86,89,173,175-177,181
	Not Reported	18	46,47,50,52,53,61,93,99,157,171,172,174,179,180,182,186,190,191
Region			
	Asia	6	53,171,174,176,179,181
	Europe	20	1,46-48,50-52,54,57,59,70,86,89,170,172,173,175,180,190,191
	North America	2	93,177

Table 20. Summary characteristics of TPMT allelic determination and thiopurine toxicity association studies (continued)

Characteristic		Number of studies	Studies
	Oceania	1	81
	Not Reported	5	61,99,157,182,186
Risk of Bias			
	Good	1	1
	Fair	29	46-48,50-54,57,59,61,70,81,86,89,157,170-177,179-181,190,191
	Poor	4	93,99,182,186

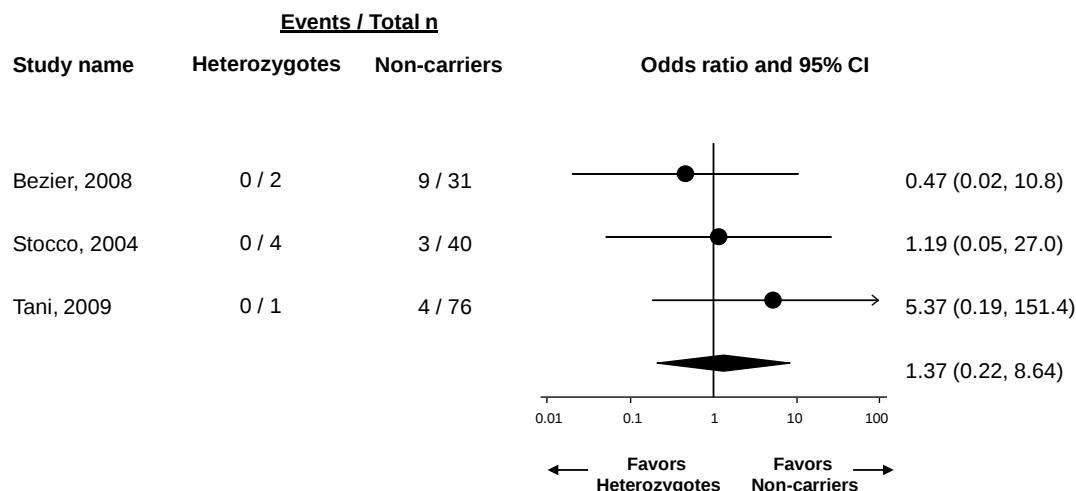
Abbreviations: 6-MP = 6-mercaptopurine; AZA = azathioprine; TPMT = thiopurine methyltransferase

Figure 25. Risk of bias of TPMT allelic determination and thiopurine toxicity association studies



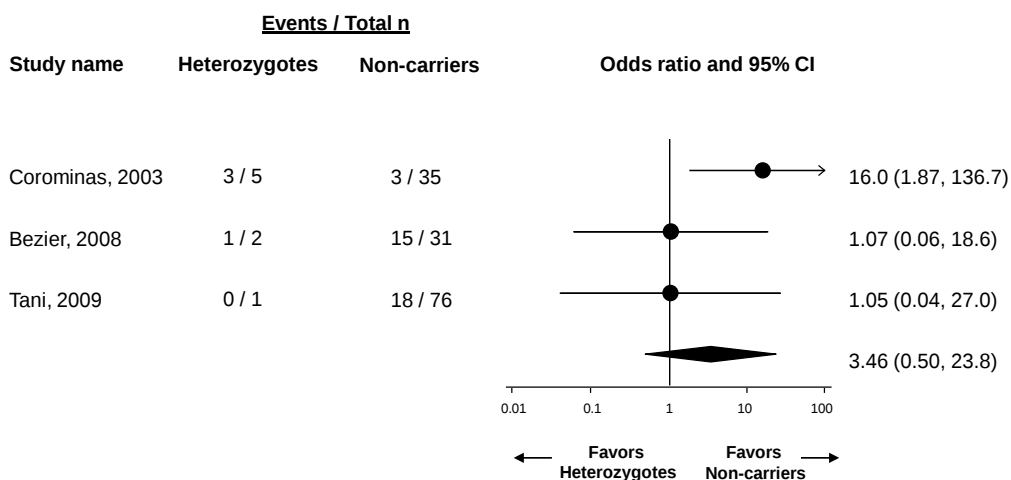
Infection. Three studies of cross-sectional study design reported infection in association with thiopurine treatment, including 155 patients with chronic autoimmune diseases.^{59,170,190} No significant association was noted between heterozygous and noncarrier carrier states when most common variant alleles (TPMT*2, *3A, *3B, *3C) were tested (OR 1.37, 95 percent CI 0.22 to 8.64). Insufficient data were available to evaluate homozygous carrier state (Figure 26).

Figure 26. Meta-analysis of odds ratios of infections during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers



Withdrawal due to adverse events. Four studies with an analyzable sample size of 317 reported this outcome. There were no withdrawals due to adverse events in 166 patients on azathioprine for 4 months administered in doses not guided by prior TPMT status determination.¹ The remaining studies of cross-sectional design, that tested for the most common variant alleles (TPMT*2, *3A, *3B, *3C) in 151 participants demonstrated no significant difference between heterozygous carriers and noncarriers in withdrawal due to adverse events on thiopurine treatment (OR 3.46, 95 percent CI 0.50 to 23.80) (Figure 27).^{57,170,190} Insufficient data were available to investigate the homozygous carrier state.

Figure 27. Odds ratio of withdrawal due to adverse events during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers



Myelotoxicity. Myelotoxicity was reflected in at least two cell lines (e.g. leukopenia and thrombocytopenia). The pooled estimate from four studies in 193 participants testing for the most common allelic variants (TPMT*2, *3A, *3B, *3C) demonstrated no significant difference between heterozygous carriers and noncarriers (OR 0.77, 95 percent CI 0.22 to 2.65).^{50,51,180,218}

When homozygous carriers were compared with noncarriers, the nonsignificant odds ratio was 2.20 (95 percent 0.15 to 32.80).^{50,171} (Figure 28 and Figure 29). Data were insufficient to derive a pooled estimate for the difference in rates of myelotoxicity between the two carrier states.⁵⁰

Figure 28. Odds ratio of myelotoxicity during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers

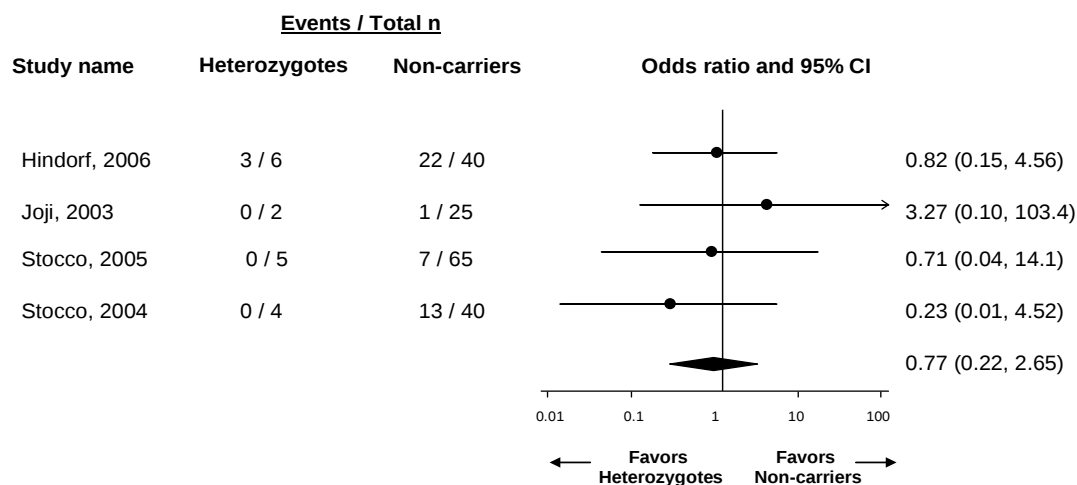
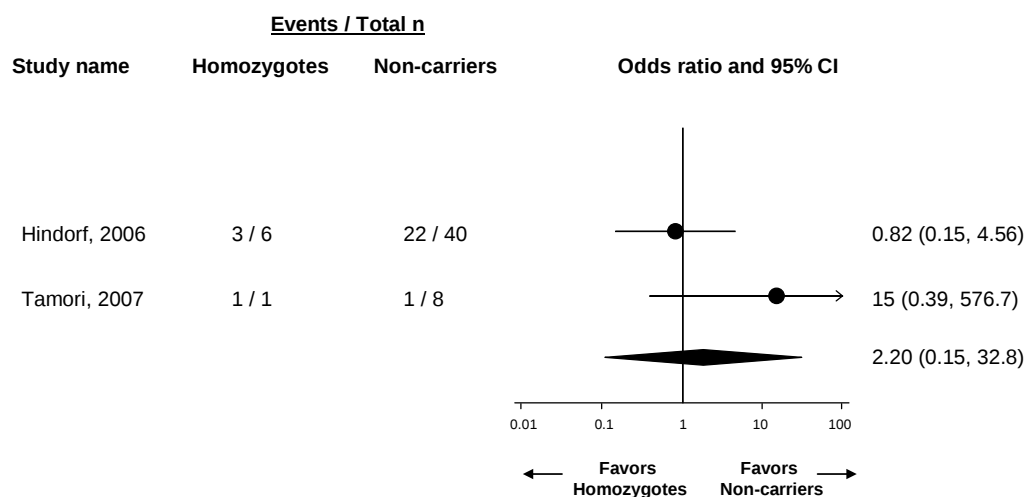


Figure 29. Odds ratio of myelotoxicity during thiopurine treatment of chronic autoimmune disease, homozygotes versus noncarriers



Leukopenia. Sixteen studies including a total of 1483 analyzable patients of whom 281 (19 percent) experienced leukopenia tested the four common variant alleles with or without additional variants.^{48,53,54,70,157,170,172-174,176,179-181,186,190,191} Odds of leukopenia were significantly higher when genotypes were heterozygous for TPMT mutations compared with noncarriers or wild type, and individual study results were quite consistent across studies (OR 4.62, 95 percent CI 2.34 to 9.16) (Figure 30). Meta-regression indicated significant differences in odds ratios with the method of genotyping (PCR versus HPLC). The study by Jae Hak et al., 2008 was the only study that used HPLC method for genotyping. A dose response relationship was indicated when homozygous carriers were compared with noncarriers (OR 18.60, 95 percent CI 4.12 to 83.60) (Figure 31).^{54,172,173,176,190} However, no significant differences in likelihood of leukopenia between the two carrier states were observed from pooling four small studies (Figure 32).^{54,172,173,176}

Figure 30. Odds ratio of leukopenia during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers

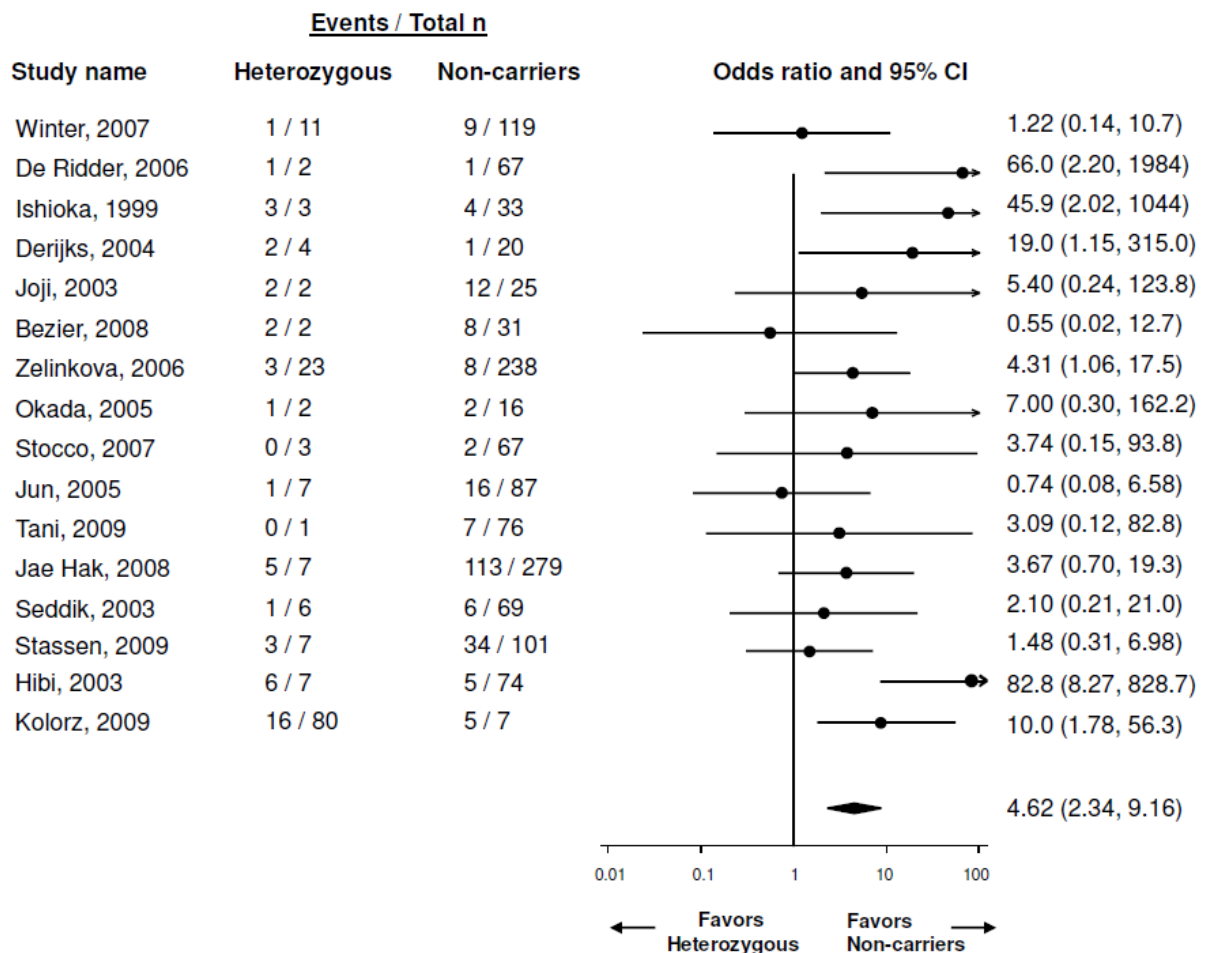


Figure 31. Odds ratio of leukopenia during thiopurine treatment of chronic autoimmune disease, homozygotes versus noncarriers

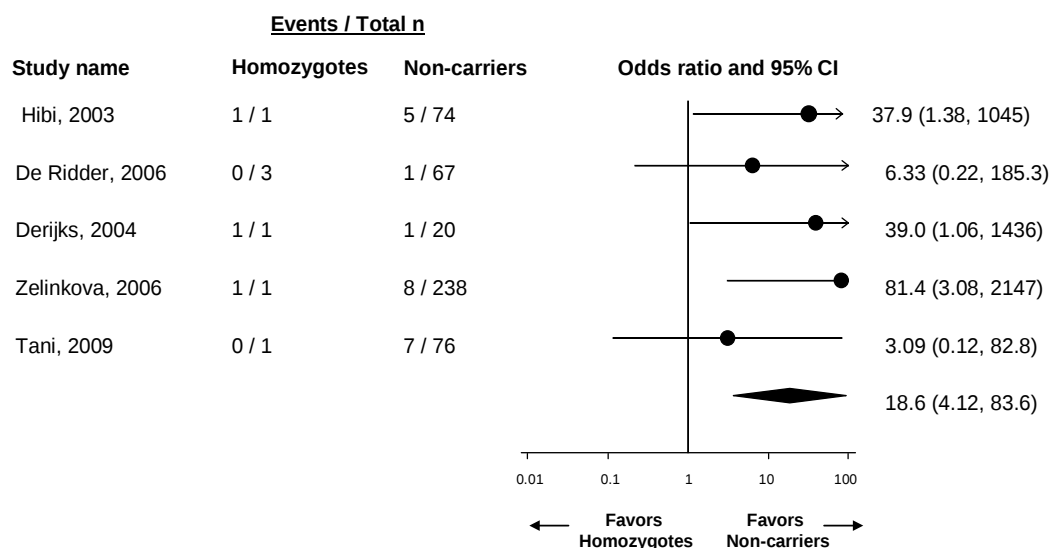
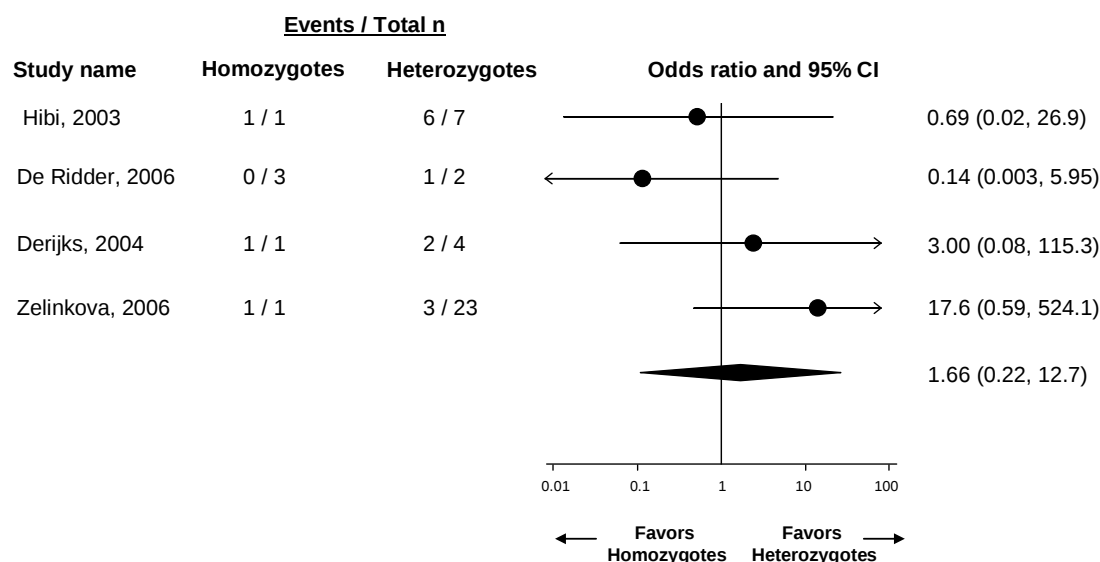
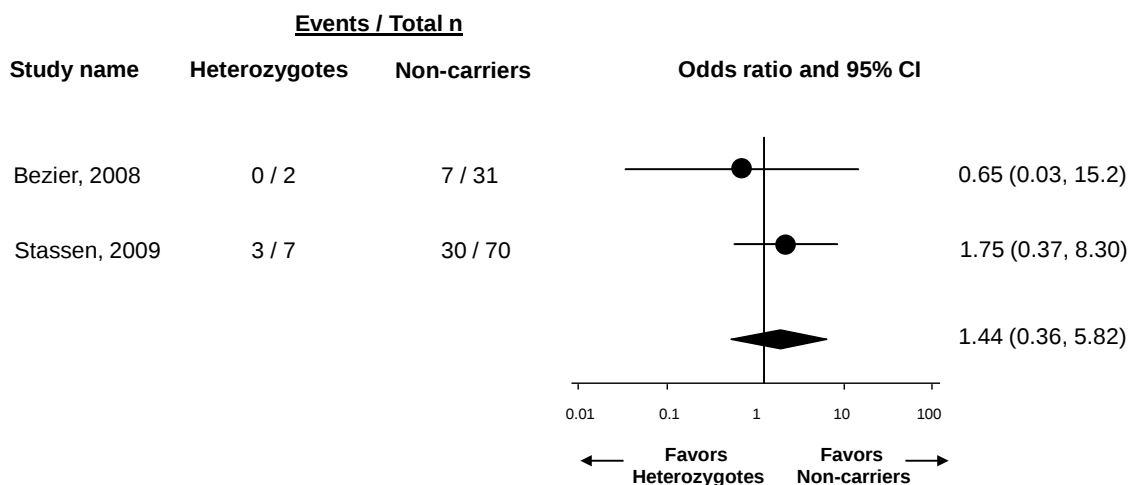


Figure 32. Odds ratio of leukopenia during thiopurine treatment of chronic autoimmune disease, homozygotes versus heterozygotes



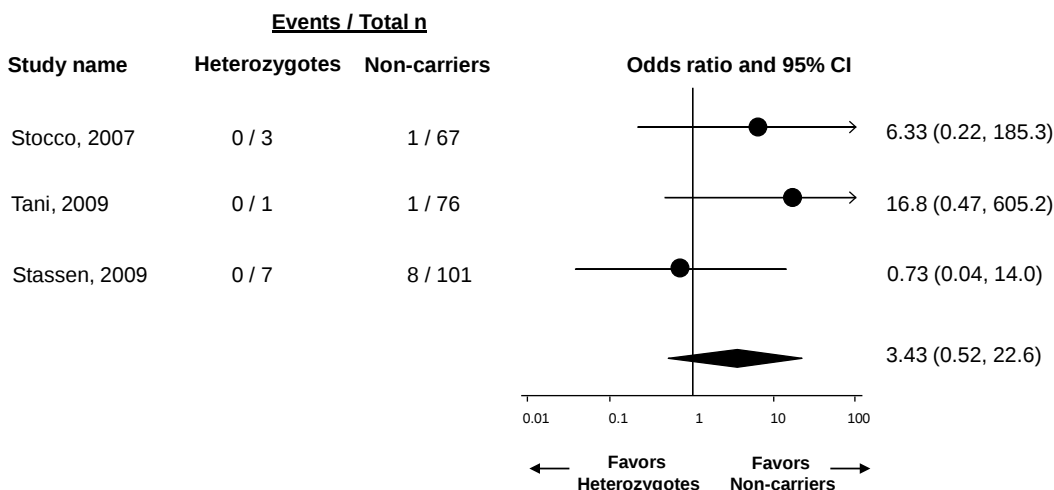
Anemia. Two studies in 140 patients did not demonstrate a significant difference in incidence of anemia when patients heterozygous for the common TPMT allelic variants were compared with noncarrier patients (OR 1.44, 95 percent CI 0.36 to 5.82) (Figure 33). There were no data pertaining to TPMT homozygosity for this outcome.

Figure 33. Odds ratio of anemia during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers



Thrombocytopenia. Based on a total of 256 patients in three studies, the pooled odds ratio for thrombocytopenia was 3.43 (95 percent CI, 0.52 to 22.60) when heterozygous genotypes were compared with noncarriers or wild type (Figure 34). Insufficient data were available to investigate the homozygous carrier state.

Figure 34. Odds ratio of thrombocytopenia during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers



Hepatitis or elevated hepatic transaminases. Based on a total of 984 patients in ten studies, comparing heterozygous carriers with noncarriers, the pooled odds ratio for hepatitis or elevated hepatic transaminases was 1.35 (95 percent CI 0.59 to 3.11) (Figure 35).^{1,48,50-52,54,59,70,173,174} Meta-regression suggested a significant relationship between the genotyping method and the outcome ($p < 0.05$). The study by Hindorf et al., which used a pyrosequencing method as opposed to the PCR used in the other studies, showed a nonsignificant point estimate in favor of heterozygosity because of zero events in the heterozygous group.⁵⁰ When homozygous participants were compared with noncarriers, similar nonsignificant odds were noted (Figure 36). Data were insufficient to compare the two carrier states.

Figure 35. Odds ratio of hepatotoxicity during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers

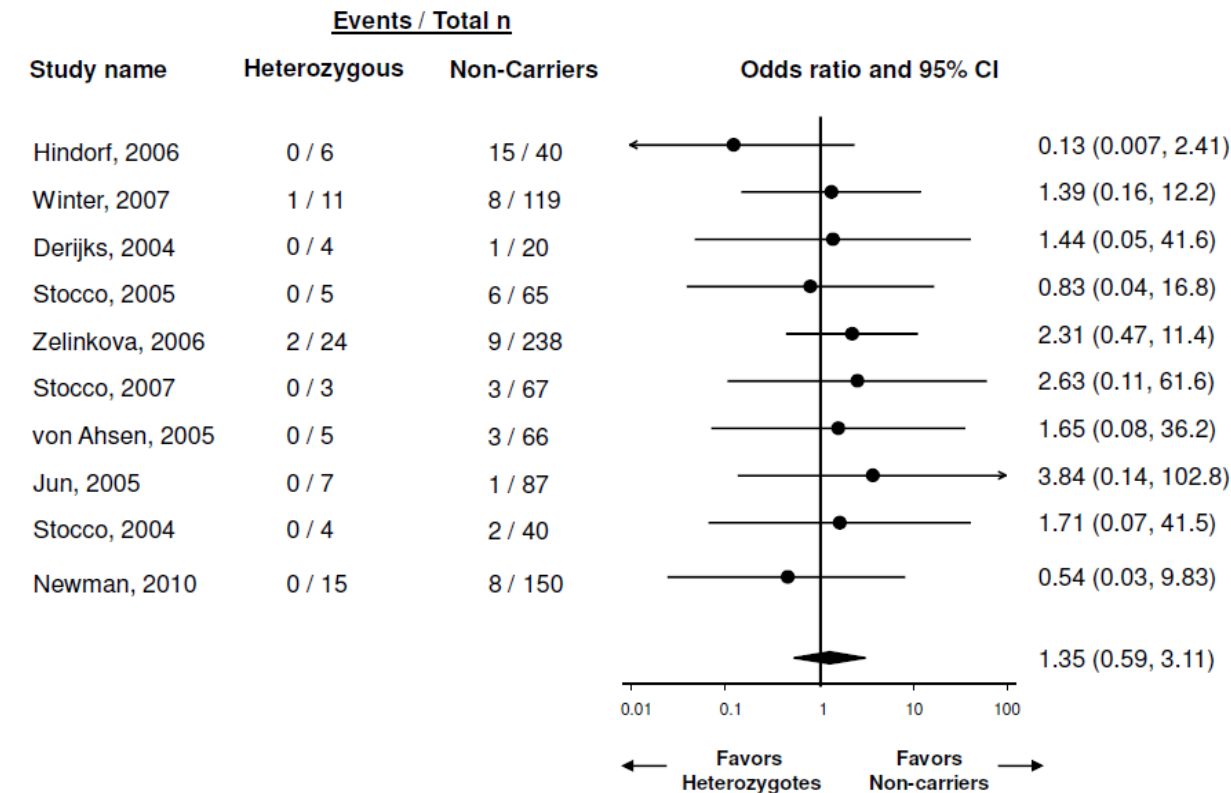
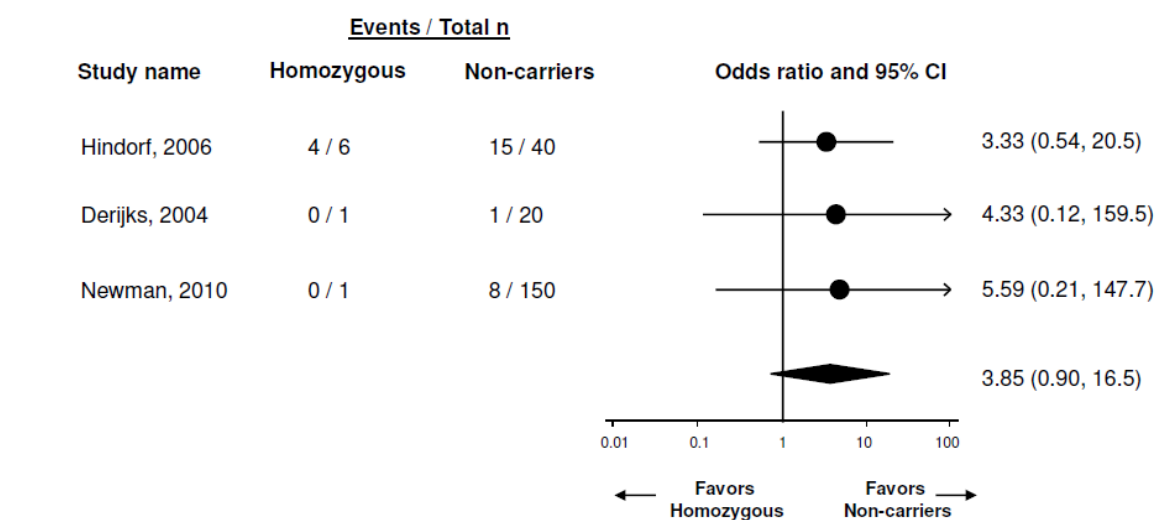


Figure 36. Odds ratio of hepatotoxicity during thiopurine treatment of chronic autoimmune disease, homozygotes versus noncarriers



Pancreatitis. when heterozygous carriers were compared with noncarriers, no significant difference in incidence of patients with pancreatitis was noted in ten studies of a total of 807 participants (OR 1.20, 95 percent CI 0.49 to 2.97) (Figure 37).

Figure 37^{1,48,50-52,54,59,70,172,182} However, meta-regression indicated a significant relationship with the overall risk of bias score with poor quality studies tended to report lower rates of

pancreatitis in heterozygous participants compared with noncarriers.¹⁸² With fewer studies, no significant difference was noted in pancreatitis events when homozygous carriers were compared with noncarriers (Figure 38). Data were insufficient to compare the two carrier states of homozygosity and heterozygosity.

Figure 37. Odds ratio of pancreatitis during thiopurine treatment of chronic autoimmune disease, heterozygotes versus noncarriers

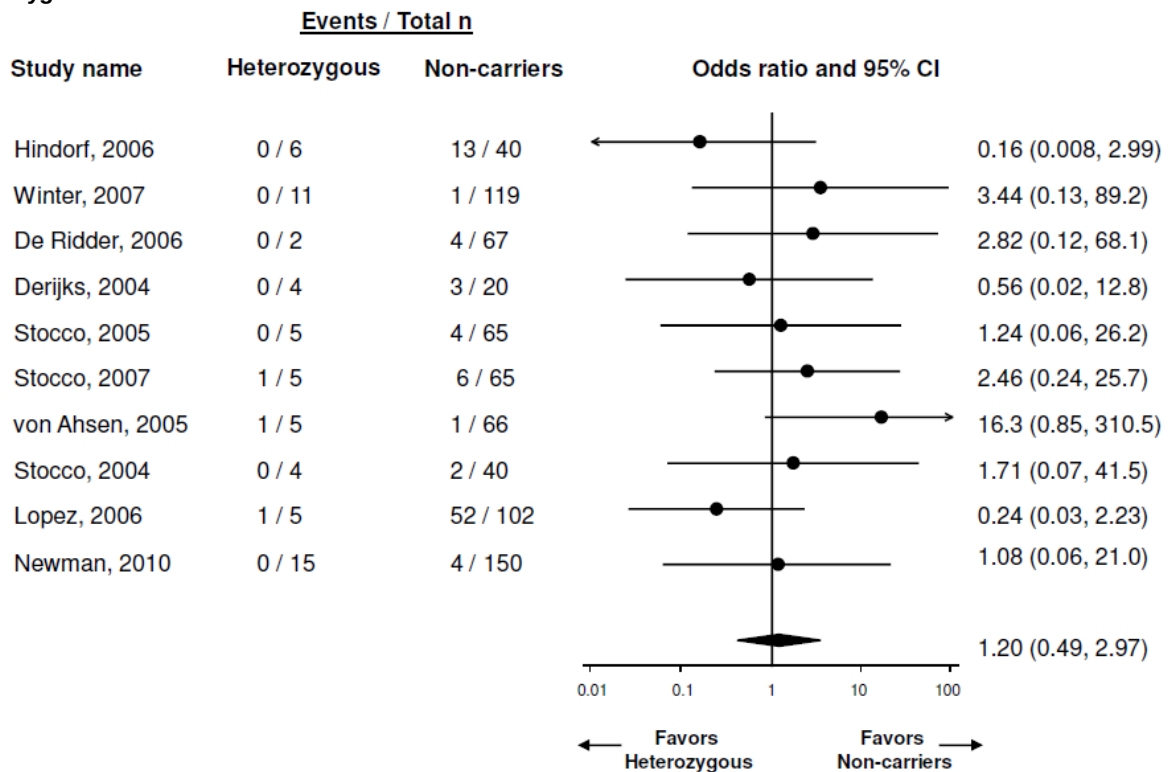
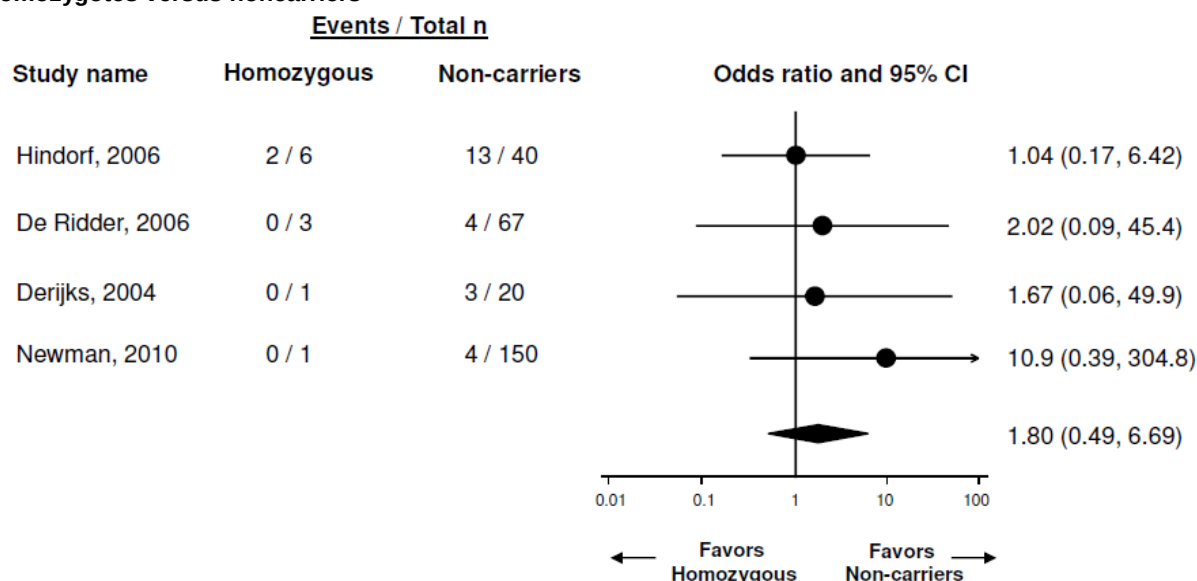


Figure 38. Odds ratio of pancreatitis during thiopurine treatment of chronic autoimmune disease, homozygotes versus noncarriers



Subgroup meta-analyses by specific sets of alleles tested. We also analyzed subgroups of studies testing specific sets of variant alleles (Table 21). Concordant with the main meta-analysis above in which studies that tested for TPMT*2, *3A, *3B, *3C with or without additional variants were pooled, studies that exclusively tested for TPMT *2, *3A, *3B, * 3C also demonstrated a pooled odds of leukopenia significantly greater with either carrier states in comparison with noncarriers. For other outcomes, comparatively fewer studies contributed subgroup specific evidence, with odds ratios failing to reach statistical significance.

Table 21. Pooled odds ratios of outcomes on thiopurine treatment between TPMT variant allele carriers and noncarriers, as well as between the two carrier states (allele specific subgroups)

TPMT alleles tested	Outcome	Homozygous carriers vs. noncarriers		Heterozygous carriers vs. noncarriers		Homozygous carriers vs. heterozygous	
		N of studies meta-analyzed	Odds Ratio (95% CI)	N of studies meta-analyzed	Odds Ratio (95% CI)	N of studies meta-analyzed	Odds Ratio (95% CI)
TPMT *2, *3A, *3B, * 3C							
	Infection	X		3 ^{59,170,190}	1.37 (0.22 to 8.64)	X	
	WDAE	X		3 ^{1,170,190}	1.06 (0.12 to 9.06)	X	
	Myelotoxicity (11% of patients with events)	X		2 ^{51,59}	0.40 (0.05 to 3.33)	X	

TPMT alleles tested	Outcome	Homozygous carriers vs. noncarriers		Heterozygous carriers vs. noncarriers		Homozygous carriers vs. heterozygous	
	Leukopenia (13% of patients with events)	4 ^{54,172,176,190}	<u>12.50</u> (<u>2.29</u> to <u>68.00</u>) Favors noncarrier	12 ^{48,53,54,70,99,157,170,172,176,186,190,191}	5.60 (<u>2.35</u> to <u>13.30</u>) Favors noncarriers	3 ^{54,172,176}	0.68 (0.08 to 5.70)
	Anemia	X		2 ^{157,170}	1.44 (0.36 to 5.82)	X	
	Thrombocytopenia	X		3 ^{48,157,190}	3.43 (0.52 to 22.60)	X	
	Hepatitis or raised hepatic transaminases	2 ^{1,54}	4.98 (0.44 to 56.30)	8 ^{1,48,51,52,54,59,70,99}	1.11 (0.39 to 3.10)	X	
	Pancreatitis	3 ^{1,54,172}	3.27 (0.49 to 21.60)	8 ^{48,51,52,54,59,70,172}	2.22(0.78 to 6.32)	X	
TPMT *3A, *3B, *3C							
	Leukopenia	X		2 ^{46,47,177}	5.13 (0.82 to 32.00) I ² > 50% and p-value for test of heterogeneity <0.10	X	
	Hepatitis or raised hepatic transaminases			2 ^{46,47}	0.98 (0.18 to 5.33)	X	
	Pancreatitis	X		2 ^{46,47}	0.48 (0.06, 3.60)	X	
TPMT*1, *2, *3A, *3B, *3C							
	Leukopenia	X		4 ^{173,179-181}	<u>5.23</u> (<u>1.99</u> to <u>13.70</u>)	X	

Notes: underlined odds ratios reached statistical significance.

Abbreviations: TPMT = thiopurine methyltransferase; vs. = versus; X =absent or insufficient evidence

Key points. Thirty studies contributed to quantitative syntheses.^{1,46-48,50-54,57,59,70,99,157,170-177,179-182,186,190,191,219}

A dose response relationship was suggested between TPMT genotypic status and leukopenia. In studies testing TPMT *2, *3A, *3B, * 3C plus/minus additional variants, homozygosity for the variant alleles yielded the highest odds ratio for leukopenia when compared with noncarrier status. Lower, but still significantly increased odds were seen for heterozygous patients versus noncarriers. However, direct comparison between the two carrier states did not yield statistically significant results.

For all other outcomes of mortality, hospitalization, serious adverse events (SAE), health related quality of life (HQOL), neutropenia, infection, withdrawal due to adverse events, myelotoxicity, anemia, thrombocytopenia, hepatitis or elevated hepatic transaminases, and pancreatitis, evidence was either absent, insufficient or lacked power to demonstrate significant differences between heterozygous and homozygous carriers in comparisons with noncarriers, and between themselves.

Key Question 4: What are the costs of determining TPMT enzyme activity and/or genotyping for patients with chronic autoimmune disease being considered for thiopurine-based therapy (e.g., costs of testing, costs of care, and costs of treating drug-associated complications)?

Eleven studies relevant to question 4 were included (Table 22). The studies were conducted in Canada,^{204,207,210} Korea,²⁰⁸ Europe,^{83,204,211} New Zealand,²⁰⁵ and the U.S.A.^{40,206,212} Five of the included studies were costing studies,^{40,204,207,210,211} while the rest were cost-effectiveness analyses. All of the studies examined the costs associated with azathioprine (AZA).

Table 22. Characteristics of TPMT testing, care and drug-associated complications costs studies

Study	Country of conduct	Type of study	Population characteristics	Perspective
Prakshar 1995 ⁴⁰	USA	Costing study	Theoretical population of RA patients receiving AZA	NR
Tavadia 2000 ²¹⁰	Canada	Costing study	Based on one BP patient with AZA toxicity	NR
Marra 2002 ²⁰⁹	Canada	CEA	Theoretical population of RA and SLE patients receiving AZA	Third-party payer
Oh 2004 ²⁰⁸	Korea	CEA	Theoretical cohort of adults with moderate to severe RA or SLE receiving AZA	Societal
Winter 2004 ⁸³	Scotland	CEA	Theoretical population of 1000 patients receiving AZA for IBD	NR
Dubinsky 2005 ²⁰⁶	USA	CEA	Theoretical population of adults with moderate to severe CD patients treated with steroids and AZA	Third-party payer
Sayani 2005 ²⁰⁷	Canada	Costing study	IBD patients participating in a randomized controlled trial	NR
Priest 2006 ²⁰⁵	New Zealand	CEA	Theoretical population of 1000 Caucasian patients with moderate to severe IBD receiving AZA	Payer's perspective (New Zealand government and IBD patients)
Compagni 2008 ²⁰⁴	Italy	SR of costing studies; costing study	Theoretical population of RA or IBD patients receiving AZA	NR
Gurwitz 2010 ²¹¹	UK and Spain	Costing study	RA or IBD patient cohorts from Spain and the UK	NR

Table 22.. Characteristics of TPMT testing, care and drug-associated complications costs studies (continued)

Study	Country of conduct	Type of study	Population characteristics	Perspective
Hagaman 2010²¹²	USA	CEA	Theoretical population of IPF patients	NR

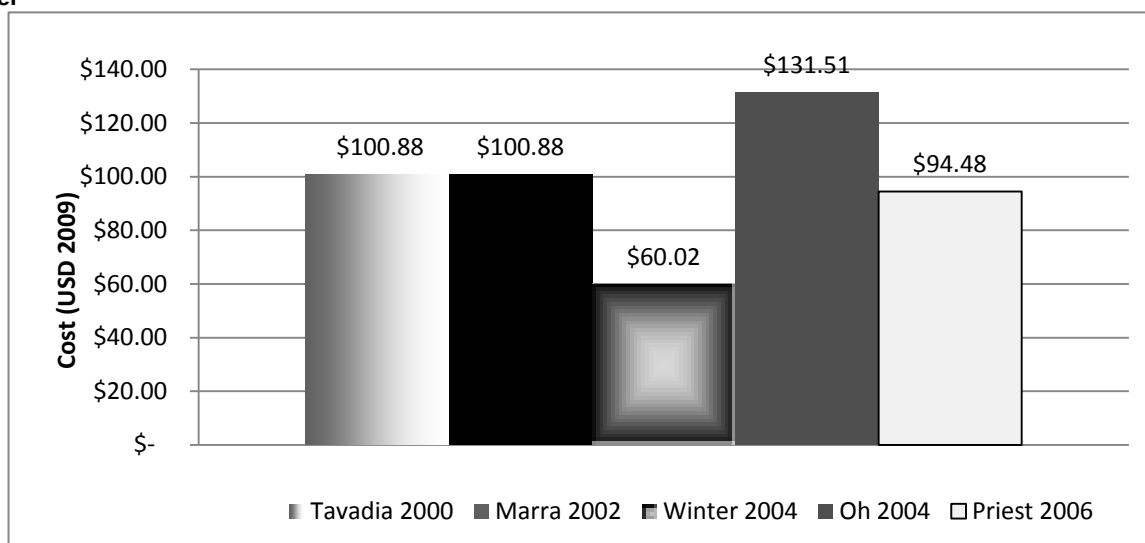
Abbreviations: AZA = azathioprine; BP = bullous pemphigoid; CD = Crohn's disease; CEA = cost-effectiveness analysis; IBD = inflammatory bowel disease; IPF=idiopathic pulmonary fibrosis; NR = not reported; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; SR = systematic review; UK=United Kingdom, USA = United States of America.

One study based its information on a patient with bullous pemphigoid,²¹⁰ while the other studies based their information on rheumatoid arthritis,⁴⁰ inflammatory bowel disease,^{83,205,207} Crohn's disease,²⁰⁶ rheumatoid arthritis or inflammatory bowel disease,^{204,211} idiopathic pulmonary fibrosis,²¹² and rheumatoid arthritis or systemic lupus erythematosus^{208,209} patients. For the costing analysis, one of the studies used the societal perspective,²⁰⁸ one used the payer's perspective,²⁰⁵ two used the third-party payer perspective,^{206,209} and the rest did not report their perspective.

Costs of adverse events and care. One included study provided costs associated with treatment failure.²⁰⁹ This study obtained information from the Canadian provincial guide to medical fees. Treatment failure with AZA was estimated to cost \$578.04 USD (2009) per rheumatoid arthritis or systemic lupus erythematosus patient. Another study provided a cost estimate for idiopathic pulmonary fibrosis disease progression, which was \$15,805.94.²¹²

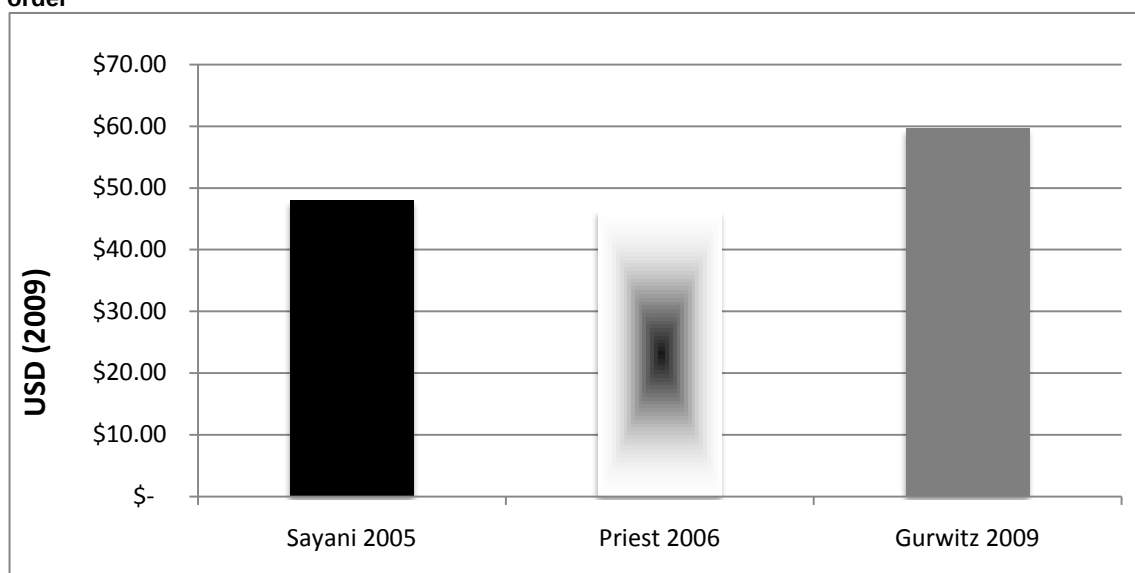
Costs of testing. Eight studies reported nine cost estimates for TPMT genotyping (Appendix C, Table C-26)^{83,204-212} Most of the genotyping cost information was obtained from public laboratories, while one was based on data from a hospital²⁰⁸ and another was from a literature search of studies reporting costs from private laboratories.²⁰⁶ The cost of obtaining a genotype test per patient ranged from \$28.03 USD to \$617.03 USD (2009). The highest cost was to obtain the TPMT genotype test from private laboratories.²⁰⁶ Excluding the costing item from the private laboratory, the average cost for the genotype test per patient was \$89.94 USD (Figure 39). One study also provided other costing estimates related to TPMT genotyping.²⁰⁴ The pharmacogenetic kits for detecting TPMT*1, *2, *3A, *3B, *3C cost \$46.25 USD/patient, pharmacogenetic kits for detecting TPMT*2, *3A, *3B, *3C cost \$29.43 USD/patient.²⁰⁴

Figure 39. Costs of genotype test per patient from public laboratory data in USD (2008), in chronological order



Four studies provided five estimates for the cost of TPMT phenotyping,^{205,207,211,212} which were obtained from laboratories or the government. The cost of obtaining the TPMT phenotype test per patient ranged from \$46.36 to \$320.98. It was not reported where the highest cost was obtained. Excluding the highest cost, the average cost for TPMT phenotyping per patient was \$53.13 (Figure 40).

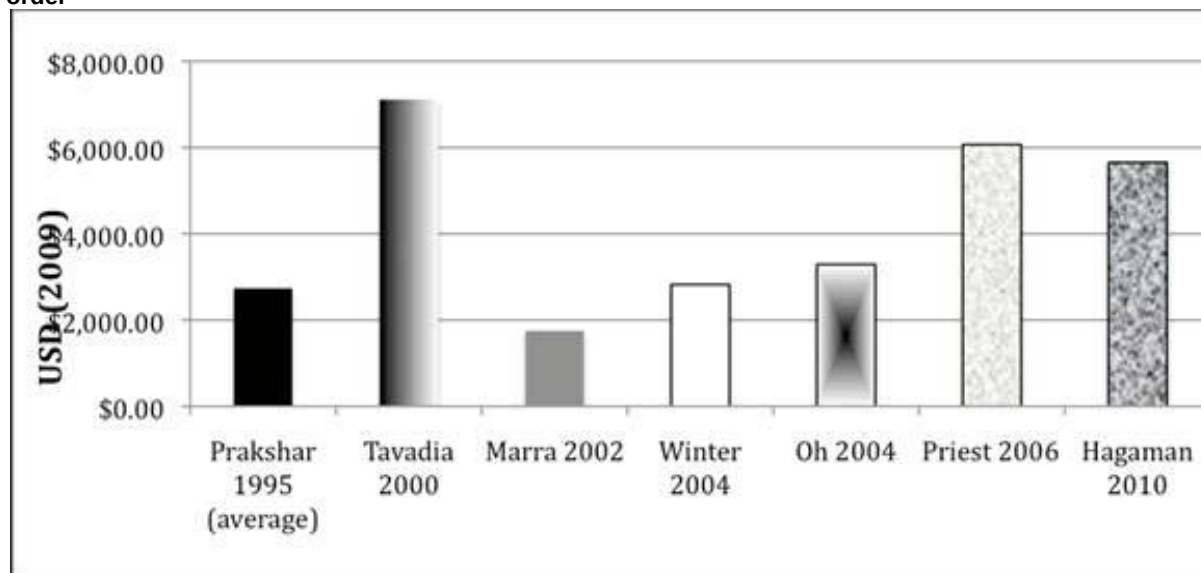
Figure 40. Costs of phenotype test per patient from public laboratory data in USD (2009), in chronological order



Costs of treating azathioprine-related complications. Seven of the included studies reported eight cost estimates related to the costs associated with treating AZA-related complications (Appendix C, Table C-27).^{40,83,205,208-210,212} All costs were obtained from hospitals or from government agencies. In order to compare across studies, the one-time cost of adverse

events associated with AZA were computed.²⁰⁴ This cost was substantial and ranged from \$1,366.82 to \$7,110.02 USD (2009) (Figure 41). The average one-time cost of AZA-induced adverse events was \$4,019.29.

Figure 41. One-time cost estimates of adverse events associated with AZA in USD (2009), in chronological order



One study reported the costs of leucopenia leading to death, which was \$15,691.46.²¹² In addition, another study reported two cost estimates for the average cost per identified TPMT-deficient individual, which was \$11,982.10 in a hospital in the United Kingdom and \$11,714.92 in UCB Pharma in Spain.²¹¹

Key points. The costs related to TPMT genotyping range from \$28.03 to \$617.80 USD (2009). This heterogeneity was likely due to different methodological choices.

TPMT phenotyping usually costs less than TPMT genotyping and ranges from \$46.36 to \$320.98. Similar to TPMT genotype testing costs, the heterogeneity observed across studies likely reflected methodological choices.

The one-time cost of adverse events associated with AZA was substantial, ranging from \$1,366.82 to \$7,110.02 across studies.

The costs related to identifying a TPMT-deficient individual is approximately \$11,848.51.

Survey of Laboratories Conducting Analyses of TPMT Enzymatic Activity and Genotyping

Responses were received from six out of the seven laboratories that were sent a link to the online questionnaire: three in Canada; and three in the United Kingdom. Results are summarized in Table .

Among the responding laboratories, yearly volumes of TPMT measurements range from 50 to 1500 for allelic determinations, and from 600 to 19,000 for enzymatic activity analyses.

Analytical methodologies. One laboratory conducts only genotyping and two laboratory conduct only phenotyping. The two labs also refer samples to other laboratories for genotyping. One lab refers out low enzymatic activity (i.e. below 10 U/g Hb) specimens for confirmatory genotyping. The other three laboratories conduct both types of analyses.

Between one and four specific genotypes are determined: TPMT*2 (four laboratories); TPMT*3A (six laboratories); TPMT*3B (four laboratories); and TPMT*3C (five laboratories). Genotype determination is performed by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP), genetic sequencing and site-directed PCR. The methods are equally popular, each being used by two laboratories.

TPMT enzymatic activity is determined on red blood cell (RBC) lysates using enzymatic assay followed by high performance liquid chromatography (HPLC) (three laboratories), or mass spectrometry (one laboratory). Three laboratories report results as nmol/g Hb/h, and one laboratory reports results as pmol/h/mg Hb. Both are numerically equivalent to 1 U/g Hb, the unit we are using in the present report.

Preanalytical requirements. All laboratories collect whole blood, with EDTA anticoagulant. Specimens are stored at 4°C or room temperature for between one and eight days.

One of the laboratories requires a list of patients' current medications prior to conducting analyses.

Quality control. All six participating laboratories report a procedure for internal quality control, which involves the inclusion of positive and negative controls within each run, in some cases in duplicate (two laboratories report conducting multiple runs per day). Results of internal quality control procedures are similar across participating laboratories, with enzymatic analysis repeatability ranging from three to ten percent within runs, and from five to ten percent between runs. Two of the laboratories conducting both genotyping and phenotyping analyses reported between 95 and 100 percent concordance between genotyping and phenotyping overall. One laboratory clarified that concordance was lower (60 percent) for intermediate carriers.

Positive and negative internal control samples are obtained from known staff, patients or pooled known samples.

Two of the six laboratories participate in external quality assurance, consisting of either specimen exchange with a comparable laboratory or participation in a formal external quality assurance program. The United Kingdom National External Quality Assessment Service has received funding for global availability for TPMT activity analyses in 2010.

Reporting of results. All of the laboratories report abnormal results to the responsible physician; however, one laboratory only reports homozygous (severe deficiency) results. Furthermore, of the five laboratories that perform enzymatic activity analyses, four also highlight suspected heterozygous patients.

Enzymatic activity reference intervals differ between laboratories. Three laboratories employ three reference intervals with two of the laboratories defining the intervals as: (low, intermediate

and normal) and one laboratory defining them as: (normal, heterozygous, deficient); One laboratory uses four reference intervals (deficient, low, normal and high). Two laboratories define low activity as below 10 U/g Hb, while the third defines low activity as between 6 and 34 U/g Hb. Two laboratories define normal activity as above 35 U/g Hb, while three other laboratories define normal activity as between 26 and 50 U/g Hb; between 25 and 120 U/g Hb; and between 38.5 and 62.5 U/g Hb respectively. The two laboratories that include a deficient interval define deficient TPMT activity as below 5 U/g Hb and below 19.2 U/g Hb. The laboratory that includes a heterozygous interval defines heterozygous as between 19.2 – 33.7 U/g Hb. Finally the laboratory that includes a high reference interval defines high TPMT activity as above 80 U/g Hb. In each case, reference intervals have been determined by the individual laboratories from large population-based studies.

Analysis turnaround times range from one day to less than three weeks, with four labs reporting a turnaround time of a week.

Costs associated with testing. Two laboratories reported on costs of testing. One reported a cost of £29 for both TPMT enzymatic activity plus genotyping if required for low activity values. The other charges £33 for TPMT enzymatic activity and £96 for genotyping.

Key points. A questionnaire, developed with expert input, was completed by six laboratories that provide TPMT analytic services, in Canada and the United Kingdom.

The preferred specimen type across laboratories is whole blood, with EDTA anticoagulant. Specimens are stored at 4°C or room temperature for between one and eight days.

Each laboratory follows a procedure for internal quality control. Two also participate in external quality control programs.

Enzymatic analysis repeatability ranges from three to ten percent within runs, and from five to 20 percent between runs.

Genotyping concordance with phenotyping up to 100 percent has been reported, although lower concordance was reported for intermediate carriers.

Analysis turnaround times range from one day to less than three weeks.

All laboratories report abnormal results to the responsible physician on an urgent basis.

Table 23. Laboratory questionnaire responses

Questionnaire item	Answer		N affirmative responses
Thiopurine methyltransferase (TPMT) analyses	Phenotyping, with low enzymatic activity specimens referred to external laboratory for genotyping		2
	Genotyping only		1
	Both genotyping and phenotyping		3
TPMT analytical method	Enzymatic activity assay	Mass spectrometry	1
		HPLC	3
	Genetic analysis	PCR/RFLP	2
		Sequencing	2
		Site-directed PCR	2
Alleles targeted in mutation specific genetic analysis	TPMT*2, *3A, *3B, *3C		3
	TPMT*2, *3A, *3C		1
	TPMT*3A, *3B, *3C		1 (external lab)
	TPMT*3A		1
Yearly TPMT testing volume	Enzymatic activity assay	600	1
		3000	1
		4000	1
		15,000	1
		19,000	1
	Genetic analysis	50	1
		550	1
		1500	1
		Unknown referrals for low enzymatic activity samples	1
Preferred specimen for analysis	EDTA whole blood		6
Preanalytical requirements a) Specimen stability prior to analysis	24-48 Hr at (4°C)		1
	Stable 4 days (temperature not specified)		1
	Stable 5 days at room temperature		1
	Stable 1 week at room temperature		1
	Stable 8 days refrigerated (4°C)		1
	N/A		1
Preanalytical requirements b) List of patients' current medications	No		5
	Yes		1
Internal quality control	Positive and negative controls with each run		4 (2 specify duplicates)
Proficiency of TPMT analyses	Enzymatic activity analysis repeatability	Within runs: 3-10%	4

Table 23. Laboratory questionnaire responses (continued)

Questionnaire item	Answer	N affirmative responses
	Between runs: 5-20%	5
	Genotyping concordance: 100% (comparator not reported)	1
	97% -100% (compared with phenotyping, overall)	2
	60% for intermediate carrier samples	1
External quality control	Yes (externally supplied / exchanged unknowns)	2
	No	4
Abnormal results called to the physician	Yes	6 (1 calls only for homozygotes)
	No	0
Reference intervals for TPMT enzymatic activity	Deficient <5 U/g Hb	1
	Deficient <19.2 U/g Hb	1
	Low 6-34 U/g Hb	1
	Low <10 U/g Hb	2
	Carrier 11-25 U/g Hb	1
	Intermediate 10-35 U/g Hb	1
	Heterozygous 19.2-33.7 U/g Hb	1
	Normal 26-50 U/g Hb	1
	Normal 38.5-62.5 U/g Hb	1
	Normal 35-79 U/g Hb	1
	Normal >35 U/g Hb	1
	High >80 U/g Hb	1
Highlight suspected heterozygous patients from enzymatic assay	Yes	4
	No	1
	N/A	1
Turn around time for testing	1-2 days	1
	1 week	4
	<3 weeks	1
Charges associated with laboratory testing	Enzymatic activity - £33 / Genotyping - £96	1
	Enzymatic activity - £29 (the price includes genotyping if required)	1

Abbreviations: EDTA = ethylenediaminetetraacetic acid; HPLC = high performance liquid chromatography; N/A = not applicable; PCR/RFLP = polymerase chain reaction/restriction fragment length polymorphism TPMT = thiopurine methyltransferase; U/g Hb = Units per gram of hemoglobin = nanomoles of product per hour per gram hemoglobin.

Chapter 4. Discussion

This review was nominated by the American Association for Clinical Chemistry (AACC), and commissioned by the Agency for Healthcare Research and Quality (AHRQ), to examine testing for thiopurine methyltransferase (TPMT) enzymatic activity (phenotype), and allelic polymorphism determination (genotype) in chronic autoimmune disease, with an overall view potentially to optimize use of thiopurine medications. The objectives were to examine the analytical aspects of TPMT status determination (genotype or phenotype); and resultant change in patient management and clinical outcomes of thiopurine toxicity in light of pretreatment knowledge of TPMT status; and costs associated with TPMT testing, along with costs of adverse events arising from thiopurine toxicities. Inclusion of evidence to meet these objectives was restricted to chronic autoimmune disease populations.

TPMT Status Determination

TPMT status may be determined through either genetic or TPMT enzymatic activity analysis. Genetic analysis involves detection of variant alleles coding for TPMT enzymes with reduced enzymatic activity, while enzymatic assays are able to determine directly the activity of the TPMT enzyme. Both genotyping and phenotyping can be determined from routine blood specimens, as white blood cells providing the required genetic material and red blood cells as the source of the TPMT enzyme. From a clinical laboratory perspective, determination of TPMT status encompasses preanalytical, analytical and postanalytical phases of testing.

Preanalytical testing requirements are often overlooked while focusing on analytical performance. However, the majority of errors in laboratory medicine occur during the preanalytical phase.²²⁰ The preanalytical phase of testing covers from the moment a specimen is collected to the point that it is analyzed, and includes sample collection method, anticoagulant used, transportation conditions, time between specimen collection and analysis, storage, specimen preparation, and preanalysis storage time and conditions. If appropriate preanalytical conditions are not met, then significant error can be introduced. These sources of error are often not recognized, as they are not identified by routine quality control monitoring of the assay. We therefore included examination of relevant preanalytical requirements, and potential confounders.

The analytic phase of testing involves the actual analysis of enzymatic activity or detection of variant TPMT alleles. Each method has individual analytical performance characteristics including precision, reproducibility, and diagnostic sensitivity and specificity, which are reviewed here.

Postanalytical requirements generally involve procedures to report results.

Preanalytic Requirements and Sample Stability

Thirteen studies examined relationships between storage conditions and TPMT enzymatic activity, with mixed results.^{108,115,116,118,121,124,127-129,131,134,136,137} Six studies reported TPMT to be stable at room temperature in anticoagulated whole blood for periods up to seven days,^{108,118,121,124,127,134} whereas in one study, reported only as an abstract, TPMT activity decreased by 25 percent over 24 hours.¹³⁷ Similarly, in red blood cell (RBC) lysate stored at -

20°C for 3 months, two studies reported TPMT activity to be stable,^{108,124} while one study reported a seven percent decrease.¹²⁸ Four studies reported that TPMT activity was stable in heparinised whole blood,^{127,134} EDTA whole blood¹²⁴, or an unspecified anticoagulant¹³⁶ at 4°C. When RBC lysate was stored at -80°C, TPMT enzymatic activity was reported to be stable for up to 25 days, whereas a 15 percent decrease in activity was measured after 16 months.^{128,129,131}

One explanation for these disparate results is that only one study was actually designed to evaluate the effect of storage on TPMT activity.¹³⁷ The available data suggests that TPMT activity is stable in EDTA and heparin anticoagulated whole blood for up to 7 days at room temperature or 4°C. RBC lysate is stable for 3 months at -20 °C. Longer storage should be at -80°C, although in the range of 15 percent of TPMT enzymatic activity may be lost after 16 months. Repeated freeze-thaw cycles were reported to decrease the results by up to 16 percent, although the drop was not statistically significant.¹¹⁵

No studies were identified that addressed any preanalytical requirements for TPMT allelic polymorphism determination. However, since preanalytical requirements are common for genetic testing, previously published guidelines can be used. The Clinical and Laboratory Standards Institute (CLSI) has published excellent guidelines covering all preanalytical requirement for collection, transportation, preparation and storage of specimens for genetic testing.²²¹

Six of the seven laboratories asked to participate in the survey returned responses, three from Canada and three from the United Kingdom. Among the responding, yearly volumes ranged from 50 to 1500 allelic determinations and 600 to 19,000 enzymatic TPMT determinations.

TPMT Variation Amongst Patient Populations

Gender, age, and race. All studies reported no gender difference for TPMT. One study of a small sample size reported a difference between TPMT enzyme activity of whites and mixed race that was not statistically significant.¹²⁹ Of ten studies, a single report showed a significant difference (p less than 0.001) in TPMT enzymatic activity between 192 children (12.0 U/mL RBCs (range 0.6 to 25.4 U/mL RBC)) and 959 adults (12.9 U/mL RBCs (range 0.2 to 24.6 U/mL RBCs)).¹¹¹ However this difference was small and not clinically relevant. Two studies observed no significant differences in TPMT activity across races, including blacks, whites, mixed races, and Japanese.^{109,129} However, more races with appropriate sample sizes should be included to confirm the lack of racial differences. One large study published in July 2004 in the journal *Pharmacogenetics* was excluded from the review on population. It analyzed 1200 healthy German individuals and demonstrated a statistically significant difference in TPMT activity between males and females. They also showed a statistical difference between smokers vs non-smokers; male and female smokers. However, clinically the differences are likely unimportant.

Coadministered drugs. Fifteen drugs (5-aminosalicylate, sulfasalazine, mesalazine, azathioprine, mesalamine, ac-5-aminosalicylate, syringic acid, prednisone, prednisolone, 6-methylprednisolone, cyclophosphamide, methotrexate, trimethoprim-sulphamethoxazole, SKF 525-A, 3,4-dimethoxy-5-hydroxybenzoic acid, trimethoprim, vincristine, dexamethasone, L-asparaginase) have been evaluated in ten studies.^{102,104,105,110,114,117,119,120,128,131} Only six of the studies were conducted in vivo,^{104,117,119,120,131} in which no clinically relevant interactions were demonstrated.

Hematocrit. Three studies investigated the effect of hematocrit on TPMT enzymatic activity.^{104,121,126} Two reported a positive correlation of hematocrit with TPMT enzyme

activity^{121,126} and one¹⁰⁴ observed no difference when comparing high and low hematocrit levels. Although two studies did demonstrate a correlation of hematocrit with TPMT activity, the effect was small (less than 7 percent in the normal hematocrit range) and likely not clinically relevant.¹²⁶ Standardizing TPMT measurement to grams of hemoglobin or milliliters of packed RBCs should correct for any significant effect of hematocrit on TPMT measurement.

Morbidities. Two studies assessed the effect of concomitant diseases on TPMT activity.^{104,106} Inflammatory bowel disease (ulcerative colitis, Crohn disease, or indeterminate colitis), autoimmune hepatitis, multiple sclerosis, myasthenia gravis, pemphigus and chronic renal failure were shown to influence TPMT activity. Although the differences between disease groups showed statistical significance, the differences were minor and not clinically relevant, with the exception of patients requiring dialysis. Patients with renal failure showed elevated TPMT enzymatic levels prior to hemodialysis, which dropped by approximately 50 percent following hemodialysis to levels comparable to normal individuals'. The mechanism responsible for the elevated TPMT activity prehemodialysis is unclear, but may involve unidentified TPMT activating uremic compounds.¹⁰⁶ Although there are no comparative studies of harms in dialysis population directly evaluating TPMT testing pre- and postdialysis, the available evidence suggests that dialysis patients should be measured postdialysis, as the levels most closely match those that would otherwise be seen in them as healthier individuals. Measurement of TPMT activity prior to dialysis may result in falsely identifying a low/absent or intermediate metabolizer as a normal metabolizer, potentially placing them at increased risk of drug toxicity. The remaining disease states studied to date are organ transplant and acute lymphoblastic leukemia, which were not included in this review.

Analytic Performance

The enzymatic measurement of TPMT was originally developed by Weinshilboum et al¹³⁴ and has since undergone only minor modifications. In brief, RBCs are concentrated by centrifugation, washed, resuspended and lysed to release the TPMT enzyme. The lysate is added to a buffered solution of radioactively labeled S-adenosyl-L-[¹⁴C]methionine and substrate 6-mercaptopurine (6-MP). TPMT methylates 6-MP to form radioactively labeled 6-methylMP which can then be measured. Modifications include use of 6-thioguanine monophosphate (6-TG) as substrate, or nonradioactive detection by high performance liquid chromatography (HPLC). The rate of product formation is dependent upon TPMT enzymatic activity, and is independent of the detection method - radiochemical or HPLC. Enzymatic assays using either 6-MP or 6-TG, regardless of the detection method (radiochemical or HPLC) were reasonably precise, with inter-assay coefficients of variance (CVs) of less than 10 percent in all cases. With an analytical coefficient of variance less than 50 percent of the biological variability, the amount of variation added to the true test variability is 11.8 percent.²²² In comparison with other enzymatic assays, the currently achievable intra-laboratory CV for TPMT enzymatic analysis is better than the minimal acceptable performance for routine enzymatic analysis specified by the U.S. Department of Health and Human Services: Clinical Laboratory Improvement Amendments of 1988 (e.g. total creatinine kinase (CK) below 30 percent, or aspartate aminotransferase (AST) below 20 percent).²²³

Three studies that partially addressed the reproducibility and accuracy of variant TPMT allelic polymorphism detection reported 100 percent concordance between denaturing HPLC and restriction fragment length polymorphism (RFLP) genotyping tests. The dichotomous nature of

genetic results, reported as either present or absent, does not allow for traditional precision and accuracy determination as is done for enzymatic determination. However, a number of guidelines are available that address the complex issues. Recently, the Centers for Disease Control released a Morbidity and Mortality Weekly Report detailing good laboratory practices for molecular genetic testing. It reviewed the need for adequate quality control of genetic testing and put forward recommendations for laboratories performing molecular genetic testing.²²⁴ The Clinical and Laboratory Standards Institute (CLSI) has also published guidelines for Molecular Diagnostic Methods for Genetic Disease²²⁵ and Validation and Verification of Multiplex Nucleic Acid Assays.²²⁶ It is recommended that any laboratories performing allelic polymorphism detection of TPMT review the above guidelines to ensure the accuracy of their results.

Diagnostic Sensitivity and Specificity

Enzymatic analysis was selected as the reference standard, as this method should identify all patients with reduced or absent TPMT activity, regardless of the mechanism. To date at least 30 mutant alleles have been identified within the coding region^{14,27-29,213,227-231}. Others have been identified in the 3' untranslated and promoter regions.²⁹ These mutations likely do not directly affect the activity of the TPMT enzyme molecule, but may influence the quantity of enzyme present and thereby indirectly decrease the overall *in vivo* TPMT enzymatic activity.

Reporting units and ranges of enzymatic activity are not standardized, so in our analyses the activity cutoff values stated in each article were used to assign patients to one of three groups: low/absent; intermediate; or normal/high. None of the studies were specifically designed to determine the diagnostic accuracy of genotyping in comparison to enzymatic activity. Thus, not surprisingly, using the **Quality Assessment of Diagnostic Accuracy Studies (QUADAS)** tool³⁵ a substantial (37 percent) of the studies were rated poor quality.

Diagnostic groups were organized in our analyses according to TPMT allelic variant(s) tested rather than by specific point mutation, to correspond to reporting in clinical practice. The relative abundance of specific allelic variants in a population has a direct impact on the diagnostic sensitivity and specificity of genetic testing, and therefore should be considered when developing genetic testing strategies. Sahasranaman et al. reviewed the relative frequency of the four most common alleles, *2A, *3A, *3B, and *3C.²²⁷ Pooled data suggests that the most common allele in Caucasians is *3A, with a mean frequency of 3.89 percent in a general population of 5076 (range 2.1 to 8.6 percent), while the most common in Africans is *3C, with a mean frequency of 4.7 percent in a population of 884 (range 2.4 - 7.6 percent). Pooled frequencies in a general population of 356 Asians and South Asians were lower than those seen in Caucasians and Africans, with a mean frequency of 1.0 percent (range zero to 2.3 percent) for *3C, and 0.17 percent (range zero to one) for *3A.

A total of 16 studies were included in the quantitative syntheses, assessing diagnostic sensitivity and specificity.^{1,46,49-53,56,59,70,93,157,158,161,162,167} From seven studies, the pooled sensitivity of genotyping for homozygosity or heterozygosity of the common TPMT *2, *3A, *3B, and *3C alleles, to correctly identify patients with absent to intermediate TPMT enzymatic activity, was 70.7 percent (95 percent confidence interval (CI) 37.9 to 90.5 percent) (meta-analysis 1). The pooled specificity of noncarrier genotype to correctly identify those with normal or high enzymatic activity approached 100 percent. With other combinations of alleles, pooled specificities of genotyping remained close to 100 percent, but sensitivity did not improve convincingly, as inadequate relevant evidence led to substantial imprecision in estimates

[sensitivity ranged from 70.70 to 82.10 percent (95 percent CI, lower bound range 37.90 to 54.00 percent; upper bound range 84.60 to 96.90 percent]. Studies by Okada et al,⁵³ and von Ahsen et al⁵² reported markedly lower sensitivities compared with the other studies. Okada et al analyzed a Japanese cohort, previously shown to have a low frequency of the common alleles, which suggest that other relatively common unidentified alleles may be present in the Japanese population. von Ahsen et al examined a German Caucasian cohort, and remarked upon the lower sensitivity observed, relative to previous reports. No plausible explanation could be identified for the heterogeneity in effect estimates.

Few individuals exhibited homozygous TPMT variant alleles. The pooled sensitivity of a homozygous TPMT genotype to correctly identify patients with low to absent enzymatic activity was based on two small studies with two percent of 341 patients identified as homozygous for variant allele. The pooled sensitivity was 87.10 percent (95 percent CI 44.30 to 98.30 percent). The pooled specificities of the noncarrier and heterozygous carrier states to correctly identify those without low or absent TPMT enzymatic activity were determined for the different combinations of tested allelic variants (meta-analysis 2). The specificities were high, approaching 100 percent.

For a screening test, genotyping appears to have moderate sensitivity to detect those with subnormal (i.e. intermediate plus low plus absent) enzymatic activities while possibly high sensitivity of 87 percent to identify only those with low to absent activities. However, the available evidence is imprecise and of uncertain validity given that 37 percent of studies were rated as poor during risk of bias categorization. These limitations in diagnostic sensitivity of genotyping are not unexpected as genetic analysis of TPMT most often targets only the common polymorphisms and will fail to identify new or rare mutations. Furthermore, the commonly employed genotypic tests while able to identify a specific SNP, are unable to determine the allelic location of it. Therefore, a patient typed as a heterozygote for TPMT*3A (i.e. wild type/*3A) may have been misdiagnosed as such while actually being a compound heterozygote TPMT*3B/*3C for the observed TPMT activity.^{232,233}

As discussed later, with a dearth of relevant primary literature, it remains unclear how incidence rates of thiopurine related adverse events may be affected by pretreatment genotyping. Therefore, this evidence should not be interpreted to conclude that prior genotyping is not effective in reducing thiopurine related drug toxicity in the treatment of chronic autoimmune diseases, especially when data associated with homozygosity were scant.

Currently, there is insufficient data to determine the optimum combination of TPMT alleles that must be tested in order to identify patients with reduced or absent enzymatic activity. There is also a lack of well powered, good quality studies comparing the diagnostic accuracy and relative effectiveness of the two methods of genotyping and phenotyping to determine TPMT status.

Postanalytic Requirements

This review did not identify any relevant studies that addressed postanalytic requirements in terms of reporting units, common reference intervals, or result reporting for either enzymatic testing or allelic polymorphism measurement. In general, results should be communicated to ordering practitioners as soon as possible after testing is completed. However, as this test is normally used prior to administration of thiopurine drugs, there is no critical requirement to contact practitioners directly to communicate abnormal results. Ideally, reports should include

both lower and upper reference limits, information on how the reference interval was determined, and an indication of overlap that is seen between normal and intermediate metabolizers. At a minimum, information on reference interval determination and overlap between intermediate and normal activities should be available on request.

Enzymatic assays are currently reported using one of two commonly used units, nmol/h/g Hb and Unit/mL RBC. One Unit is defined as generation of 1 nmol of product per hour (6-methylMP or 6-methylTG). Thus, the key difference is the standardization of the product generation, per gram of hemoglobin in RBC lysate, or per milliliter of packed RBCs. Hence, conversion between the two is neither easy, nor exact. It is recommended that a single preferred unit of measure be identified and used in the future to simplify reporting and interpretation, and to allow comparisons between laboratories.

Interpretation of enzymatic testing results is highly dependent on the stated reference interval provided by the performing laboratory. Individuals with very low or absent TPMT enzymatic activity (homozygous abnormal) are relatively easily identified, as they are clearly separated from patients with normal activity. Those patients with intermediate activity, however, are more difficult to identify as theirs' often overlap with the enzymatic activity of normal metabolizers. Therefore, determination of the lower reference limit for normal metabolizers is most important. Currently, there is no universally agreed upon lower limit of normal for TPMT activity, however many studies used similar lower limits. Standardization of analytical methods and reporting units will aid in identifying a universal lower limit of normal, and should be a future goal.

Clinical Laboratory Survey

A survey of laboratories was conducted to gather information regarding current clinically available TPMT analysis. Following identification of potential laboratories, two organizations and seven laboratories were contacted to determine their willingness either to complete a questionnaire or to disseminate the questionnaire to other relevant laboratories. Six of seven laboratories invited to participate returned a completed questionnaire. Preanalytical requirements, acceptable specimen type, storage times and conditions, were in keeping with the results of this review. Stated analytical precision of enzymatic analysis by the surveyed laboratories, as expected, was similar to that reported within published articles and ranged from 3 to 10 percent within runs, and from 5 to 20 percent between runs. Among the surveyed laboratories, reported concordance between enzymatic analysis genotyping ranged from 60 percent to 100 percent. Although the range is in keeping with the published reports, no data was provided to directly support these conclusions.

Knowledge of TPMT Status to Guide Therapy

A single fair quality randomized trial in 333 patients demonstrated that over a four month observation period, pretreatment genotyping did not significantly alter prescribing practice compared with no pretesting. There was no significant difference between the two groups in terms of starting doses and mean AZA prescribed dose at the end of the study period. Despite prior knowledge of noncarrier TPMT status in the tested group of patients, most patients were administered starting doses lower than 2mg/kg/day, similar to the nontested group. This was because physicians were free to practice as per routine, and which they did just as cautiously as the nontested group despite prior knowledge obtained from genotyping. Knowledge of

heterozygous status, however, did result in prescription of lower starting doses compared with noncarriers in the group tested before therapy. There is limited applicability of this evidence because there was just one homozygous carrier in the whole sample of mostly IBD patients. The finding conforms with an earlier national survey in the United Kingdom in which the uptake of prior TPMT testing differed substantially by clinical specialty – there was a higher uptake of TPMT enzyme-level testing by dermatologists, compared with gastroenterologists and rheumatologists, and this might explain why the group that underwent prior genotyping still ended up receiving doses of azathioprine similar to the nongenotyped control group.²³⁴ Since most patients had inflammatory bowel disease, it appears that gastroenterologists, specifically, tend to exercise a cautious prescribing approach over one primarily guided by prior knowledge of the TPMT status.

When compared with no pretesting for TPMT status, testing did not demonstrate a significant difference in the odds of mortality and serious adverse events in 333 randomized patients. The evidence was rated as insufficient given a medium risk of bias and strong possibility of type II error. The applicability of the evidence was deemed limited as there was just one homozygous carrier of TPMT variant allele in the entire sample of mostly IBD patients, the followup period was just 4 months and eligibility criteria of the trial excluded patients who would most likely have experienced adverse events. Evidence was also rated insufficient for the outcomes of health-related quality of life and myelotoxicity as no data were available.

Evidence from one RCT with low event rate showed no significant advantage of prior genotyping with respect to the intermediate outcomes of neutropenia and pancreatitis. The applicability of this evidence is quite limited because there was just one homozygous carrier in the whole sample of 333 patients, the followup period was just 4 months and eligibility criteria of the trial excluded patients who would most likely have experienced adverse events. Also, type II error cannot be ruled out. As TPMT status determination may not identify all individuals at increased risk of drug toxicity,²³⁵ direct and conclusive evidence of the utility of pretesting in terms of drug related harms reduction is wanting for evidence-based guidelines on thiopurine therapy. For the outcome of liver toxicity, significantly higher odds were observed in the group that underwent prior TPMT genotyping, odds ratio 2.54 (1.08, 5.97)]. There was no significant difference in starting or mean doses received between the tested and nontested group. Although this finding merits further investigation, but it appears to be a type I error and unrelated to the intervention of pretreatment genotyping.

Various recent guidelines, as well as the product monograph for azathioprine, have advocated determination of TPMT status prior to treatment with thiopurine drugs.^{30,243} The proposition that knowledge of TPMT status prior to 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 of limited validity. Compared with non-carriers and heterozygous carriers, homozygous are considered to be most at risk of developing neutropenia. However, from an evidence-based perspective, guideline recommendations of pretreatment TPMT testing are premature for several reasons. First and foremost, the direct evidence base for these recommendations is lacking – especially the crucial evidence that TPMT pretesting before thiopurine therapy decreases myelotoxicity specific mortality. Also, given just one homozygous carrier in the only available direct evidence investigating usefulness of pretreatment, evidence is equally lacking for this particular subgroup of patients. Second, patients on thiopurine drugs are required to undergo complete blood count monitoring on a regular basis in an attempt to prevent severe myelotoxicity by early detection. Third, azathioprine and 6-MP had been used

successfully for a number of years prior to the availability of TPMT testing and management (i.e. testing or not before therapy) varies across clinical specialties. Fourth, thiopurine related toxicities are also partially explained by mutations in other enzymes, drug interactions, intercurrent infections, and immune mediated drug reactions. Fifth, direct evidence of effectiveness of pretesting in the specific subpopulation of patients homozygous or compound heterozygous for the TPMT variant alleles is lacking the most, albeit not surprisingly, because of the low prevalence of homozygosity. Furthermore, the use of TPMT status to guide treatment has the potential to reduce the efficacy of thiopurine drugs if physicians are overzealous in reduction of thiopurine dosage. Indeed, the 2004 guidelines from the British Society of Gastroenterology recognized this and stated, “It cannot yet be recommended as a prerequisite to therapy, because decades of experience has shown clinical [azathioprine] to be safe in [ulcerative colitis] or [Crohn’s disease]”.²⁴⁴ As far as the utility of pretesting for TPMT status before thiopurine treatment is concerned, our review is indeterminate because of insufficient evidence and calls for urgent further research. This is at odds with previously published economic evaluations recommending testing. However, those evaluations have been criticized for incorporating clinical data from retrospective studies and expert opinion instead of prospective empiric evidence – the latter, as our review shows, are lacking²³⁶

Association of TPMT Status With Thiopurine Toxicity

In the presence of insufficient direct evidence of prior knowledge of TPMT status to guide thiopurine therapy, possible associations between TPMT status and the clinical outcomes of mortality, infections, hospitalization, withdrawals due to adverse events, serious adverse events and health-related quality of life, as well as the surrogate outcomes of myelotoxicity, liver toxicity, and pancreatitis were examined.

Toxicity of thiopurine drugs is thought to be mediated primarily through their pharmacologically active metabolites, 6-tGNs, which can be considered a dose-dependent toxicity. Incorporation of 6-tGN into DNA triggers cell-cycle arrest and apoptosis through the mismatch repair pathway.⁸ Recent evidence has also shown that thiopurine drugs can induce apoptosis in T-cells through modulation of Rac1 activation upon CD28 costimulation. Therefore accumulation of 6-tGNs can clearly induce various degrees of myelosuppression. Dose-independent toxicity, until recently was not understood, and appears to be either immune mediated or due to metabolites previously thought to be inactive. Both hepatotoxicity and pancreatitis are thought to be caused through dose-independent toxicity. Immune mediated reactions with AZA include hepatitis, pancreatitis, rash etc. and usually occur with 4 weeks of initiation of therapy. In some patients, this reaction can be overcome by switching to 6-MP, implying a role for the imidazole moiety in toxicity.²³⁷ The mechanisms of hepatotoxicity have been studied in most depth. A link between hepatotoxicity and increased levels of 6-methylMP ribonucleotide (6-MMPR) has been suggested. Seidman et al. identified a link between TPMT activity and levels of 6-MMPR, suggesting that TPMT may play a role in hepatotoxicity.²³⁸ However, in this case higher TPMT activity may be more relevant to induction of toxicity than lower activity (see Figure 1). In a subset of patients with subtherapeutic levels of 6-tGNs, dose escalation of 6-MP did not increase 6-tGN levels, however levels of 6-MMPR did increase. In one study, 24 percent of patients with elevated 6-MMPR levels showed higher rates of hepatotoxicity.¹¹⁹ Mardini et al also found that elevated levels of 6-MMPR correlated with

hepatotoxicity.²³⁹ While others have found no relation between levels of 6-MMP and hepatotoxicity.^{240,241}

Thirty-four studies were identified that provided relevant data on allelic variants and 16 studies were identified that provided data on TPMT enzymatic activity in relation to clinical outcomes. There is insufficient evidence examining association of TPMT status, as determined by either allelic determination or enzymatic activity, with the outcomes of mortality, hospitalization rates, serious adverse events, health related quality of life and neutropenia precluding meaningful conclusions. Furthermore, insufficient data were available for infection and thrombocytopenia by enzymatic analysis; however limited data showing no effect were available for allelic determination. The majority of the studies were of cross-sectional design and fair quality.

The available evidence confirms our previous understanding that there is strong association between the outcome of leukopenia and presence of variant TPMT alleles or subnormal enzymatic activity, and dose response relationships with both allelic variants and TPMT enzymatic activity.²²⁷ The strongest association was for homozygous carriers or low to absent enzymatic activity, compared with noncarrier or normal enzymatic activity patient groups. There is also some indication that lower levels of TPMT enzymatic activity may be associated with the composite outcome of myelotoxicity, defined as decreased levels of at least two hematopoietic cell lines. For most other outcomes, there was no significant association with either a presence of a TPMT allelic variant or subnormal enzymatic activity. Given the small number of studies involving few patients with events, type II error cannot be ruled out for most outcomes other than hepatotoxicity and pancreatitis. For these two outcomes, our findings of no association of these outcomes with either low enzymatic activity or presence of TPMT carrier states are consistent with extant literature.^{72,82,163}

Costs of Determining TPMT Status Versus Costs of Treating Drug-Associated Complications

Global interest in costs of TPMT phenotyping and genotyping is reflected in studies from around the world, published between 1995 and 2010.

Most cost estimates were based on theoretical populations, although one study was based on a bullous pemphigoid patient with AZA toxicity.²¹⁰ Across all studies, there was some consensus on the cost of genotype and phenotype testing, although the cost perspective was often not reported. The one study reporting costs from a societal perspective showed higher costs than the others.²⁰⁸ Heterogeneous estimates of the total cost likely arose from differing methodological choices. For example, the cost of treating AZA-associated complications was estimated to be between \$1,325 and \$5,877 in USD. This four-fold difference has the potential to result in disparate total cost estimates. The average cost of TPMT phenotyping was approximately half of the average cost of TPMT genotyping, but these costs may not be generalizable to all TPMT tests. These costs will have to be taken into consideration, along with the relative sensitivity and specificity of TPMT genotyping and TPMT phenotyping, when deciding upon which test to use.

Strengths and Limitations

This is the first comprehensive systematic review answering the question whether testing TPMT status prior to thiopurine therapy changes management and thiopurine toxicity outcomes. It is also the first review of the analytical performance characteristics of enzymatic measurement of TPMT activity and determination of TPMT allelic polymorphisms. These key questions were developed from a conceptual framework of the topic with input from the Technical Expert Panel. This panel included clinical, genetics, biochemistry and systematic review methodology experts. We contacted authors and obtained additional data that we incorporated in meta-analyses. A survey of laboratories, although not part of the original work plan, further added to our understanding of laboratory practices related to TPMT testing.

Despite our rigorous methodological approach, this review has several limitations. From a clinical perspective, the most important equipoise about the utility of prior TPMT testing remains insufficiently answered due to a dearth of comparative effectiveness literature on TPMT testing and its limited applicability. Evidence relating to other key questions originated in observational studies of poor to fair quality, so is of limited strength. The genetic associations established in this review, while confirming previous literature, are still of limited reliability.²⁴² Lastly, we pooled diverse studies, with the assumption that most thiopurine toxicity is determined genetically and biochemically. There was insufficient primary evidence to identify important effect modifiers or to carry out separate subgroup meta-analyses on studies with lower risk of bias.

Recommendations and Future Research

There is insufficient evidence examining the effectiveness of TPMT pretreatment enzymatic or genetic testing, to minimize thiopurine related toxicity in patients with chronic autoimmune diseases. As a priority, well powered, good quality, randomized controlled studies need to be conducted, in diverse and representative patient populations, to compare the effectiveness of TPMT genotyping and phenotyping with one another, and with no TPMT testing. These studies

should be large enough to include a sizable number of patients homozygous for the variant alleles and should be pragmatic in conduct, mimicking routine clinical practice. Outcomes would include both treatment efficacy and harms associated with thiopurine therapy. Another objective would be to establish the optimum initial dose adjustment for a given TPMT status. These studies should ensure that outcomes are truly assessed without prior knowledge of results of TPMT testing and administered drug dose, by employing appropriate blinding procedures. The recently concluded pragmatic TARGET study by Newman and associates was under-powered to detect differences in clinically important outcomes, largely because it faced recruitment problems. In future such recruitment problems may be mitigated by educating the public and clinicians that the evidence base for pretreatment TPMT testing is lacking and that it is unclear whether pretreatment testing does more good (i.e. reduction in thiopurine related toxicity) than harm (i.e. reduction in thiopurine efficacy because of overzealous dose reductions based on prior testing).

Until such experimental high quality evidence becomes available, alternative evidence may be sought in prospectively designed observational studies that estimate health related quality of life, drug prescription patterns, and myelotoxicity related mortality as important outcomes associated with and with no pretreatment TPMT testing. With availability of empiric evidence from such studies, decision-analytic modeling that comprehensively consider alternative strategies such as regular blood cell count and liver enzyme testing, metabolite monitoring, and dose adjustments for concomitant medications that impact the TPMT enzymatic pathway can help guide practice until evidence becomes available from well powered pragmatic trials. Subsequent models might also need to consider new information as technologies develop and knowledge evolves.

TPMT genotyping should test for the most common TPMT polymorphisms in the population of interest. There is little direct evidence identifying the optimum set of alleles to be tested, and this may need to be established for specific populations if TPMT genotyping turns out to be effective in future studies.

TPMT activity analyses are reported on one of two bases: per milliliter of packed red blood cells; or per gram of hemoglobin. These are not readily or exactly comparable. Common reporting units are needed, as well as cutoffs for low/absent, intermediate, normal TPMT enzymatic activity, and high enzymatic activities.

Future studies should clearly report numbers of uninterpretable or equivocal test results.

Conclusions

This is the first comprehensive systematic review answering the question whether testing TPMT status prior to thiopurine therapy changes management and thiopurine toxicity outcomes, including leukopenia and myelotoxicity. There is currently insufficient evidence regarding effectiveness of determining TPMT status prior to thiopurine treatment in terms of improvement in clinical outcomes and incident myelotoxicity in comparison with routine monitoring of full blood counts and adverse events. It is also unclear whether pretesting guides appropriate prescribing. Indirect evidence confirmed previously known strong associations between lower levels of TPMT enzymatic activity and the presence of TPMT variant alleles with thiopurine related leukopenia.

Sufficient preanalytical data are available to recommend preferred specimen collection, stability and storage conditions for determination of TPMT status. There was no clinically

significant effect of age, gender, various coadministered drugs, or most comorbid conditions (with the exception of renal failure and dialysis). The currently available methods for determination of TPMT enzymatic activity show good precision, with coefficients of variation generally below 10 percent. Based upon limited evidence, the reproducibility of TPMT allelic polymorphism determination is acceptable. However, the sensitivity of genetic testing to identify patients with low and/or intermediate TPMT enzymatic activity cannot be precisely estimated. Thus, if knowledge of TPMT status is desired, and if recent RBC transfusion is excluded, the available evidence suggests that enzymatic assay should be preferred over the determination of allelic polymorphism.

Despite widespread interest, precise costs associated with TPMT phenotyping are unknown. More research has been conducted examining TPMT genotyping but the cost estimates are heterogeneous, likely due to different methodological choices.

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List of Acronyms/Abbreviations

6-MMP	6-methylmercaptopurine
6-MMPR	6-methylMP ribonucleotide
6-MP	6-mercaptopurine
6-MTG	6-methylthioguanine
6-TG	6-thioguanine
6-tGN	deoxy-6-thioguanosine 5' triphosphate
6-tIMP	6-thiomercaptopurine
AACC	American Association for Clinical Chemistry
AHRQ	Agency for Health Research Quality
ALL	acute lymphoblastic leukemia
AO	aldehyde oxidase
AST	aspartate aminotransferase
AZA	azathioprine
CI	confidence interval
CK	creatinine kinase
CLSI	Clinical and Laboratory Standards Institute
CV	coefficient of variance
CLIB	Cochrane Library
FN	false negative
FP	false positive
g	gram
GD	guanine deaminase
h	hour
Hb	haemoglobin
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HPLC	high performance liquid chromatography
HQOL	health related quality of life
HWE	Hardy-Weinberg equilibrium
IBD	inflammatory bowel disease
IC50	concentration of inhibitor at which enzyme activity is 50 percent of uninhibited activity
ICU	intensive care unit
IMPDH	inosine monophosphate dehydrogenase
MALDI-TOF	Matrix-assisted laser desorption/ionization, with time-of-flight mass spectrometry
mg	milligram
n	number in group with particular characteristic
N	total number in study or group
nmol	nanomole
OR	odds ratio
p	probability
PCR	polymerase chain reaction
pmol	picomol
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
RBCs	red blood cells

RFLP	restriction fragment length polymorphism
SAE	serious adverse events
SAM	S-adenosyl-L-methionine
SNPs	single nucleotide polymorphisms
TARGET	TPMT: Azathioprine Response to Genotyping and Enzyme Testing study
TN	true negative
TP	true positive
TPMT	thiopurine methyltransferase
U	unit (nmol/h production in an enzymatic reaction)
WDAE	withdrawal due to adverse events
XO	xanthine oxidase

Appendix A

Exact Search Strings

Ovid MEDLINE(R) 1950 to May Week 3 2010

- 1 (TPMT* or thiopurine methyltransferase* or thiopurine s-methyltransferase* or thiopurine methyl-transferase* or thiopurine s-methyl-transferase* or Thiopurinemethyltransferase*).mp. [mp=title, original title, abstract, name of substance word, subject heading word]
- 2 animal/
- 3 human/
- 4 2 not (2 and 3)
- 5 1 not 4

EMBASE 1980 to 2010 Week 21

- 1 exp Thiopurine Methyltransferase/
- 2 (TPMT* or thiopurine methyltransferase* or thiopurine s-methyltransferase* or thiopurine methyl-transferase* or thiopurine s-methyl-transferase* or Thiopurinemethyltransferase*).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name]
- 3 1 or 2
- 4 human.sh.
- 5 nonhuman.sh.
- 6 animal.sh. (18273)
- 7 animal experiment.sh.
- 8 or/5-7
- 9 8 not (4 and 8)
- 10 3 not 9

Central (CLIB 2009 2)

(Thiopurine Methyltransferase*):ti,ab,kw or (TPMT* OR Thiopurinemethyltransferase*):ti,ab,kw or (thiopurine s-methyltransferase*):ti,ab,kw or (thiopurine methyl-transferase*):ti,ab,kw or (thiopurine s-methyl-transferase*):ti,ab,kw

Ovid Healthstar 1966 to April 2010

- 1 (TPMT* or thiopurine methyltransferase* or thiopurine s-methyltransferase* or thiopurine methyl-transferase* or thiopurine s-methyl-transferase* or Thiopurinemethyltransferase*).mp.
- 2 animal/
- 3 human/
- 4 2 not (2 and 3)
- 5 1 not 4

Genetic Abstracts. May 7 2009.

Query: KW=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*) OR TI=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*) OR AB=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*) OR DE=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*)

BioSYS May 5 2009

1 Topic=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*) OR Title=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*)
Databases=PREVIEWS Timespan=All Years

2 DE=(TPMT* OR thiopurine methyltransferase* OR thiopurine s-methyltransferase* OR thiopurine methyl-transferase* OR thiopurine s-methyl-transferase* OR Thiopurinemethyltransferase*) Databases=PREVIEWS
Timespan=All Years

3 #2 OR #1 Databases=PREVIEWS Timespan=All Years

4 TS=(econom* or cost or costs or costly or costing or costed or cost-benefit* or price or prices or pricing or priced or discount or discounts or discounted or discounting or expenditure or expenditures or budget* or afford* or pharmacoeconomic* or pharmaco-economic*) Databases=PREVIEWS Timespan=All Years

5 TI=(econom* or cost or costs or costly or costing or costed or cost-benefit* or price or prices or pricing or priced or discount or discounts or discounted or discounting or expenditure or expenditures or budget* or afford* or pharmacoeconomic* or pharmaco-economic*) Databases=PREVIEWS Timespan=All Years

6 DE=(econom* or cost or costs or costly or costing or costed or cost-benefit* or price or prices or pricing or priced or discount or discounts or discounted or discounting or expenditure or expenditures or budget* or afford* or pharmacoeconomic* or pharmaco-economic*) Databases=PREVIEWS Timespan=All Years

7 TI=(markov or markow or monte carlo) Databases=PREVIEWS
Timespan=All Years

- # 8 TI=(markov or markow or monte carlo) Databases=PREVIEWS
Timespan=All Years
- # 9 DE=(markov or markow or monte carlo) Databases=PREVIEWS
Timespan=All Years
- # 10 TS=sensitivity analys* Databases=PREVIEWS Timespan=All Years
- # 11 TI=sensitivity analys* Databases=PREVIEWS Timespan=All Years
- # 12 DE=sensitivity analys* Databases=PREVIEWS Timespan=All Years
- # 13 TS=quality adjusted life Databases=PREVIEWS Timespan=All Years
- # 14 TI=quality adjusted life Databases=PREVIEWS Timespan=All Years
- # 15 DE=quality adjusted life Databases=PREVIEWS Timespan=All Years
- # 16 TS=CE analys?s Databases=PREVIEWS Timespan=All Years
- # 17 TI=CE analys?s Databases=PREVIEWS Timespan=All Years
- # 18 Databases=PREVIEWS Timespan=All Years
- # 19 TS=(decision tree* or decision analy* or decision model*)
Databases=PREVIEWS Timespan=All Years
- # 20 TI=(decision tree* or decision analy* or decision model*)
Databases=PREVIEWS Timespan=All Years
- # 21 DE=(decision tree* or decision analy* or decision model*)
Databases=PREVIEWS Timespan=All Years
- # 22 TS="quality of life" Databases=PREVIEWS Timespan=All Years
- # 23 TI="quality of life" Databases=PREVIEWS Timespan=All Years
- # 24 DE="quality of life" Databases=PREVIEWS Timespan=All Years
- # 25 TS="willingness to pay" Databases=PREVIEWS Timespan=All Years
- # 26 TI="willingness to pay" Databases=PREVIEWS Timespan=All Years
- # 27 DE="willingness to pay" Databases=PREVIEWS Timespan=All Years

28 Major Concepts=(Economics) Databases=PREVIEWS Timespan=All Years

29 #28 OR #27 OR #26 OR #25 OR #24 OR #23 OR #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 Databases=PREVIEWS Timespan=All Years

30 TS=(markov or markow or monte carlo) Databases=PREVIEWS Timespan=All Years

31 #30 OR #29 Databases=PREVIEWS Timespan=All Years

32 #31 AND #3 Databases=PREVIEWS Timespan=All Years

Appendix B

Data extraction and related forms

Contents

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Key Questions 1a and 1b

Pre-analytical requirements for enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms; and
Within and between laboratory precision and reproducibility of enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms

Data element	Comments, coding for data extraction	
Ref id		
First author, year		
Companion ref id	<i>If applicable</i>	
Contains information about (select all that apply)	<i>1= pre-analytic requirement; 2 = post-analytic requirement, 3 = within laboratory precision and reproducibility, 4 = between laboratory precision and reproducibility</i>	
Full or partial industry funding	<i>0 = no, 1 = yes, 2 = not reported</i>	
Study region	<i>1= North America; 2 = Europe, 3 = Central and South America, 4 = sub Saharan Africa, 5 = Middle East and North Africa, 6 = South Asia, 7 = Asia, 9 = Oceania, 10 = 1+ 2, 11 = mixed /others, 12 = not reported</i>	
Summary description of TPMT activity assay		
Summary description of TPMT genotyping assay		
Summary of pertinent paper (capture as much as possible in as few words)		
Study limitations		
If not eligible for extraction, provide reasons		

Abbreviations: Ref id = unique reference identification number; TPMT = thiopurine methyltransferase

Key question 1c

Diagnostic sensitivity and specificity of TPMT allelic polymorphism measurement compared to the measurement of TPMT enzymatic activity

1. General information for key question 1c

Data element	Comments, coding	
Ref id		
First author, year		
Companion ref id		
Author contacted	1 = yes, 0 = no	
Full or partial industry funding	0 = no, 1 = yes, 2 = not reported	
Region of participants residence	1 = North America; 2 = Europe, 3 = central and South America, 4 = sub Saharan Africa, 5 = Middle East and north Africa, 6 = South Asia, 7 = Asia, 9 = Oceania, 10 = 1+ 2, 11 = mixed others, 12 = not reported	
Diagnostic study design	1 = cross-sectional, 2 = case-control, 3 = prospective observational (including cohort), 4 = RCT, 5 = can't tell, 6 = other (specify)	
Is enzymatic activity assay the reference/gold standard?	1 = yes, 0 = No	
Enzymatic activity assay: testing method?	1 = Radioassay, 2 = HPLC, 3 = other (name the method), 4 = NR	
Enzymatic activity assay: manufacturer?		
Enzymatic activity: categories?	1 = high, normal, intermediate, low, absent; 2 = high, normal, intermediate, low/absent; 3 = high/normal, intermediate, low, absent; 4 = high/normal, intermediate, low/absent; 5 = other (specify)	
Enzymatic activity: cut off values?	specify different cut-offs or state NR	
Genotyping: method	specify (e.g. RFLP, RT-PCR (probe hybridization or fluorescence), INVADER-PCR etc.) 1 = PCR, 2 = pyrosequencing, 3 = other	
Source of DNA for genotyping?	specify	
Number of SNPs genotyped		
Specify genotyped SNPs		

1. General information for key question 1c (continued)

Data element	Comments, coding	
Setting	1= inpatient, 2 = outpatient primary care, 3 = outpatient specialty clinic, 4 = NR or can't tell	
Participants	(a few words to describe the study population)	
Participants, summary inclusion/exclusion criteria		
Underlying disease (s)	including severity	
Thiopurine treatment	0= no; 1 = AZA, 2 = 6-MP, 3 = mixed thiopurines, 4 = other, 5 = NR	
Concomitant treatment potentially affecting TPMT activity	1 = no, 2 = allopurinol, 3 = 5-ASA (mesalazine/mesalamine/sulphasalazine, olsalazine), 4 = furosemide/frusemide, 5 = NSAID, 6 = other (specify), 7 = NR	
Age group	1 = adults (≥ 16) only, 2 = children only (< 16), 3 = adolescence only, 4 = mixed, 5 = not sure/ NR	
Age in years	mean or median or range	
Females %		
Caucasians %		
African descent %		
Hispanic %		
Asians %		
Other %	specify race and % of participants	
This report also has relevant subgroup data of genotyping and phenotyping results	specify subgroups	
Applicability based on population characteristics and testing methodology	1 = applicable, 2 = not applicable (explain why), 3= questionable applicability (explain why)	

Abbreviations: 6-MP = 6-mercaptopurine; ASA = acetylsalicylic acid or aspirinTM; AZA = azathioprine; NR = not reported; NSAID = nonsteroidal anti-inflammatory drug; Ref id = unique reference identification number; TPMT = thiopurine methyltransferase

2. Quantitative evidence for key question 1c

Data element	Comments, coding for data extraction	
Ref id		
First author, year		
Total number included in the study		
Dropouts and withdrawals or untested		
Activity assay: number of patients with non-interpretable test results		
Genotyping: number of patients with non-interpretable test results		
SNPs tested	<i>Specify SNPs</i>	
<i>First comparison</i>		
Genotypes	<i>Heterozygous or homozygous for variant(s) / wild type</i>	
Normal or hyperactivity (or controls)	<i>Numbers of participants with particular enzyme activity, with genotype</i>	
Intermediate or low/absent activity (or cases)	<i>Numbers of participants with particular enzyme activity, with genotype</i>	
<i>Second comparison</i>		
Genotype	<i>Homozygous for variant(s) / Wild type or heterozygous for variants</i>	
Normal/hyperactivity/intermediate activity (or controls)	<i>Numbers of participants with particular enzyme activity, with genotype</i>	
Low/absent activity (or cases)	<i>Numbers of participants with particular enzyme activity, with genotype</i>	
Number of allele specific subgroups		

Abbreviations: Ref id = unique reference identification number; TPMT = thiopurine methyltransferase

3. Quality assessment for key question 1c

Data element	Comments, coding for data extraction	
Ref id		
First author, year		
Is the spectrum of patients representative of those with one or more chronic autoimmune disease in terms of eligibility criteria, demographics, disease severity?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Clearly describes I/E criteria?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was the reference test (activity assay) likely to correctly identify enzymatic activity?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Did the whole or a random sample of patients receive TPMT activity test?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was the TPMT activity test administered regardless of genotyping results?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Were the TPMT activity and genotyping tests independent of each other?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was the genotyping method clearly described for replication?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was the activity assay clearly described for replication?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Activity test results were not interpreted with prior knowledge of genotyping results?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Genotyping results were not interpreted with prior knowledge of enzymatic activity?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Were the clinical data availability before testing was undertaken similar to that in usual practice setting in which the testing will be performed?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Were non-interpretable or equivocal test results reported?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Were withdrawals from the study explained?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was deviation from Hardy-Weinberg equilibrium reported?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Reviewer's overall risk of bias assessment	<i>1 = Good; 2 = Fair, 3 = Poor</i>	
Reason for overall risk of bias assessment (explain why good if good, explain why not good if fair or poor)	<i>specify</i>	

Abbreviations: I/E = inclusion / exclusion; Ref id = unique reference identification number.

Laboratory Survey Introductory Letter

Sent by email attachment, on Ottawa Health Research Institute letterhead

DATE

Dear Dr. X,

The University of Ottawa Evidence-based Practice Center (EPC) is conducting a systematic review on the assessment of Thiopurine Methyltransferase (TPMT) in patients prescribed Azathiopurine or other Thiopurine-based Drugs.

In addition to obtaining relevant data from the literature, we are conducting a survey to obtain specific data from laboratories that are involved in measuring TPMT. We are very interested in gathering data from your organization and invite you to participate in our survey. We would be happy to share with you the results of the survey as well as the final report. All data from the survey will be pooled ensuring anonymity. No organization will be identified in the report. The survey should take no more than 15-20 minutes to complete and we will give you three weeks to complete it. Please note all data will be housed for 15 years after termination of the study.

At this time we'd like to inquire into whether you are interested in participating in the survey. If so, we would greatly appreciate a response on this matter by DATE.

In your response, please include the proper name of your organization as well as the contact person/details.

Name of Organization: _____

Contact name and details: _____

If you have any questions about your rights as a research participant, you may contact the Board Chairman at 613-798-5555 ext 14902.

Sincerely,

Sophia Tsouros

Research Coordinator, University of Ottawa Evidence-Based Practice Centre

Clinical Epidemiology Program, Ottawa Hospital Research Institute

501 Smyth Road W0575

Box 208

Ottawa, Ontario, CANADA K1H 8L6

Ph: 613.737.8899 ext. 73920

Fax: 613.737.8781

Email: stsouros@ohri.ca

On behalf of Dr. Ronald Booth, Clinical Chemist, Ottawa Hospital Research Institute, Clinical Lead, TPMT review and Dr. David Moher, Director of EPC

Survey for “Thiopurine Methyltransferase” Laboratories

The University of Ottawa Evidence-based Practice Center is conducting a systematic review on assessment of Thiopurine Methyltransferase (TPMT) in patients prescribed Azathiopurine or other Thiopurine-based Drugs.

In addition to obtaining relevant data from the literature, we are conducting a survey to obtain specific data from laboratories that are involved in measuring TPMT. We are very interested in gathering data from your organization and invite you to participate in our survey. We would be happy to share with you the results of the survey as well as the final report. All data from the survey will be pooled ensuring anonymity. No organization will be identified in the report. The survey should take no more than 15-20 minutes to complete.

Name of Organization (text box)

Contact name (text box)

- 1) Does your laboratory perform analysis of Thiopurine Methyltransferase (TPMT) in-house?

No

Genotyping only

Phenotyping (Enzymatic analysis) only

Both Genotyping and Phenotyping

- a. If no, do you refer specimens out for analysis of TPMT?

Yes

No

- b. If yes to 1a, where do you send your specimens for analysis of TPMT, and what type of analysis is done (genotyping or phenotyping)?

(Text box)

- 2) What method is used for analysis of TPMT?

- a. Enzymatic assay - please select detection method (select all that apply):

- i. **Radiometric,**
- ii. **HPLC,**
- iii. **Mass Spec.,**
- iv. **Other (please specify) (Text box)**

- b. Genetic analysis - please select method (select all that apply):

- i. **Sequencing**
- ii. **PCR/RFLP**
- iii. **Real Time PCR**
- iv. **Site directed PCR (please indicate mutations screened for)**
- v. **Other (please specify) (Text box)**

- c. If mutation specific genetic analysis is performed please indicate all alleles targeted (select all that apply):
 - i. **TPMT*2**
 - ii. **TPMT*3A**
 - iii. **TPMT*3B**
 - iv. **TPMT*3C**
 - v. **Other (please list) (Text box)**
- 3) What is your yearly TPMT testing volume (indicate separately for both genotyping and phenotyping if applicable)? **(Text box)**
- 4) What is the preferred specimen type for analysis
 - a. Enzymatic analysis
 - i. **Not applicable**
 - ii. **EDTA whole blood**
 - iii. **Heparinized whole blood**
 - iv. **Other (please indicate) (Text box)**
 - b. Genotyping
 - i. **Not applicable**
 - ii. **EDTA whole blood**
 - iii. **Heparinized whole blood**
 - iv. **Other (please indicate) (Text box)**
- 5) What are the pre-analytical requirements for analysis
 - a. Specimen stability prior to analysis
 - i. Enzymatic analysis
 - 1. **Not applicable**
 - 2. **Specimen stability time (please indicate number of hours) (Text box)**
 - 3. **Storage temperature (room temperature, 4 C, -20 C, or -70 C) (Text box)**
 - ii. Genotyping
 - 1. **Not applicable**
 - 2. **Specimen stability time (please indicate number of hours) (Text box)**
 - 3. **Storage temperature (room temperature, 4 C, -20 C, or -70 C) (Text box)**
 - b. Do you require a list of medications the patient is currently taking?
 - i. **Yes**
 - ii. **No**
- 6) What are the quality control results for:
 - a. Enzymatic analysis
 - i. Coefficient of Variation
 - 1. **Within run (Text box)**

2. Between runs (Text box)

- b. Genotyping
 - i. Percent concordance with another genotyping method and/or genotyping standards from an external source. Please indicate the percentage the comparator genotyping methods and the basis of the comparison.
(Text box)
 - c. Enzymatic analysis in comparison with genotyping
 - i. Percent concordance between TPMT phenotyping and for each range of enzymatic activity reported by your laboratory.
(Text box)
- 7) Do you participate in an External Quality Assurance (EQA) program?
(Please indicate details of the program) (Text box)
- 8) What type of quality control is performed?
- a. **In-house (patient pool or other – please indicate) (Text box)**
 - b. **Commercial (please indicate supplier) (Text box)**
 - c. **Please indicate number of quality control levels (1, 2, 3, more) and quality control frequency (per-run, every day, multiple per day) (Text box)**
- 9) Reporting of results
- a. Are abnormal results called to the physician
 - i. **No**
 - ii. **Yes (under what circumstances) (Textbox)**
 - b. Please indicate your reference intervals for normal (homozygous wild type), heterozygote, and deficient as well as units of measure. **(Text box)**
 - c. How was your reference interval determined?
 - i. **Correlation with known homozygous normal patients**
 - ii. **Other (please explain) (Text box)**
 - d. Do you highlight suspected heterozygous patients when analyzed by enzymatic assay
 - i. **No**
 - ii. **Yes (under what circumstances) (Text box)**
- 10) Please provide your expected turn around time of the laboratory testing you provide.
(Text box)
- 11) Please provide details of the charges associated with laboratory testing in your native currency. (Please specify currency)
- a. **TPMT enzymatic activity test (Text box)**
 - b. **TPMT genotyping(Text box)**

Key Question (2), 3a, 3b

(No studies were identified to answer Key Question 2)

TPMT status to guide therapy - Cohort studies

Data element	Comments, coding for data extraction	
Ref id		
First author, Year		
Study site and setting		
Full or partial industry funding	0 = no, 1 = yes, 2 = not reported	
Total number of participants included in the study		
Describe population characteristics and setting	(a few words to describe the study population in terms of age, ethnicity, underlying diseases and their severity, and practice setting)	
Followup period		
Number of person weeks by groups		
Number lost to followup		
Intervention group description (including type of TPMT testing and treatment modification). n	Intervention group is the group which had TPMT pre-testing followed by thiopurine treatment adjustment	
Control group description (no testing and no treatment modification <u>OR</u> testing but no treatment modification based on its results). n	Control group had no treatment adjustment based on TPMT pre-testing	
Treatment and dosage intervention group	0 = no; 1 = AZA, 2 = 6-MP, 3 = mixed thiopurines, 4 = other, 5 = NR	
Treatment and dosage in the control	0 = no; 1 = AZA, 2 = 6-MP, 3 = mixed thiopurines, 4 = other, 5 = NR	
Outcome, and definition	mortality, SAE, WDAE, all infections, sepsis, hospitalization, ICU admission, myelotoxicity, leukopenia, neutropenia	
n experiencing the outcome		
Confounding factors controlled for	state factors	
Crude RR (intervention/control)		
Adjusted RR (adjusted for?) (intervention/control)		
Risk difference		
Proportion of those with outcome of interest in the intervention group n/N	N = total number in the group	
Proportion of those with the outcome of interest in the control group, n/N	N = total number in the group	
Were investigators and participants blinded to testing?	0 = no, 1 = yes, 2 = unclear	

Data element	Comments, coding for data extraction	
Was there a blinded assessment of outcomes?	<i>0 = no, 1 = yes, 2 = unclear</i>	
Were groups similar in characteristics at baseline	<i>0 = not similar, 1 = similar, 2 = unclear</i>	
Were outcomes adequately described?	<i>1 = reasonably adequately, 0 = inadequately, 2 = unclear</i>	
Was there an intention to treat analysis?	<i>0 = no, 1 = yes, 2 = unclear</i>	
Is there a conflict of interest?	<i>1 = yes, 2 = likely, 3 = unlikely</i>	
Was there a high loss to follow-up or important differential loss to follow-up between groups?	<i>0 = no, 1 = yes, 2 = unclear</i>	
Is the sample size adequate?	<i>0 = no, 1 = yes, 2 = unclear</i>	
Was the selection of participants unlikely to be biased? (e.g. inception cohort or method to avoid selection bias)	<i>0 = no, 1 = yes, 2 = unclear</i>	
Were methods to control confounding (e.g. matching, restriction to subgroups, analytic methods of stratification, propensity scores etc.) employed?	<i>0 = no, 1 = yes, 2 = unclear</i>	
Were the method of TPMT testing (as applicable, e.g. genotyping, or phenotyping or both) appropriate?	<i>0 = no, 1 = yes, 2 = unclear</i>	
Reviewer's overall risk of bias assessment for the study	<i>1 = Good; 2 = Fair, 3 = Poor</i>	
Reason for overall risk of bias assessment (explain why good if good, explain why not good if fair or poor)	<i>specify</i>	
Applicability based on population characteristics and testing methodology	<i>1 = applicable, 0 = not applicable (explain why), 2 questionable applicability (explain why)</i>	

Abbreviations: 6-MP = 6-mercaptopurine; AZA = azathioprine; ICU = intensive care unit; n = number with characteristic of interest; N = total number in group or trial or study; NR = not reported; RR = relative risk; Ref id = unique reference identification number.

Key question 3c - phenotyping

Association between TPMT status as determined by phenotyping, and adverse events

Data element	Comments, coding for data extraction	
Ref id		
First author, year		
Thiopurine treatment tailored according to TPMT genotype?	0 = no, 1 = yes (if yes do not data extract the study on this sheet. That is, the study does not qualify for Q3c)	
Companion ref id	If applicable	
Author contacted	1 = yes, 0 = no	
Full or partial industry funding	0 = no, 1 = yes, 2 = not reported	
Region of participants residence	1 = North America; 2 = Europe, 3 = central and South America, 4 = sub Saharan Africa, 5 = Middle East and north Africa, 6 = South Asia, 7 = Asia, 9 = Oceania, 10 = 1+ 2, 11 = mixed others, 12 = not reported	
Setting	1 = inpatient, 2 = outpatient primary care, 3 = outpatient specialty clinic, 4 = NR or can't tell	
Participants	(a few words to describe the study population)	
Participants, summary inclusion/exclusion criteria summary		
Underlying disease(s)	including severity	
Thiopurine treatment following TPMT testing?	0 = no; 1 = AZA, 2 = 6-MP, 3 = mixed thiopurines, 4 = other, 5 = NR	
Concomitant treatment affecting TPMT activity	1 = no, 2 = allopurinol, 3 = 5-ASA (mesalazine/mesalamine/sulphasalazine, olsalazine), 4 = furosemide/frusemide, 5 = NSAID, 6 = other (specify), 7 = NR	
Drug dosage	e.g. X mg/day	
Age group	1 = adults (>=16) only, 2 = children only (<16), 3 = adolescence only, 4 = mixed, 5 = not sure/ NR	
Age in years	mean or median or range	
Females %		
Caucasians %		
African descent %		
Hispanic %		
Asians %		
Other %	specify race and % of participants	
This report also has relevant subgroup data for	specify subgroup	
Study duration, or duration of followup for outcome assessment	weeks	
Describe TPMT activity assay	1 = RA; 2 = Mass spectrometry; 3 = HPLC, 4 = Other (specify)	
Cut of, or range, for TPMT high activity	state NR if not reported	

Key question 3c – phenotyping (continued)

Data element	Comments, coding for data extraction	
Cut of, or range, for TPMT normal activity	<i>state NR if not reported</i>	
Cut of, or range, for TPMT intermediate activity	<i>state NR if not reported</i>	
Cut of, or range, for TPMT low/absent activity	<i>state NR if not reported</i>	
Study design	<i>1 = cohort (retrospective or prospective), 2 = case-control, 3 = cross-sectional, 4 = other (specify)</i>	
In this report, was there clearly no biased assessment of outcomes and/or phenotyping results based on a prior knowledge of each other?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Were the groups similar at baseline?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Were outcomes described adequately?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Was there an intention to treat analysis?	<i>0= no, 1 = yes, 2 = can't tell</i>	
Is there a potential for financial conflict of interest?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Was there a high loss to follow-up or important differential loss to follow-up between groups?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Is the sample size adequate?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Was the selection of participants unlikely to be biased? (e.g. inception cohort or method to avoid selection bias)	<i>1 = yes, 2 = no, 3 = unclear</i>	
Were methods to control confounding (e.g. matching, restriction to subgroups, analytic methods of stratification, propensity scores etc.) employed?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Were the method of TPMT testing (as applicable, e.g. genotyping, or phenotyping or both) appropriate?	<i>1 = yes, 2 = no, 3 = unclear</i>	
Reviewers overall risk of bias assessment for the study	<i>1 = Good; 2 = Fair, 3 = Poor</i>	
Reason for overall risk of bias assessment (explain why good if good, explain why not good if fair or poor)	<i>specify</i>	
Applicability based on population characteristics and testing methodology	<i>1 = applicable, 2 = not applicable (explain why), 3= questionable applicability (explain why)</i>	
N total recruited (i.e. included in the study)		

Key question 3c – phenotyping (continued)

Data element	Comments, coding for data extraction	
N total analyzed		
Dichotomous Outcome	<i>1 = mortality, 2 = any infection (including sepsis), 3 = sepsis, 4 = hospitalization, 5 = ICU admission, 6 = WDAE, 7 = SAE, 8 = HQoL (describe instrument), 9 = hepatitis, 10 = pancreatitis, 11 = myelotoxicity, 12 = leukopenia, 13 = neutropenia, 14 = anemia, 15 = thrombocytopenia, 16 = number in need of thiopurine dose reduction, 17 = number in need of switching to another immunomodulating treatment</i>	
Definition (or details) of dichotomous outcomes where applicable		
Continuous outcomes	<i>1 = any infection (including sepsis), 2 = sepsis, 3 = HQoL (describe instrument), 4 = hepatitis, 4 = pancreatitis, 6 = myelotoxicity, 7 = leukopenia, 8 = neutropenia, 9 = anemia, 10 = thrombocytopenia, 11 = number in need of thiopurine dose reduction, 12 = number in need of switching to another immunomodulating treatment</i>	
Definition (or details) of continuous outcomes where applicable		
Low or absent activity: n with events		
Low or absent activity: n without events		
Intermediate activity: n with events		
Intermediate activity: n without events		
Normal activity: n with events		
Normal activity: n without events		
Continuous outcome mean (specify whether its is mean change, post treatment mean, or percentage mean change) with unit		
Continuous outcome -- applicable group	<i>e.g. all homozygotes or all heterozygotes etc.</i>	
Measure of dispersion (SD or SE)		
N analyzed for the group		
If not eligible for extraction, provide reasons here		

Abbreviations: HPLC = high performance liquid chromatography; HQoL = health related quality of life; ICU = intensive care unit; n = number with characteristic of interest; N = total number in group or trial or study; NR = not reported; RA = radiolabel assay; Ref id = unique reference identification number; SD = standard deviation; SE = standard error; SEA = serious adverse events; TPMT = thiopurine methyltransferase; WDAE = withdrawal due to adverse events.

Key question 3c - genotyping

Association between TPMT status as determined by genotyping, and adverse events

Data element	Comments, coding for data extraction	
Ref id		
First Author, year		
Thiopurine treatment tailored according to TPMT genotype?	0 = no, 1 = yes (if yes do not data extract the study on this sheet. That is, the study does not qualify for Q3c)	
Companion ref id (if applicable)		
Author contacted?	1 = yes, 0 = no	
Full or partial industry funding	0 = no, 1 = yes, 2 = not reported	
Region of participants residence	1 = North America; 2 = Europe, 3 = central and South America, 4 = sub Saharan Africa, 5 = Middle East and north Africa, 6 = South Asia, 7 = Asia, 9 = Oceania, 10 = 1+ 2, 11 = mixed others, 12 = not reported	
Setting	1 = inpatient, 2 = outpatient primary care, 3 = outpatient specialty clinic, 4 = NR or can't tell	
Participants	(a few words to describe the study population)	
Participants, summary inclusion/exclusion criteria summary		
Underlying disease (s)	including severity	
Type of thiopurine treatment?	0 = no; 1 = AZA, 2 = 6-MP, 3 = mixed thiopurines, 4 = other, 5 = NR	
Drug dosage	e.g. x mg/day	
Concomitant treatment potentially affecting TPMT activity	1 = no, 2 = allopurinol, 3 = 5-ASA (mesalazine/mesalamine/sulphasalazine, olsalazine), 4 = furosemide/frusemide, 5 = NSAID, 6 = other (specify), 7 = NR	
Age group	1 = adults (>=16) only, 2 = children only (<16), 3 = adolescence only, 4 = mixed, 5 = not sure/ NR	
Age in years	mean or median or range	
Percentage females		
Caucasians %		
African descent %		
Hispanic %		
Asians %		
Other %	specify race and % of participants	
This report also has relevant subgroup data for	specify subgroup	
Study duration, or duration of follow-up for outcome assessment	weeks	

Key question 3c – genotyping (continued)

Describe genotyping assay	<i>1 = pyrosequencing; 2 = PCR, 3 = other (specify)</i>	
Number of TPMT alleles tested		
Specify TPMT Alleles tested		
Study design	<i>1 = cohort (retrospective or prospective), 2 = case-control, 3 = cross-sectional, 4 = other (specify)</i>	
Were genotype groups comparable?	<i>1 = yes, 0 = no, 2 = unclear (Subjects should have been matched by ethno-geographic and ancestral origin and confounding variables (e.g. social indicators) or by clearly describing techniques of matching by genetic background to avoid confounding by population stratifications. If not, genomic control to test for patterns in unlinked markers should be undertaken (i.e. results were adjusted for additional genetic markers associated with ancestry in the population). Also, subjects should be matched for characteristics that are also likely to be genetically determined and also associated with the outcome of interest (e.g. weight). Consider control for clinical and other modifiers (e.g. diet and smoking) between genotypes)</i>	
Were participants enrolled in studies without prior knowledge of genotype?	<i>1 = yes, 0 = no, 2 = unclear</i>	
In this report, was there clearly no biased assessment of outcomes and/or genotyping results based on prior knowledge of each other? (i.e. blinded for outcomes and/or genotyping results)	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was genotyping method described adequately?	<i>1 = yes, 0 = no, 2 = unclear (how samples were handled, what genotyping methods was used, any quality checks, rules set to say when genotyping results would be considered valid)</i>	
Was reliability of genotyping method established?	<i>1 = yes, 0 = no, 2 = unclear (i.e. reference to a paper where genotyping assay reliability was assessed or re-genotyping of all/random subset of samples)</i>	
Is the extent of ambiguous results due to genotyping error reported?	<i>1 = yes, 0 = no, 2 = unclear (i.e. reference to a paper where genotyping assay reliability was assessed or re-genotyping of all/random subset of samples)</i>	
Was Hardy-Weinberg equilibrium tested?	<i>1 = yes, 0 = no, 2 = unclear (in cohort studies, HWE should have been tested in the whole study population, but in the case-control in the controls only, which represent the general population)</i>	
Was gene-gene interaction assessed?	<i>1 = yes, 0 = no, 2 = unclear</i>	

Key question 3c – genotyping (continued)

Did studies assess compliance with thiopurine treatment (and correct for differences where applicable)?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Did studies utilize different sources of DNA for all patients, introducing potential measurement bias?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was lost to followup reported?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Was there possibility of survival bias? For example, hyper-normal population (older people, free of disease) can introduce survival bias	<i>1 = yes, 0 = no, 2 = unclear</i>	
Is there a potential for financial conflict of interest?	<i>1 = yes, 0 = no, 2 = unclear</i>	
Reviewers overall risk of bias assessment for the study	<i>1 = Good; 2 = Fair, 3 = Poor</i>	
Reason for overall risk of bias assessment (explain why good if good, explain why not good if fair or poor)	<i>specify</i>	
Applicability based on population characteristics and testing methodology	<i>1 = applicable, 2 = not applicable (explain why), 3 = questionable applicability (explain why)</i>	
N total recruited (i.e. included in the study)		
N total analyzed		
Dichotomous Outcome	<i>1 = mortality, 2 = any infection (including sepsis), 3 = sepsis, 4 = hospitalization, 5 = ICU admission, 6 = WDAE, 7 = SAE, 8 = HQoL (describe instrument), 9 = hepatitis, 10 = pancreatitis, 11 = myelotoxicity, 12 = leukopenia, 13 = neutropenia, 14 = anemia, 15 = thrombocytopenia, 16 = number in need of thiopurine dose reduction, 17 = number in need of switching to another immunomodulating treatment</i>	
Definition (or details) of outcomes where applicable		
TPMT*2 homozygous: n with events		
TPMT*2 homozygous: n without events		
TPMT*2 heterozygous: n with events		
TPMT*2 heterozygous: n without events		
TPMT*3A homozygous: n with events		
TPMT*3A homozygous: n without events		
TPMT*3A heterozygous: n with events		

Key question 3c – genotyping (continued)

TPMT*3A heterozygous: n without events		
TPMT*3C homozygous: n with events		
TPMT*3C homozygous: n without events		
TPMT*3C heterozygous: n with events		
TPMT*3C heterozygous: n without events		
TPMT wild type: n with events		
TPMT wild type: n without events		
All homozygotes for TPMT allelic variant: n with events		
All homozygotes for TPMT allelic variant: n without events		
All heterozygotes for TPMT allelic variant: n with events		
All heterozygotes for TPMT allelic variant: n without events		
If not eligible for extraction, provide reasons here		
Allele specific subgroups		
Across alleles		

Abbreviations: HPLC = high performance liquid chromatography; HQoL = health related quality of life; ICU = intensive care unit; n = number with characteristic of interest; N = total number in group or trial or study; NR = not reported; PCR = polymerase chain reaction; Ref id = unique reference identification number; SEA = serious adverse events; TPMT = thiopurine methyltransferase; WDAE = withdrawal due to adverse event

Key Question 4

Costs of TPMT testing, costs of care, and costs of treating drug-associated complications

Data element	Comments, coding for data extraction	
Ref id		
First author, year		
Companion ref id (if applicable)		
Author contacted?	1 = yes, 0 = no	
Country		
Type of Study	E.g. Cost-effectiveness analysis, cost analysis	
Population Characteristics	E.g. type and severity of chronic autoimmune disease, ethnic and age distribution	
Perspective		
Costs of adverse events and care		
Currency		
Year of costing data		
Source of cost data		
Costing item	E.g. myelosuppression, ICU stay, etc.	
Type of costing data	E.g. one time annual cost	
Cost		
Converted to USD 2008 (inflation and conversion rate)	Must use the PPP for this	
Costs of testing		
Currency	E.g. USD	
Year of costing data		
Source of cost data		
Type of test		
Costing details		
Cost		
Converted to USD 2008 (inflation and conversion rate)		
Cost of treating drug-associated complications		
Currency		
Year of costing data		
Source of cost data		
Type of drug	e.g., AZA, 6-MP, AZA & 6-MP	
Costing item	e.g., myelosuppression due to AZA	
Type of costing data	E.g. one time annual cost	
Cost	Denomination and year	
Converted to USD 2008 (inflation and conversion rate)	Must use the PPP for this	
Other costs reported in study?		

Abbreviations: AZA = azathioprine; 6-MP = 6-mercaptopurine; ICU = intensive care unit; PPP = purchasing power parities and the consumer price index, as described in the methods; Ref id = unique reference identification number; TPMT = thiopurine methyltransferase; USD = United States dollars

Appendix C: Evidence Tables

Additional data are presented here for Key Questions 1b, 1c, 2, 3a, 3b, 3c and 4
Reference list is provided at the end of the document

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Key Question 1b: Within and between laboratory precision and reproducibility of enzymatic measurement of TPMT and determination of TPMT allelic polymorphisms

Table C-1. KQ 1b: Precision and reproducibility of TPMT status determinations

Study	Full or partial industry funding	Study region	TPMT activity assay method particulars*	Inter-assay coefficient of variance (%)	Intra-assay coefficient of variance (%)
HPLC TPMT assays Unless otherwise noted, TPMT is measured as the formation of 6-MMP from 6-MP, using nonradio-labelled SAM for the methyl donor, in RBC lysates. ^{1,2}					
Lennard 2001 ³	No	Europe	HPLC TPMT assay previously described ^{2,4}	5-9%	6.3%
Jacqz-Aigrain, 1994 ¹	NR	Europe	HPLC TPMT assay	6.2% (n=5)	8.9% (n=5)
Indjova 2003 ⁵	NR	Europe	HPLC TPMT assay	4.4-4.9%	6.8%
Shipkova 2004 ⁶	NR	Europe	HPLC TPMT assay	Pooled RBC: 5%; Control: 2.4%	Pooled RBC: 7.6%; Control: 2.4%
Zhang 2007 ⁷	No	Asia	HPLC TPMT assay	Control: 4.29%; Author control: 3.29%	Control: 4.29%; Author control: 3.25%
Indjova 2003 ⁸	NR	Europe	HPLC TPMT assay previously described ⁵ .	Pooled RBC: <5% Control: <2.4%	Pooled RBC: <7.6% Control: <2.4%
Anglicheanu 2002 ⁹	NR	Europe	HPLC TPMT assay - No extraction step.	6.0% (n=5)	9.5% (n=5)
Dervieux 2001 ¹⁰	No	Europe	HPLC TPMT assay previously described ¹ .	2.6% (n=5)	5.2% (n=8)
Dervieux 1999 ¹¹	No	Europe	HPLC TPMT assay previously described ^{1,12}	2.6% (n=5)	5.2% (n=8)
Menor 2001 ¹³	No	Europe	HPLC TPMT assay	2.4-2.8%	4.2-6.3%
Ganiere-Monteil 1999 ¹⁴	NR	Europe	HPLC TPMT assay with liquid-liquid extraction using a pH 9.5 NH ₄ Cl buffer	6.1% (n=56)	9,5% (n=56)

Table C-1. Precision and reproducibility of TPMT status determinations

Study	Full or partial industry funding	Study region	TPMT activity assay method particulars*	Inter-assay coefficient of variance (%)	Intra-assay coefficient of variance (%)
Escousse 1998 ¹⁵	NR	Europe	HPLC assay previously described ^{1,16}	8.4-9.4% (n=3)	0-8.2% (n=5)
Medard 1997 ¹²	NR	Europe	HPLC TPMT assay previously described ¹ . - Following incubation, liquid-liquid extraction replaced liquid-solid extraction. - Improved chromatographic conditions.	6.7-10.2% (n=21)	2.7-6.9% (n=5)
Johnson-Davis 2008 ¹⁷	NR	North America	HPLC TPMT assay	0.2-0.8% (n=20)	3.2-8.7% (n=20)
Khalil 2005 ¹⁸		Europe	HPLC TPMT assay - Simplified by eliminating time consuming extraction steps with organic solvents, a heating step and gradient elution.	1.4-4.9% (n=10)	1% (n=12)
Ganiere-Monteil 2004 ¹⁹	NR	Europe	HPLC TPMT assay previously described ^{1,14} .	1.7-4.3% (n=6)	0.8-6.0% (n=6)
Zhang 2006 ²⁰	NR	Asia	HPLC TPMT assay previously described. ^{1,14}	4.29%	4.29%

Table C-1. Precision and reproducibility of TPMT status determinations

Study	Full or partial industry funding	Study region	TPMT activity assay method particulars*	Inter-assay coefficient of variance (%)	Intra-assay coefficient of variance (%)
Keizer-Garritsen 2003 ²¹	NR	Europe	HPLC TPMT assay	Between day (n=5): Supernatants were stored at -23°C. Initial activities: 17.2±1.6, 38.5±2.4, 17.6±2.1, 15.9±1.4, 12.2±2.1 pmol/h/10 ⁷ RBCs. After 7 days: 19.2±1.4, 36.4±2.1, 17.0±1.8, 19.1±2.5, 12.8±2.4 pmol/h/10 ⁷ RBCs. After 25 days stored at -80°C: 17.0±2.2, 34.0±2.6, 15.5±2.4, 16.6±1.8, 10.6±1.6 pmol/h/10 ⁷ RBCs.	15.0±1.4 and 22.4±2.0 pmol/h/10 ⁷ RBCs (n=2).
Oselin 2006 ²²	No	Europe	HPLC TPMT assay	Precision 4%	Accuracy 96–103%.
Kroplin 1998 ²³	NR	Europe	HPLC TPMT assay to measure 6-MTG in RBC lysates.	5% (n=20)	6.7% (n=36)
Lentz 2000 ²⁴	NR	North America	HPLC TPMT assay to measure 6-MTG formed from 6-TG in whole blood and RBC lysates.	2–3%	4–10%
Ford 2006 ²⁵	No	Europe	HPLC TPMT assay to measure 6-MTG formed from 6-TG in whole blood and RBC lysates.	Standard RBCs: 3.6% (n=10) Whole blood: 2.7% (n=10)	Standard RBCs: 8.0% (n=20); Whole blood: 7.6% (n=20)
Ford 2004 ²⁶	Yes	Europe	HPLC TPMT assay to measure 6-MTG in RBC lysates.	3.4% (n=20)	9.1% (n=37)
Decaux 2001 ²⁷	Yes	Europe	HPLC TPMT assay to measure 6-MTG in RBC lysates.	4.1-4.5% (n=6)	6.9-8% (n = 17)

Abbreviations: 6-MP = 6-mercaptopurine; 6-MMP = 6-methyl mercaptopurine; 6-MTG = 6-methylthioguanine; HPLC = high pressure liquid chromatography; RBCs = red blood cells; SAM = S-adenosyl-L-methionine; SEM = standard error of means; TPMT = thiopurine methyl transferase.

Table C-2. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – study characteristics, part 1 (continued)

Key Question 1c: Diagnostic sensitivity and specificity of TPMT allelic polymorphism measurement, compared with the measurement of TPMT enzymatic activity

Table C-2. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – patient and study characteristics, part 1

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Genotyping method	SNPs Genotyped
Ansari, 2008 ²⁸	Europe Outpatient specialty clinic Non-randomized intervention study	Exclusion: 18-80 years of age; previous history of thiopurine or biologics use; very low TPMT activity	AZA 5-ASA, steroids	Mass spectrometry	Low 0-10 U/g Hb Intermediate 11-25 U/g Hb Normal 26-50 U/g Hb	NR	TPMT*3A, *3B, *3C
Ansari, 2002 ²⁹	NR Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; current or past treatment with AZA. Exclusion: Reliable data on clinical response or adverse effects not available.	AZA 5-ASA, steroids	Radioassay	Low <2.5 U/mL RBC Intermediate 2.5- 7.5 U/mL RBC High >7.5 U/mL RBC	Polymerase chain reaction	TPMT*2, *3A, *3C
Gardiner, 2008 ³⁰	Oceania Inpatient and outpatient specialty clinics Prospective observational	Inclusion: Diagnosis of IBD; normal or intermediate TPMT activity; first time treatment with AZA or 6-MP. Exclusion: Pre-existing neutropenia	AZA, 6-MP 5-ASA	Radioassay	Normal 9.3- 17.6 U/mL RBC Intermediate 5- 9.2 U/mL RBC	Polymerase chain reaction	TPMT*2, *3A, *3C
Haglund, 2004 ³¹	Europe NR	NR	Mixed thiopurines	Radioassay	Low <5.0 U/mL RBC Intermediate	Pyro-sequencing	TPMT*2, *3A, *3B, *3C, *3D,

Table C-2. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Genotyping method	SNPs Genotyped
	Cross-sectional		5-ASA, steroids		5.0- 9.0 U/mL RBC Normal >9.0 U/mL RBC		*4, *5, *6, *7, *8
Hindorf, 2006 ³²	Europe NR Cross-sectional	Inclusion: Diagnosis of IBD; history of Metabolite or TPMT measurements between 2003 and 2007; current or past treatment with thiopurines.	AZA 5-ASA, steroids	Radioassay	Low <2.5 U/mL RBC Intermediate 2-5-8.9 U/mL RBC Normal >9.0 U/mL RBC	Pyro-sequencing	TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, 10, *14, *15
Hindorf, 2004 ³³	Europe NR Prospective observational	Inclusion: Diagnosis of IBD; current treatment with AZA or 6-MP for at least 4 months and unchanged dose for at least 3 months. Exclusion: Repeatedly low or immeasurable metabolite levels.	AZA, 6-MP 5-ASA	Radioassay	NR	Polymerase chain reaction	TPMT*3A, *3B, *3C, *3D
Langley, 2002 ³⁴	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of autoimmune hepatitis; hypergammaglobulinemia, elevated serum aminotransferase and liver biopsies. Exclusion: Possible causes of liver disease.	AZA steroids	Radioassay	Low <5.0 U/mL RBC Intermediate 5.0-13.7 U/mL RBC High >13.7 U/mL RBC	Polymerase chain reaction	TPMT*3A, *3B, *3C
Lindqvist, 2006 ³⁵	Europe Outpatient	Exclusion: Recent thiopurine therapy; history of	AZA, 6-MP 5-ASA,	Radioassay	Low <3 U/mL RBC Intermediate	Pyro-sequencing	TPMT*2, *3A, *3B, *3C, *3D,

Table C-2. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Genotyping method	SNPs Genotyped
	specialty clinic Prospective observational	pancreatitis with thiopurines; pregnancy; breastfeeding	steroids		3-8.9 U/mL RBC Normal/high >8.9 U/mL RBC		*4, *5, *6, *7, *8, 10, *14, *15
Marinaki, 2003 ³⁶	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; East Indian or Pakistani heritage	AZA NR	Radioassay	Low >2.5 U/g Hb Intermediate 2.5–7.5 U/g Hb Normal 7.6–14.5 U/g Hb	Polymerase chain reaction	TPMT*2, *3A, *3C
Okada, 2005 ³⁷	Asia NR Cross-sectional	Inclusion: Diagnosis of SLE; current or past treatment with AZA	AZA NR	High performance liquid chromatography	NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C
Schwab, 2002 ³⁸	Europe Outpatient specialty clinic Retrospective review of records	Inclusion Diagnosis of IBD; current or past treatment with AZA.	AZA 5-ASA, steroids, other	High performance liquid chromatography	Low <5 U/mL RBC Intermediate 5-10 U/mL RBC Normal/high: >10 U/mL RBC	Denatured high performance liquid chromatography	TPMT*2, *3A, *3B, *3C, *3D
Snow, 1995 ³⁹	North America NR Cross-sectional	NR	AZA NR	Radioassay	Low <5 U/mL RBC Intermediate 5-13.7 U/mL RBC High 13.8 to 25.1 U/mL RBC	NR	NR

Table C-2. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Genotyping method	SNPs Genotyped
Stassen, 2009 ⁴⁰	NR NR Retrospective review of records	NR	AZA NR	High performance liquid chromatography	Low <2 U/g Hb Intermediate 2-23.5 U/g Hb Normal/high >23.5 U/g Hb	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C
Stocco, 2005 ⁴¹	Europe Outpatient specialty clinic Prospective observational	Inclusion: Diagnosis of IBD; current treatment with a thiopurine for at least 3 months or having experienced an adverse effect during thiopurine treatment	Mixed thiopurines NR	High performance liquid chromatography	Low <4 U/mL RBC Intermediate 4-8U/mL RBC Normal 8-12 U/mL RBC High >12U/mL RBCs	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C
Stocco, 2004 ⁴²	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; child or young adult; current or past treatment with thiopurines	AZA NR	High performance liquid chromatography	Intermediate <8.5 U/mL RBC High >8.5 U/mL RBC	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C
von Ahsen, 2005 ⁴³	Europe NR Randomized controlled trial	Inclusion: Diagnosis of active Crohn's disease; >18 years; prednisone treatment >300mg during last 4 weeks or relapse within 6 months after steroid pulse therapy. Exclusion: History of cancer; preexisting renal or hepatic	AZA 5-ASA, other	Radioassay	Low <5 U/mL RBC Intermediate 5-9.9 nmol/mL Normal/high >=10 U/mL RBC	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C

Table C-2. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Genotyping method	SNPs Genotyped
		disease; pregnancy; breastfeeding					
Winter, 2007 ⁴⁴	Europe NR Cross-sectional	Inclusion: Diagnosis of IBD; current or past treatment with thiopurines.	AZA 5-ASA	Mass spectrometry	Low <10 U/g Hb Intermediate 10-25 U/g Hb Normal 26-50 U/g Hb High >50 U/g Hb	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C
Newman, 2010 ⁴⁵	Europe Outpatient Specialty Clinic Cross-sectional	Nursing and pregnant women, and those likely to experience adverse events were excluded	AZA NR	High performance liquid chromatography	NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C
Hindorf, 2010 ⁴⁶	Europe NR Cross-sectional	Inclusion: post treatment AIH scores > 11	Mixed thiopurines NR	High performance liquid chromatography	Normal: equal or above 9.0 U/mL RBC; Intermediate: 2-5-8.9 U/mL RBC; Low: below 2.5 U/mL RBC	Pyro-sequencing	TPMT *2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, and 10 Also *14 and *15

Abbreviations: 5-ASA = 5-aminosalicylates; 6-MP = 6-mercaptopurine; AZA = azathioprine; IBD = inflammatory bowel disease; NR = not reported; RBCs = red blood cells; SLE = systemic lupus erythematosus; SNP = single nucleotide polymorphism; TPMT = thiopurine methyltransferase.

Table C-3. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – patient and study characteristics, part 2

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Number of dropouts, withdrawals and untested participants	TPMT Activity: Number with non-interpretable test results	Genotyping: Number with non-interpretable test results
Ansari, 2008 ²⁸	IBD Adults	40.3 (18 - 80)	54	192	23	NR	NR
Ansari, 2002 ²⁹	IBD Adults	44	46	40	0	NR	NR
Gardiner, 2008 ³⁰	IBD Adult	39.2 (35.4- 42.9)	51	68	9	NR	NR
Haglund, 2004 ³¹	IBD Mixed	38 (12-61)	43	30	0	0	0
Hindorf, 2006 ³²	IBD Adults	28 (18-42) (median)	47	52	NR	NR	NR
Hindorf, 2004 ³³	IBD Adults	36 (18-63)	47	55	6	0	0
Langley, 2002 ³⁴	AIH Mixed	45 (13-72) (median)	74	53	19	NR	NR
Lindqvist, 2006 ³⁵	IBD Adults	40.5 (18-76)	47	60	NA	NR	NR
Marinaki, 2003 ³⁶	IBD NR	NR	NR	85	NR	NR	NR
Okada, 2005 ³⁷	SLE NR	NR	NR	55	NR	NR	NR

Table C-3. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – patient and study characteristics, part 2 (continued)

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Number of dropouts, withdrawals and untested participants	TPMT Activity: Number with non-interpretable test results	Genotyping: Number with non-interpretable test results
Schwab, 2002 ³⁸	IBD Adults	41 (17-71) (median)	52	93	NR	NR	NR
Snow, 1995 ³⁹	Autoimmune dermatologic conditions Adults	NR (26-88)	39	26	2	NR	NR
Stassen, 2009 ⁴⁰	Anti-neutrophil cytoplasmic antibody associated vasculitis NR	NR	NR	108	0	NR	NR
Stocco, 2005 ⁴¹	IBD Mixed	14.2 (0.8-38.8) (median)	51	28	NR	NR	NR
Stocco, 2004 ⁴²	IBD Mixed	16.4 (4-38)	45	27	NR	NR	NR
von Ahsen, 2005 ⁴³	IBD Adults	36 (SD 11.6)	56	71	0	0	0
Winter, 2007 ⁴⁴	IBD NR	45	46	130	14	14	14
Newman, 2010 ⁴⁵	Majority of IBD patients Adults	42	50	333	11	NR	NR

Table C-3. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – patient and study characteristics, part 2 (continued)

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Number of dropouts, withdrawals and untested participants	TPMT Activity: Number with non-interpretable test results	Genotyping: Number with non-interpretable test results
Hindorf, 2010⁴⁶	Autoimmune hepatitis Adults	55 (35-67)	80	229	9	NR	0

Abbreviations: AIH = autoimmune hepatitis; IBD = inflammatory bowel disease; NR = not reported; SD = standard deviation; SLE = systemic lupus erythematosus; TPMT = thiopurine methyltransferase.

Table C-4. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 1

Study	Representative spectrum of patients	Clear Description of selection criteria	Activity assay correctly measures enzyme activities	Whole or random sample administered activity test	TPMT activity test administered independent of genotype results	Independence of TPMT activity and genotyping tests	Genotyping method description clear for replication	TPMT activity test method description clear for replication
Ansari, 2008 ²⁸	Yes	Clear	Yes	Unclear	Unclear	Yes	Unclear	Clear
Ansari, 2002 ²⁹	Unclear	Clear	Yes	Yes	Yes	Yes	Clear	Clear
Gardiner, 2008 ³⁰	No	Clear	Yes	No	Yes	Yes	Clear	Clear
Haglund, 2004 ³¹	No	Partially clear	Yes	Yes	Yes	Yes	Clear	Clear
Hindorf, 2006 ³²	Unclear	Clear	Yes	Yes	Yes	Yes	Clear	Clear
Hindorf, 2004 ³³	No	Clear	Yes	Yes	Yes	Yes	Clear	Clear
Langley, 2002 ³⁴	Unclear	Clear	Yes	Unclear	Yes	Yes	Clear	Clear
Lindqvist, 2006 ³⁵	Unclear	Clear	Yes	Yes	Yes	Yes	Clear	Clear
Marinaki, 2003 ³⁶	Unclear	Unclear	Yes	Yes	Yes	Yes	Clear	Clear
Okada, 2005 ³⁷	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Clear
Schwab, 2002 ³⁸	Unclear	Partially clear	Yes	Yes	Yes	Yes	Clear	Clear
Snow, 1995 ³⁹	Unclear	Unclear	Yes	Yes	Yes	Yes	Unclear	Clear
Stassen, 2009 ⁴⁰	Unclear	Unclear	Yes	Yes	Yes	Yes	Clear	Clear

Table C-4. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 1 (continued)

Stocco, 2005⁴¹	Unclear	Clear	Yes	Unclear	Unclear	Yes	Clear	Clear
Stocco, 2004⁴²	Unclear	Partially clear	Yes	Unclear	Unclear	Yes	Clear	Clear
von Ahsen, 2005⁴³	Unclear	Clear	Yes	Yes	Yes	Yes	Clear	Clear
Winter, 2007⁴⁴	Unclear	Unclear	Yes	Yes	Yes	Yes	Clear	Clear
Newman, 2010⁴⁵	Unclear	Clear	Yes	Yes	Yes	Yes	Yes	Yes
Hindorf, 2010⁴⁶	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Yes

Abbreviations: TPMT = thiopurine methyltransferase.

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Ansari, 2008²⁸	Unclear	Unclear	Unclear	No	Yes	Yes	Applicable	Fair - Unclear if TPMT testing was blinded.
Ansari, 2002²⁹	Unclear	Unclear	Unclear	No	No	No	Unclear - Uncertain representativeness of adult IBD patients presenting to a specialty clinic. Excluded patients with low TPMT enzymatic activity	Fair - Unclear if TPMT testing was blinded. HWE not tested.

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Gardiner, 2008 ³⁰	Unclear	Unclear	Unclear	No	Yes	No	Unclear - Well representative of adult IBD patients presenting to a gastroenterology clinic, but excluded patients with low TPMT enzymatic activity	Poor - HWE not tested. Sample not representative. Unclear if phenotyping influenced by genotyping results, and vice versa.
Haglund, 2004 ³¹	Unclear	Unclear	Unclear	No	Yes	No	Unclear - Uncertain representativeness of adolescent and adult IBD patients. Patients were pre-selected based on their TPMT activity.	Poor - HWE not tested. Exclusion criteria not reported. Samples selected based on TPMT activity. TPMT testing not blinded.

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Hindorf, 2006 ³²	Unclear	Unclear	Unclear	No	No	No	Unclear - Not representative of IBD patients, including only those currently or previously on thiopurines	Fair - HWE not reported. Unclear if genotyping was blinded.
Hindorf, 2004 ³³	Unclear	Unclear	Unclear	Unclear	Yes	No	Unclear - Not representative of adult IBD patients, including only adult IBD patients on long-term thiopurine treatment	Fair - HWE not reported. Unclear if TPMT testing was blinded.
Langley, 2002 ³⁴	Yes	Yes	Yes	Unclear	No	No	Unclear - Not representative of AIH patients, including only those in a tertiary care setting	Poor - HWE not reported. Unclear representativeness of sample and unclear random genotyping of a subset of patients .

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Lindqvist, 2006 ³⁵	Unclear	Unclear	Yes	Unclear	Yes	No	Unclear - Not representative of adult IBD patients, excluding those likely to experience adverse events.	Fair - Sample not representative. Unclear if TPMT testing was blinded.
Marinaki, 2003 ³⁶	Unclear	Unclear	Unclear	No	No	No	Unclear - Uncertain representativeness of IBD patients. Includes only those of South Asian ancestry.	Fair - Unclear representativeness of sample. Unclear if TPMT testing was blinded.
Okada, 2005 ³⁷	Unclear	Unclear	Unclear	No	No	No	Unclear - Not representative of Japanese SLE patients, including only those in a tertiary care setting.	Poor - Unclear representativeness of sample. Unclear if TPMT testing was blinded and whether testing was non-selective.

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Schwab, 2002 ³⁸	Unclear	Unclear	Unclear	No	No	No	Unclear - Not representative of IBD patients, including only those in a tertiary care setting.	Fair - Unclear representativeness of sample. Unclear if TPMT testing was blinded.
Snow, 1995 ³⁹	Unclear	Unclear	Unclear	No	Yes	No	Unclear - Uncertain representativeness of autoimmune dermatologic patients. No description of testing methodology.	Poor - Poorly reported testing methodology and inclusion criteria.
Stassen, 2009 ⁴⁰	Unclear	Unclear	Unclear	Unclear	No	No	Unclear - Little to no reporting of population characteristics.	Fair – Unclear if TPMT testing was blinded.

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Stocco, 2005 ⁴¹	Unclear	Unclear	Yes	No	Yes	No	Unclear - Uncertain representativeness of IBD patients.	Poor - Unclear representativeness of sample. Unclear if TPMT testing was blinded. Unclear if phenotyping influenced by genotyping results or other factors.
Stocco, 2004 ⁴²	Unclear	Unclear	Unclear	No	No	No	Unclear - Not representative of IBD patients, including only those in a tertiary care setting.	Poor - Unclear representativeness of sample. Unclear if TPMT testing was blinded.
von Ahsen, 2005 ⁴³	Unclear	Unclear	Unclear	Unclear	Yes	No	Unclear - Unrepresentative of active Crohn's disease patients.	Fair - Unclear representativeness of sample. Unclear if TPMT testing was blinded.

Table C-5. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – risk of bias assessment of included studies, part 2

Study	TPMT activity test results assessed independent of genotyping results	Genotyping results assessed independent of TPMT activity test results	Clinical data availability before assessing activity or genotyping was routine	Reporting of uninterpretable test results	Explanation provided for study withdrawals	HWE Tested	Applicability	Summary risk of bias assessment
Winter, 2007 ⁴⁴	Unclear	Unclear	Unclear	Yes	Yes	No	Unclear - Uncertain representativeness of patients with IBD. All had previously received thiopurines	Fair - Unclear representativeness of sample. Unclear if TPMT testing was blinded.
Newman, 2010 ⁴⁵	Unclear	Unclear	Yes	No	Yes	No	Unclear - Uncertain representativeness of patients with IBD. excluded patients with important adverse events due to previous thiopurine exposure	Fair - Unclear blinded assessment, and exclusion of participants likely to experience SAE
Hindorf, 2010 ⁴⁶	Unclear	Unclear	Unclear	No	Yes	No	Little reporting of patient characteristics. Unclear representativeness of AIH patients	Fair - Unclear blinded assessment

Abbreviations: HWE = Hardy-Weinberg equilibrium; IBD = inflammatory bowel disease; TPMT = thiopurine methyltransferase.

Table C-6. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – summary data

Study	Genotypes	Number of participants		Genotypes	Number of participants		Normal and hyperactivity		Normal, hyperactivity or intermediate activity	
		Normal or hyper-activity	Intermediate or low activity		Normal, hyper-activity or intermediate activity	Low activity	Sensitivity	Specificity	Sensitivity	Specificity
Ansari, 2008 ²⁸	Heterozygous or homozygous for variant(s)	1	17	Homozygous for variant(s)	0	0	73.9 (52.8, 87.8)	99.4 (95.9, 99.9)	0/0	99.7 (96, 100)
	Non-carriers	168	6	Non-carriers or heterozygous for variant(s)	192	0				
Ansari, 2002 ²⁹	Heterozygous or homozygous for variant(s)	0	10	Homozygous for variant(s)	0	0	95.5 (55.2, 99.7)	98.4 (78.9, 99.9)	0/0	98.8 (83.3, 99.9)
	Non-carriers	30	0	Non-carriers or heterozygous for variants	40	0				
Gardiner, 2008 ³⁰	Heterozygous or homozygous for variant(s)	2	5	Homozygous for variant(s)	0	0	55.6 (25.1, 82.3)	96.6 (87.4, 99.2)	0/0	99.3 (89.5, 100)
	Non-carriers	57	4	Non-carriers or heterozygous for variants	68	0				
Haglund, 2004 ³¹	Heterozygous or homozygous for variant(s)	0	21	Homozygous for variant(s)	0	6	97.7 (72.3, 99.9)	95 (52.5, 99.7)	75.0 (37.7, 93.7)	97.8 (73.2, 99.9)
	Non-carriers	9	0	Non-carriers or heterozygous for variant(s)	22	2				

Table C-6. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – summary data (continued)

Study	Genotypes	Number of participants		Genotypes	Number of participants		Normal and hyperactivity		Normal, hyperactivity or intermediate activity	
		Normal or hyper-activity	Intermediate or low activity		Normal, hyper-activity or intermediate activity	Low activity	Sensitivity	Specificity	Sensitivity	Specificity
Hindorf, 2006 ³²	Heterozygous or homozygous for variant(s)	1	11	Homozygous for variant(s)	0	6	91.7 (58.7, 98.8)	97.5 (84.3, 99.6)	92.9 (42.3, 99.6)	98.9 (85.1, 99.9)
	Non-carriers	39	1	Non-carriers or heterozygous for variant(s)	46	0				
Hindorf, 2004 ³³	Heterozygous or homozygous for variant(s)	0	5	Homozygous for variant(s)	0	1	91.7 (37.8, 99.5)	99 (86.2, 99.9)	100%	99.1 (87.1, 99.9)
	Non-carriers	50	0	Non-carriers or heterozygous for variant(s)	54	0				
Langley, 2002 ³⁴	Heterozygous or homozygous for variant(s)	4	6	Homozygous for variant(s)	0	0	66.7 (33.3, 88.9)	90.9 (78.2, 96.5)	0%	99.1 (86.6, 99.9)
	Non-carriers	40	3	Non-carriers or heterozygous for variant(s)	52	1				
Lindqvist, 2006 ³⁵	Heterozygous or homozygous for variant(s)	1	7	Homozygous for variant(s)	0	1	63.6 (33.9, 85.7)	98 (86.9, 99.7)	1/1	99.2 (88, 99.9)
	Non-carriers	48	4	Non-carriers or heterozygous for variant(s)	59	0				

Table C-6. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – summary data (continued)

Study	Genotypes	Number of participants		Genotypes	Number of participants		Normal and hyperactivity		Normal, hyperactivity or intermediate activity	
		Normal or hyper-activity	Intermediate or low activity		Normal, hyper-activity or intermediate activity	Low activity	Sensitivity	Specificity	Sensitivity	Specificity
Marinaki, 2003 ³⁶	Heterozygous or homozygous for variant(s)	0	5	Homozygous for variant(s)	0	0	91.7 (37.8, 99.5)	99.4 (90.9, 100)	0/0	99.4 (91.4, 100)
	Non-carriers	80	0	Non-carriers or heterozygous for variant(s)	85	0				
Okada, 2005 ³⁷	Heterozygous or homozygous for variant(s)	1	3	Homozygous for variant(s)	0	0	23.1 (7.6, 52.2)	97.6 (84.9, 99.7)	0/0	99.1 (87.3, 99.9)
	Non-carriers	41	10	Non-carriers or heterozygous for variant(s)	55	0				
Schwab, 2002 ³⁸	Heterozygous or homozygous for variant(s)	3	5	Homozygous for variant(s)	0	1	91.7 (37.8, 99.5)	96.6 (90, 98.9)	1/1	99.5 (92, 100)
	Non-carriers	85	0	Non-carriers or heterozygous for variant(s)	92	0				
Snow, 1993 ³⁹	Heterozygous or homozygous for variant(s)	0	5	Homozygous for variant(s)	0	0	91.7 (37.8, 99.5)	97.7 (72.3, 99.9)	0/0	98.1 (76.4, 99.9)
	Non-carriers	21	0	Non-carriers or heterozygous for variant(s)	26	0				

Table C-6. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – summary data (continued)

Study	Genotypes	Number of participants		Genotypes	Number of participants		Normal and hyperactivity		Normal, hyperactivity or intermediate activity	
		Normal or hyper-activity	Intermediate or low activity		Normal, hyper-activity or intermediate activity	Low activity	Sensitivity	Specificity	Sensitivity	Specificity
Stassen, 2009 ⁴⁰	Heterozygous or homozygous for variant(s)	0	7	Homozygous for variant(s)	0	0	93.8 (46.1, 99.6)	99.5 (92.7, 100)	0/0	99.5 (93.1, 100)
	Non-carriers	101	0	Non-carriers or heterozygous for variant(s)	108	0				
Stocco, 2005 ⁴¹	Heterozygous or homozygous for variant(s)	0	3	Homozygous for variant(s)	0	0	87.5 (26.6, 99.3)	98.1 (75.6, 99.9)	0/0	98.3 (77.7, 99.9)
	Non-carriers	25	0	Non-carriers or heterozygous for variant(s)	28	0				
Stocco, 2004 ⁴²	Heterozygous or homozygous for variant(s)	0	4	Homozygous for variant(s)	0	0	90 (32.6, 99.4)	97.9 (74.1, 99.9)	0/0	98.2 (77, 99.9)
	Non-carriers	23	0	Non-carriers or heterozygous for variant(s)	27	0				
von Ahsen, 2005 ⁴³	Heterozygous or homozygous for variant(s)	0	5	Homozygous for variant(s)	0	0	23.8 (10.3, 46)	99 (86.2, 99.9)	0/0	99.3 (89.9, 100)
	Non-carriers	50	16	Non-carriers or heterozygous for variant(s)	71	0				

Table C-6. KQ1c: Diagnostic sensitivity and specificity of TPMT determinations – summary data (continued)

Study	Genotypes	Number of participants		Genotypes	Number of participants		Normal and hyperactivity		Normal, hyperactivity or intermediate activity	
		Normal or hyper-activity	Intermediate or low activity		Normal, hyper-activity or intermediate activity	Low activity	Sensitivity	Specificity	Sensitivity	Specificity
Winter, 2007 ⁴⁴	Heterozygous or homozygous for variant(s)	0	11	Homozygous for variant(s)	0	0	64.7 (40.4, 83.2)	99.6 (93.4, 100)	0/0	99.6 (94.2, 100)
	Non-carriers	113	6	Non-carriers or heterozygous for variant(s)	129	1				
Newman, 2010 ⁴⁵	Heterozygous or homozygous for variant(s)	0	35	Homozygous for variant(s)	0	1	94.6 (80.8, 98.6)	99.8 (97.0, 100)	1/1	99.8 (97.6, 100)
	Non-carriers	296	2	Non-carriers or heterozygous for variant(s)	332	0				
Hindorf, 2010 ⁴⁶	Heterozygous or homozygous for variant(s)	2	20	Homozygous for variant(s)	0	0	87.0 (66.5, 95.7)	99.0 (96.2, 99.8)	0/0	99.8 (96.6, 100)
	Non-carriers	204	3	Non-carriers or heterozygous for variant(s)	229	0				

Abbreviations: TPMT = thiopurine methyltransferase.

Key question 2: Pre-testing for TPMT status, and change in management of patients with chronic inflammatory diseases

Table C-7 KQ 2: TPMT status and change in management – characteristics and results of included study

Study	Study Design Study Region Study Setting	Inclusion and exclusion criteria	Thiopurine treatment in group that was genotyped apriori (intervention group) N=167	Thiopurine treatment in group that was not genotyped apriori (control group) N=166	Intervention group description	Control group description Number of participants	Results
Newman, 2010 ⁴⁵	RCT Europe Outpatient specialty clinics at 19 study centers	Inclusion: Adults with chronic inflammatory diseases, mostly IBD, eligible for azathioprine therapy Exclusion: Nursing and pregnant women and those likely to experience adverse events were excluded	AZA Mean (SD) starting dose was 0.92 (0.61) in non-carriers and 0.67 (0.35) in heterozygous carriers. There were no homozygous carriers in this group	AZA Mean (SD) starting dose 0.88 (0.54) in those later found out to be non-carriers and 0.94 (0.68) in heterozygous carriers. There was one homozygous carrier in this group	Therapy was advised to be guided by genotyping results, however, all treatment decisions were at the discretion of treating physicians	Patient management was at the discretion of the treating physician	Outcome of interest (i.e. <i>number of patients requiring dose reduction</i>) was not reported. However, over a 4 month period, genotyping did not significantly alter prescribing practice. Most patients in both groups were given starting doses lower than 2mg/kg/day. 7.3% of those in whom non-carrier genotype was predisclosed received AZA doses ≥ 2 mg/kg/day as compared to 8.4% of those in whom non-carrier genotype was found out after the fact. Furthermore, there was no

							significant difference between the two groups in terms of mean AZA prescribed dose at the end of the study period.
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Table C-8 KQ 2: TPMT status and change in management – risk of bias assessment of included study, part 1

Study	Was the method of randomization appropriate?	Adequacy of allocation concealment?	Were Investigators and participants blinded to interventions?	Was there blinded assessment of outcomes?	Were groups similar at baseline?	Were outcomes described adequately?	Was there intention-to-treat analysis?	Was there a potential for financial conflict of interest?
Newman, 2010 ⁴⁵	Yes	Unclear	No	Not applicable	Yes	Yes	Yes	No

Table C-9 KQ 2: TPMT status and change in management – risk of bias assessment of included study, part 2

Was there a high loss to follow-up or important differential loss to follow-up between groups?	Was treatment adherence assessed?	Reviewers' overall risk of bias assessment for the study	Applicability based on population characteristics and testing methodology
No	Yes	Fair – concealment of allocation is not certain, potential for selection bias	Limited applicability because there was just one homozygous carrier in the whole sample of mostly IBD patients

Key question 3a: TPMT status to guide therapy, clinical outcomes

Table C-10 KQ 3a: TPMT status to guide therapy, clinical outcomes – characteristics and results of included study

Study	Study Design Study Region Study Setting	Inclusion and exclusion criteria	Thiopurine treatment in group that was genotyped apriori (intervention group) N=167	Thiopurine treatment in group that was not genotyped apriori (control group) N=166	Intervention group description	Control group description	Results (n/N)
Newman, 2010 ⁴⁵	RCT Europe Outpatient specialty clinics at 19 study centers	Inclusion: Adults with chronic inflammatory diseases, mostly IBD, eligible for azathioprine therapy Exclusion: Nursing and pregnant women and those likely to experience adverse events were excluded	AZA Mean (SD) starting dose was 0.92 (0.61) in non-carriers and 0.67 (0.35) in heterozygous carriers. There were no homozygous carriers in this group	AZA Mean (SD) starting dose 0.88 (0.54) in those later found out to be non-carriers and 0.94 (0.68) in heterozygous carriers. There was one homozygous carrier in this group	Therapy was advised to be guided by genotyping results, however, all treatment decisions were at the discretion of treating physicians	Patient management was at the discretion of the treating physician	Over a 4 month duration, there were no significant differences in the outcomes of mortality (1/167 vs. 3/166); SAE (4/167 vs. 8/166); and WDAE (0/167 vs. 0/166). Odds ratios of 0.33 (0.03, 3.18), 0.48 (0.14, 1.64) and non-estimable, respectively

Table C-11 KQ 3a: TPMT status to guide therapy, clinical outcomes – Risk of bias assessment of included study, part 1

Study	Was the method of randomization appropriate?	Adequacy of allocation concealment?	Were Investigators and participants blinded to interventions?	Was there blinded assessment of outcomes?	Were groups similar at baseline?	Were outcomes described adequately?	Was there intention-to-treat analysis?	Was there a potential for financial conflict of interest?

Newman, 2010 ⁴⁵	Yes	Unclear	No	Yes for mortality and SAE, no for WDAE and other intermediate outcomes	Yes	Yes	Yes	No
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Table C-12 KQ 3a: TPMT status to guide therapy, clinical outcomes – Risk of bias assessment of included study, part 2

Was there a high loss to follow-up or important differential loss to follow-up between groups?	Was treatment adherence assessed?	Reviewers' overall risk of bias assessment for the study	Applicability based on population characteristics and testing methodology
No	Yes	Fair – Concealment of allocation is not certain, potential for selection bias	Limited applicability because there was just one homozygous carrier in the whole sample of mostly IBD patients

Key Question 3b: TPMT status to guide therapy, intermediate outcomes

Table C-13. KQ 3b: TPMT status to guide therapy, intermediate outcomes – characteristics of included studies

Study	Study Region Study Design Study Setting	Inclusion and exclusion criteria	Thiopurine treatment in group that was pre-tested for TPM status (intervention group)	Thiopurine treatment in group that was not pre-tested (control group)	Intervention group description Number of participants	Control group description Number of participants	Outcomes assessed
Newman, 2010 ⁴⁵	RCT Europe Outpatient specialty clinics at 19 study centers	Inclusion: Adults with chronic inflammatory diseases, mostly IBD, eligible for azathioprine therapy Exclusion: Nursing and pregnant women and those likely to experience adverse events were excluded	AZA Mean (SD) starting dose was 0.92 (0.61) in non-carriers and 0.67 (0.35) in heterozygous carriers. There were no homozygous carriers in this group	AZA Mean (SD) starting dose 0.88 (0.54) in those later found out to be non-carriers and 0.94 (0.68) in heterozygous carriers. There was one homozygous carrier in this group	Therapy was advised to be guided by genotyping results, however, all treatment decisions were at the discretion of treating physicians 167	Patient management was at the discretion of the treating physician 166	A statistically significant difference was not observed for the outcomes neutropenia (2/167 vs. 1/166); hepatitis (19/167 vs. 8/166); and pancreatitis (1/167 vs. 4/166). Odds ratios of 2 (0.18, 22.27); 2.54 (1.08, 5.97); and 0.24 (0.03, 2.21), respectively

Banerjee, 2006⁴⁷	Retrospective cohort North America Tertiary care academic centre	Inclusion: Diagnosis of IBD; pediatric patients (1.7-20 years, mean 14 years)	Mixed thiopurines AZA mean dose 1.7 mg/kg/day; about 10% received 6-MP dose NR Length of followup, 68 weeks	Mixed thiopurines AZA mean dose 1.2 mg/kg/day; about 10% received 6-MP dose NR Length of followup, 108 weeks	TPMT status by enzymatic activity. Normal patients given normal starting dose. Intermediate activity patients given lower starting dose. Additional dose change based on 6-TGN levels. 64	TPMT not tested. Dose titrated based on clinical response or drug toxicity. 7	A statistically significant difference was not observed for the outcome of leukopenia (study versus control group; 4.7 percent versus 0 percent) and hepatotoxicity (study versus control group; 9.4 percent versus 16.2 percent)
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Abbreviations: 6-MP = 6-mercaptopurine; 6-TGN = 6-thioguanine nucleotide; AZA = azathioprine; IBD = inflammatory bowel disease; NR = not reported; TPMT = thiopurine methyltransferase.

Table C-14. KQ 3b: TPMT status to guide therapy – risk of bias assessment of included study, part 1

Study	Blinded participants and investigators	Blinded outcome assessment	Similarity of groups at baseline	Outcomes adequately described	Intention to treat analysis	Potential for financial conflict of interest	High loss to follow-up or differential loss to followup between groups
Newman, 2010⁴⁵	See Table C-10. KQ 3a above						
Banerjee, 2006⁴⁷	Unclear	Unclear	Yes	Yes	Yes	Unlikely	No

Abbreviation: TPMT = thiopurine methyltransferase.

Table C-15. KQ 3b: TPMT status to guide therapy – risk of bias assessment of included study, part 2

Study	Adequate sample size	Avoidance of selection bias	Methods to control confounding	Appropriate TPMT test	Applicability	Summary risk of bias assessment
Banerjee, 2006⁴⁷	Unclear	Unclear	Unclear	Yes	Unclear - Uncertain of representativeness of pediatric IBD patients. Included on those previously treated with a stable thiopurine dose.	Poor - Only intervention group received regular metabolite monitoring with accordingly regular thiopurine adjustment -- an important unaccounted for confounding in this study that limits validity of inference drawn about the comparative effectiveness of testing from this study.

Abbreviations: IBD = inflammatory bowel disease; TPMT = thiopurine methyltransferase

Key Question 3c: Association between TPMT status and thiopurine toxicity

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
Ansari, 2002 ²⁹	NR Outpatient specialty clinic Cross-sectional	Inclusion: Current or past treatment with AZA. Exclusion: Reliable data on clinical response or adverse effects not obtained.	AZA (1.69 (NR)) 5-ASA, steroids	Radioassay	Low <2.5 U/mL RBC Intermediate 2.5-7.5 U/mL RBC High >7.5 U/mL RBC	Neutropenia
Czaja, 2006 ⁴⁸	Europe NR Cross-sectional	Inclusion: Diagnosis of autoimmune hepatitis; current AZA treatment	AZA (50 mg/day (median); (50-150) steroids	Radioassay	Low <6.3 U/mL RBC Intermediate 6.3-15 U/mL RBC Normal 15.1-26.4 U/mL RBC High >26.4 U/mL RBC	Withdrawal due to adverse events

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
Firooz, 2008 ⁴⁹	Middle East and North Africa Outpatient specialty clinic Cross-sectional	Inclusion: Current or past treatment with AZA and prednisolone. Exclusion: Reliable data on clinical response or adverse effects not available; other variants of pemphigus	AZA (NR (2-3)) steroids	High performance liquid chromatography	Low undetectable Intermediate <20 ng/mL/h RBC Normal 20-130 ng/mL/h RBC High >130 ng/mL/h RBC	Anemia, hepatitis, leukopenia, pancreatitis, withdrawal due to adverse events
Gisbert, 2006 ⁵⁰	Europe NR Prospective cohort	Inclusion: Diagnosis of IBD; adult; first time treatment with AZA	AZA (2.3 (SD: 0.5)) 5-ASA, steroids	Radioassay	Low <5 U/mL RBC Intermediate 5-13.7 U/mL RBC High >13.8 U/mL RBC	Hepatitis, myelotoxicity, pancreatitis
Hindorf, 2006 ³²	Europe NR Cross-sectional	Inclusion: Diagnosis of IBD; history of metabolite or TPMT measurements between 1997 and 2003; current or past treatment with thiopurine drugs; not yet started therapy	AZA (NR (NR)) 5-ASA, steroids	Radioassay	Low <2.5 U/mL RBC Intermediate 2.5–8.9 U/mL RBC Normal >=9.0 U/mL RBC	Hepatitis, myelotoxicity, pancreatitis

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
Kader, 2000 ⁵¹	North America NR Cross-sectional	Inclusion: <21 years of age; current or past treatment with 6-MP or AZA	AZA (1.8 (1.1-2.3)); 6-MP (0.9 (0.42-1.11)) NR	NR	Low <5.0 U/mL RBC Intermediate 5.0-13.7 U/mL RBC Normal 13.8-25.1 U/mL RBC	Hepatitis, leukopenia
Lennard, 1989 ⁵²	Europe and North America NR Case-control	Inclusion (cases): diagnosis of autoimmune disorder; current AZA treatment; acute and prolonged immunosuppression during short course (<3 months) of low-dose (<3 mg/kg) AZA. Inclusion (controls): current treatment with <3 mg/kg/day AZA for more than 6 months at unchanging dose; normal renal and hepatic function; recorded white blood cell counts above 5.0 x 10 ⁹ /L. Exclusion: History of myelosuppression.	AZA (Controls 1.45 (median) (1.1-2.0); Cases 1.9 (median) (1-2.6)) steroids	Radioassay	Low <5 U/mL RBC Intermediate 5-10 U/mL RBC Normal >10 U/mL RBC	Anemia, leukopenia, myelotoxicity, thrombocytopenia

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
Okada, 2005 ³⁷	Asia NR Cross-sectional	Inclusion: Diagnosis of SLE; current or past treatment with AZA	AZA (NR (NR)) NR	High performance liquid chromatography	NR	Leukopenia
Schedel, 2006 ⁵³	Europe NR Cohort	Inclusion: Diagnosis of chronic inflammatory disease	AZA (NR (NR)) NR	Radioassay	NR	Leukopenia, myelotoxicity,
Shah, 2008 ⁵⁴	Europe NR Cross-sectional	Inclusion: Diagnosis of IBD	AZA (NR (NR)) 5-ASA	NR	NR	Withdrawal due to adverse events
Snow, 1995 ³⁹	North America NR Cross-sectional	NR	AZA (NR (0.4-2.6)) None	Radioassay	Low <5 U/mL RBC Intermediate 5.0-13.7 U/mL RBC Normal 13.8-25.1 U/mL RBC	Hepatitis, leukopenia
Stassen, 2009 ⁴⁰	NR NR Chart review	NR	AZA (NR (NR)) NR	High performance liquid chromatography	Low <2.0 U/g Hb Intermediate 2-23.5 U/g Hb Normal/high >23.5 U/g Hb	Leukopenia

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
Stocco, 2005 ⁴¹	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; thiopurine treatment for at least 3 months or having experienced an adverse effect during thiopurine treatment	AZA (2.0 (median) (1-4); 6-MP (NR (NR)) 5-ASA	High performance liquid chromatography	Low <4 U/mL RBC Intermediate 4-8 U/mL RBC Normal 8-12 U/mL RBC High >12 U/mL RBC	Hepatitis, myelotoxicity, pancreatitis

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
Stolk, 1998 ⁵⁵	Europe Outpatient specialty clinic Non-randomized intervention study	Inclusion: Diagnosis of RA; 18-75 years of age; active disease with at least 3 of the following features: ≥3 swollen joints, ≥6 joints painful on motion or pressure, morning stiffness ≥45 minutes, and erythrocyte sedimentation rate, ≥28 mm/hour; no prior treatment with AZA. Exclusion: blood transfusion within previous 4 months; hematologic disease or abnormal hematologic parameters; kidney disease; liver disease; malignancy; acute or chronic infection; insulin-dependent diabetes mellitus; pregnancy; alcohol abuse; ACR functional class IV; use of medication that may interact with AZA or affect purine metabolism; history of poor compliance with medication regimens.	AZA (1.45 (0.6-2.9)) None	Radioassay	Low <8.3 pmol/10 ⁶ erythrocytes/h Intermediate 8.3-18.0 pmol/10 ⁶ erythrocytes/h High 18.1-39.4 pmol/10 ⁶ erythrocytes/h	Any infection, hepatitis, myelotoxicity

Table C-16. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study Region Study Setting Study Design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	TPMT activity testing method	TPMT activity range and cut-off values	Outcomes assessed
von Ahsen, 2005 ⁴³	Europe NR Randomized controlled trial	Inclusion: Diagnosis of active Crohn's disease; >18 years of age; prednisone treatment >300 mg during last 4 weeks or a relapse within 6 months after steroid pulse therapy. Exclusion: malignancy; pre-existing renal or hepatic disease; pregnancy or breastfeeding.	AZA (2.5 for first two weeks; subsequently two randomized groups: one remaining on 2.5, the other with 6-TGN concentration dose adjustment) 5-ASA, other	Radioassay	Low < 5 U/mL RBC Intermediate <10 U/mL RBC Normal ≥10 U/mL RBC	Hepatitis, myelotoxicity, pancreatitis, withdrawal due to adverse events
Winter, 2007 ⁴⁴	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; current or past treatment with thiopurine drugs.	AZA (1.6 (median) (NR)) 5-ASA	Mass spectrometry	Low <10 U/g Hb Intermediate 10-25 U/g Hb Normal 26-50 U/g Hb High >50 U/g Hb	Hepatitis, leucopenia, pancreatitis

Abbreviations: 5-ASA = 5-aminosalicylates; 6-MP = 6-mercaptopurine; 6-TGN = 6-thioguanine nucleotide; AZA = azathioprine; IBD = inflammatory bowel disease; NR = not reported; RA = rheumatoid arthritis; RBCs = red blood cells; SD = standard deviation; SLE = systemic lupus erythematosus; TPMT = thiopurine methyltransferase.

Table C-17. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 2

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Duration of observation on treatment mean (range) in months unless otherwise noted
Ansari, 2002 ²⁹	IBD NR	44	46	106	6 (1–108)
Czaja, 2006 ⁴⁸	AIH Adults	44 (SD 2)	71	86	26 (1-180)
Firooz, 2008 ⁴⁹	Pemphigus vulgaris NR	40.8	73.1	138	NR
Gisbert, 2006 ⁵⁰	IBD Adults	43 (SD: 14)	50	394	38.3 (95% CI: 10.3-66.3)
Hindorf, 2006 ³²	IBD Adults	28 (18-42) (median)	47.2	364	18 (6-36) (median)
Kader, 2000 ⁵¹	IBD Mixed	13.7 (6-21)	63.6	22	7.5 (1-24)
Lennard, 1989 ⁵²	Autoimmune disorders Adults	Controls: 52 (20-78) Cases: 55 (38-63)	42.9	21	Controls: 12 Cases: <3
Okada, 2005 ³⁷	SLE Mixed	SLE: 39.8 (14- 76) Healthy volunteers 25.4 (21-57)	NR	18	NR
Schedel, 2006 ⁵³	SLE, RA, IBD, AIH and others Adults	NR	NR	96	NR
Shah, 2008 ⁵⁴	IBD NR	NR	NR	131	19 (6-96)

Table C-17. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – patient and study characteristics, part 2 (continued)

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Duration of observation on treatment mean (range) in months unless otherwise noted
Snow, 1995 ³⁹	Autoimmune dermatologic conditions Adults	NR (26-88)	38.5	26	NR
Stassen, 2009 ⁴⁰	Anti-neutrophil cytoplasmic antibody associated vasculitis NR	NR	NR	108	47
Stocco, 2005 ⁴¹	IBD Mixed	14.2 (0.8-38.8) (median)	51.4	28	19.6 (0.5–85.0)
Stolk, 1998 ⁵⁵	RA Adults	56.2 (33-74)	75	32	6
von Ahsen, 2005 ⁴³	IBD Adults	36 (SD: 11.6)	56.3	71	6
Winter, 2007 ⁴⁴	IBD NR	45	51.4	130	Adverse effects: 1.4 (median) No adverse effects: 30 (median)

Abbreviations: AIH = autoimmune hepatitis; CI = confidence interval; IBD = inflammatory bowel disease; NR = not reported; RA = rheumatoid arthritis; SD = standard deviation; SLE = systemic lupus erythematosus; TPMT = thiopurine methyltransferase.

Table C-18. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – risk of bias assessment, part 1

Study	Unbiased outcomes assessment and phenotyping	Similarity of groups at baseline	Outcomes adequately described	Intention to treat analysis	Potential for financial conflict of interest	High loss to follow-up or differential loss to followup between groups	Adequate sample size
Ansari, 2002 ²⁹	Unclear	Unclear	Yes	Yes	Unlikely	No	Unclear
Czaja, 2006 ⁴⁸	Unclear	Unclear	Yes	Yes	Unlikely	No	Unclear
Firooz, 2008 ⁴⁹	Unclear	Unclear	No	Yes	Unlikely	No	Unclear
Gisbert, 2006 ⁵⁰	Unclear	Unclear	Yes	Yes	Unlikely	No	Unclear
Hindorf, 2006 ³²	Unclear	Not similar	Yes	Unclear	Unlikely	No	Unclear
Kader, 2000 ⁵¹	Unclear	Unclear	Yes	Yes	Unlikely	No	Unclear
Lennard, 1989 ⁵²	Unclear	Unclear	Yes	No	Unlikely	No	Unclear
Okada, 2005 ³⁷	Unclear	Unclear	Yes	Unclear	Unlikely	Unclear	Unclear
Schedel, 2006 ⁵³	Unclear	Unclear	No	Yes	Unlikely	Unclear	Unclear
Shah, 2008 ⁵⁴	Unclear	Unclear	Yes	Yes	Unlikely	Yes	Unclear
Snow, 1995 ³⁹	Unclear	Unclear	Yes	No	Unlikely	No	Unclear
Stassen, 2009 ⁴⁰	Unclear	Unclear	Unclear	Yes	Unlikely	No	Unclear

Table C-18. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – risk of bias assessment, part 1 (continued)

Stocco, 2005⁴¹	Unclear	Unclear	Yes	No	Unlikely	Yes	Unclear
Stolk, 1998⁵⁵	Unclear	Unclear	No	No	Unlikely	No	Unclear
von Ahsen, 2005⁴³	Unclear	Unclear	Yes	Unclear	Likely	Yes	Unclear
Winter, 2007⁴⁴	Unclear	Unclear	No	No	Unlikely	No	Unclear

Abbreviations: TPMT = thiopurine methyltransferase.

Table C-19. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – risk of bias assessment, part 2

Study	Avoidance of selection bias	Methods to control confounding	Appropriateness of TPMT activity test	Applicability	Summary risk of bias assessment
Ansari, 2002 ²⁹	Unclear	No	Yes	Unclear - Uncertain representativeness of patients with IBD.	Fair – Unclear if outcome assessment is biased. Methods to control confounding not described. Avoidance of selection bias is unclear.
Czaja, 2006 ⁴⁸	Unclear	No	Yes	Unclear - Uncertain representativeness of patients with autoimmune hepatitis	Fair - Unclear if outcome assessment is biased. Avoidance of selection bias is unclear. Unclear reliability and suitability of TPMT activity assay.
Firooz, 2008 ⁴⁹	Unclear	No	Yes	Unclear - Uncertain representativeness of pemphigus vulgaris patients. Excluded patients for whom adverse event of clinical data were not available.	Fair - Unclear if outcome assessment is biased. No methods to control confounding. Unclear similarity of groups. Adequacy of sample size is unclear.
Gisbert, 2006 ⁵⁰	Unclear	Unclear	Yes	Unclear - Uncertain representativeness of patients with IBD.	Fair - Unclear if outcome assessment is biased. Methods to control confounding not described. Unclear similarity of groups.
Hindorf, 2006 ³²	Yes	No	Yes	Unclear – Uncertain representativeness of patients with IBD. Excluded patients for whom TPMT metabolite or enzymatic assessment had not been previously performed.	Fair - Unclear if outcome assessment is biased. Potential confounding by age, use of corticosteroids and 5-ASAs.
Kader, 2000 ⁵¹	No	No	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Poor - Unclear reliability and suitability of TPMT activity assay. Uncertain representativeness of pediatric patients. Unclear similarity of groups.

Table C-19. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Avoidance of selection bias	Methods to control confounding	Appropriateness of TPMT activity test	Applicability	Summary risk of bias assessment
Lennard, 1989 ⁵²	No	No	Yes	Unclear - Uncertain representativeness of patients with chronic autoimmune disease.	Poor - Unclear if outcome assessment is biased. Methods to control confounding not described. Avoidance of selection bias is unclear. Unclear similarity of groups. Uncertain representativeness of autoimmune disorder patients.
Okada, 2005 ³⁷	Unclear	No	Yes	Unclear - Uncertain representativeness of Japanese patients with SLE.	Fair - . Avoidance of selection bias is unclear. Small sample size. Unclear if blinded outcome assessment and TPMT activity testing.
Schedel, 2006 ⁵³	Unclear	No	Unclear	Unclear - Uncertain representativeness of patients with chronic autoimmune disease.	Fair - Unclear if outcome assessment is biased. Avoidance of selection bias is unclear. Uncertain representativeness of patients with autoimmune disease. Unclear reliability and suitability of TPMT activity assay.
Shah, 2008 ⁵⁴	Unclear	No	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Poor - No methods to control confounding. Unclear similarity of groups. Unclear reliability and suitability of TPMT activity assay. Adequacy of sample size is unclear. Unclear if blinded outcome assessment and TPMT activity testing.
Snow, 1995 ³⁹	No	No	Yes	Unclear – Uncertain representativeness of patients with autoimmune dermatologic conditions	Poor – Unclear similarity of groups. Small sample size. Unclear if blinded outcome assessment and TPMT activity testing. Methods to control confounding not described. Avoidance of selection bias is unclear.

Table C-19. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Avoidance of selection bias	Methods to control confounding	Appropriateness of TPMT activity test	Applicability	Summary risk of bias assessment
Stassen, 2009 ⁴⁰	Unclear	No	Yes	Unclear – Uncertain representativeness of patients with anti-neutrophil cytoplasmic antibody associated vasculitis	Fair - Unclear if outcome assessment is biased. Unclear similarity of groups. Methods to control confounding not described.
Stocco, 2005 ⁴¹	Unclear	No	Yes	Unclear - Uncertain representativeness of patients with IBD.	Fair - Unclear if outcome assessment is biased. Potential for selection bias.
Stolk, 1998 ⁵⁵	No	Yes	Yes	Unclear - Uncertain representativeness of patients with rheumatoid arthritis. Strict inclusion criteria.	Fair - Unclear if outcome assessment is biased. Avoidance of selection bias is unclear. Small sample size.
von Ahsen, 2005 ⁴³	Unclear	No	Yes	Unclear - Uncertain representativeness of patients with IBD. Excluded those likely to experience adverse events.	Fair – Avoidance of selection bias is unclear. Uncertain representativeness of adult IBD patients. Unclear if blinded outcome assessment and TPMT activity testing. Potential for selective outcome reporting bias.
Winter, 2007 ⁴⁴	Unclear	No	Yes	Unclear - Uncertain representativeness of patients with IBD. Included only those patients with a history of thiopurine use.	Fair - Unclear if outcome assessment is biased. Unclear similarity of groups.

Abbreviations: 5-ASA = 5-aminosalicylates; IBD = inflammatory bowel disease; SLE = systemic lupus erythematosus; TPMT = thiopurine methyltransferase.

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
Ansari, 2002 ²⁹	Neutropenia Neutrophil count <2.0 X 10 ⁹ cells	0	0	1	9	1	95	10.56 (0.61, 183.38)		
Czaja, 2006 ⁴⁸	WDAE Included a range of side effects (thrombocytopenia, leukopenia, pancytopenia, nausea and vomiting, malaise and opportunistic infection) that treating physician deemed severe enough to end treatment	0	1	2	10	9	64	1.42 (0.27, 7.56)	1.4 (0.04, 45.68)	2.26 (0.09, 59.69)
Firooz, 2008 ⁴⁹	WDAE	0	0	1	10	13	114	0.88 (0.1, 7.41)		
	NR									
	Hepatitis >3 fold increase of the upper limit of normal liver enzymes after 10 days	0	0	1	10	10	117	1.17 (0.14, 10.09)		
	Pancreatitis NR	0	0	0	11	1	126	3.67 (0.14, 95.24)		

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data (continued)

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
	Leukopenia	0	0	0	11	1	126	3.67 (0.14, 95.24)		
	NR									
	Anemia	0	0	0	11	1	126	3.67 (0.14, 95.24)		
	NR									
Gisbert, 2006 ⁵⁰	Pancreatitis	0	0	0	28	11	355	0.54 (0.03, 9.44)		
	Abdominal pain present and serum amylase >3 times upper normal limit									
	Myelotoxicity	0	0	4	24	13	353	4.53 (1.37, 14.94)		
	NR									
Hindorf, 2006 ³²	Hepatitis	0	6	4	41	15	298	1.94 (0.61, 6.12)	0.71 (0.03, 14.78)	1.48 (0.08, 27.51)
	Included all types of hepatotoxic reactions									
	Pancreatitis	0	6	2	43	13	300	1.07 (0.23, 4.92)	1.34 (0.06, 31.12)	1.71 (0.09, 31.99)
	NR									

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data (continued)

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
	Myelotoxicity Included all types of haematological toxicity (anemia, leukopenia, neutropenia, thrombocytopenia)	3	3	3	42	22	291	0.94 (0.27, 3.29)	14 (1.93, 101.72)	13.23 (2.52, 69.43)
Kader, 2000 ⁵¹	Leukopenia WBC <4000/mm ³	0	0	1	1	1	19	19 (0.62, 583.42)		
	Hepatitis Aminotransferases >2 times normal	0	0	1	1	2	18	9 (0.39, 206.54)		
Lennard, 1989 ⁵²	Leukopenia NR	4	1	0	5	0	11		33 (1.06, 1023.62)	69 (2.35, 2028.86)
	Thrombocytopenia NR	3	2	0	5	0	11		15.4 (0.56, 425.55)	32.2 (1.23, 841.87)
	Anemia NR	4	1	0	5	0	11		33 (1.06, 1023.62)	69 (2.35, 2028.86)
	Myelotoxicity NR	3	2	0	5	0	11		15.4 (0.56, 425.55)	32.2 (1.23, 841.87)
Okada, 2005 ³⁷	Leukopenia WBC <2300/mm ³	0	0	1	2	2	13	3.25 (0.19, 54.78)		

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data (continued)

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
Schedel, 2006 ⁵³	Myelotoxicity	0	3	1	27	1	64	2.37 (0.14, 39.3)	2.62 (0.09, 77.58)	6.14 (0.21, 179.81)
	Pancytopenia (not defined)									
	Leukopenia	2	1	2	26	0	65	12.36 (0.57, 266.16)	26 (1.58, 426.87)	218.33 (6.99, 6815.05)
	NR									
Shah, 2008 ⁵⁴	WDAE	NR	NR	1	11	18	101	0.51 (0.06, 4.2)		
	NR									
Snow, 1995 ³⁹	Hepatitis	0	0	0	5	1	20	1.24 (0.04, 34.93)		
	Raised aspartate transaminase and alanine transaminase									
	Leukopenia	0	0	2	3	1	20	13.33 (0.91, 196.38)		
	NR									
Stassen, 2009 ⁴⁰	Leukopenia	0	0	3	4	34	67	1.48 (0.31, 6.98)		
	NR									
	Thrombocytopenia	0	0	0	7	8	93	0.73 (0.04, 13.98)		
	NR									
	Anemia	0	0	3	4	30	71	1.78 (0.37, 8.42)		
	NR									

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data (continued)

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
Stocco, 2005 ⁴¹	Hepatitis Alanine aminotransferase, gamma-glutamyl transferase or alkaline phosphatase >2 times normal levels	0	0	0	3	0	25			
	Pancreatitis Severe abdominal pain accompanied by serum amylase level >2 times normal levels	0	0	0	3	1	24	2.33 (0.08, 69.29)		
	Myelotoxicity Leukopenia (WBC <3000/mm ³) and/or thrombocytopenia (platelets <100,000/mm ³)	0	0	0	3	3	22	0.92 (0.04, 21.86)		
Stolk, 1998 ⁵⁵	Any infection	0	0	1	7	0	24	9.8 (0.36, 266.64)		
	Upper airway infection									
	Hepatitis	0	0	1	7	1	23	3.29 (0.18, 59.6)		
	NR									
	Myelotoxicity	0	0	0	8	1	23	0.92 (0.03, 24.87)		
	Pancytopenia									

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data (continued)

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
von Ahsen, 2005 ⁴³	WDAE	0	0	14	7	18	32	3.56 (1.21, 10.42)		
	Withdrawal from study for whatever reason									
	Hepatitis	0	0	1	20	2	48	1.2 (0.1, 14)		
	Aspartate or alanine aminotransferase ≥ 2 times upper limit of reference interval									
	Pancreatitis	0	0	1	20	1	49	2.45 (0.15, 41.11)		
	Upper abdominal pain accompanied by pancreatic amylase or lipase ≥ 2 times upper limit of reference interval									
	Myelotoxicity	0	0	0	21	0	40			
Winter, 2007 ⁴⁴	Myelosuppression (leukocyte count $< 2.5 \times 10^9/L$ or platelet count $< 100 \times 10^9/L$)									
	Hepatitis	0	1	1	15	8	105	0.88 (0.1, 7.5)	3.44 (0.09, 127.7)	4.14 (0.16, 109.52)
	Deranged liver function tests									

Table C-20. KQ 3c: Association between TPMT enzymatic activity and thiopurine toxicity – summary data (continued)

Study	Outcomes and definition	Low activity		Intermediate activity		Normal activity		Intermediate vs. normal	Low vs. intermediate	Low vs. normal
		n with events	n without events	n with events	n without events	n with events	n without events	Odds ratio (95% CI)		
	Pancreatitis NR	0	1	1	15	0	113	21.97 (0.86, 563.38)	3.44 (0.09, 127.7)	
	Leukopenia Mild leukopenia (WBC 2-3 x10 ⁹ /L) and severe leukopenia (WBC < 2 x10 ⁹ /L)	1	0	1	15	8	105	0.88 (0.1, 7.5)	31 (0.84, 1149.3)	37.24 (1.41, 985.68)

Abbreviations: CI = confidence interval; n = number; NR = not reported; WBC = white blood cell count; WDAE = withdrawal due to adverse events

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Ansari, 2008 ²⁸	Europe NR Non-randomized intervention study	Inclusion: Diagnosis of IBD; 18-80 years of age. Exclusion: Past thiopurine treatment; history of use of biologics; very low TPMT activity (<10 pmol/h/mg Hb).	AZA (2.0 (1.9-2.4) (median)) 5-ASA, steroids	NR	TPMT*3A, *3B, *3C	Hepatitis, leukopenia, pancreatitis, withdrawal due to adverse events
Bezier, 2008 ⁵⁶	Europe Inpatient Cross-sectional	Inclusion: Diagnosis of bullous pemphigoid, cicatricial pemphigoid, pemphigus or epidermolysis bullosa acquista; hospitalized in a Dermatology department; history of TPMT genotyping. Exclusion: IgA bullous dermatosis linear; dermatosis herpetiformis; pemphigoid gestationis.	AZA (2.2 (NR)) steroids, others	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Anemia, any infection, leukopenia, neutropenia, withdrawal due to adverse events
Black, 1998 ⁵⁷	Europe Outpatient specialty clinic Prospective cohort	Inclusion: Current or past AZA treatment	AZA (NR (2-3)) NSAIDs	Polymerase chain reaction	TPMT*2, *3A	Hepatitis, leukopenia

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Corominas, 2003 ⁵⁸	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of rheumatoid arthritis; current treatment with low-dose AZA	AZA (NR (0.5-1.5)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C, *7, *8	Withdrawal due to adverse events
De Ridder, 2006 ⁵⁹	Europe NR Cross-sectional	Inclusion: Diagnosis of pediatric-onset IBD; current or past AZA treatment of at least 3 months unless stopped due to adverse effects; <19 years of age	AZA (NR (2-2.5)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Hepatitis, leukopenia, pancreatitis

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Derijks, 2004 ⁶⁰	Europe Outpatient specialty clinic Non-randomized intervention study	Inclusion: Diagnosis of IBD; 18-75 years of age; 6-MP indicated due to steroid tolerance, steroid resistance, or AZA intolerance. Exclusion: Pregnancy or expected pregnancy within 6 months; inadequate contraception in women; lactation; active infection; history of tuberculosis; HIV; hepatitis B or C; severe pancreatitis; malignancy; current treatment with other immunosuppressive drugs; impaired renal function; elevated liver function tests; bone marrow suppression.	6-MP (50 mg/day (NR)) 5-ASA	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Hepatitis, leukopenia, pancreatitis
Dubinsky, 2000 ⁶¹	North America Outpatient specialty clinic Prospective cohort	Exclusion: Leukopenia; increased serum hepatic or pancreatic enzyme activity	AZA (NR (NR)), 6-MP (1.25 (0.4-2.4)) 5-ASA	Polymerase chain reaction	TPMT*3A, *3B, *3C	Leukopenia

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Gearry, 2004 ⁶²	Oceania Outpatient specialty clinic Case-control	Inclusion: Past treatment with thiopurine drugs	Mixed thiopurines NR	Polymerase chain reaction	TPMT*1, *2, *3A, *3C	Hepatitis, leukopenia, pancreatitis
Hibi, 2003 ⁶³	Asia Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; past AZA or 6-MP treatment	AZA (NR (25-100 mg/day)), 6-MP (NR (30-50 mg/day)) NR	Polymerase chain reaction	TPMT*2, *3B, *3A, *3C	Leukopenia
Hindorf, 2006 ³²	Europe NR Cross-sectional	Inclusion: Diagnosis of IBD; history of metabolite or TPMT measurements; current or past treatment with thiopurine drugs. Exclusion: Patients who had not yet started therapy	AZA (NR (NR)) 5-ASA, steroids	Pyrosequencing	TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, *15	Hepatitis, myelotoxicity, pancreatitis
Ishioka, 1999 ⁶⁴	Asia NR Unclear if prospective or retrospective design	Included: Diagnosis of a rheumatic disease; past AZA treatment; Japanese	AZA (50 mg/day (NR)) NR	Polymerase chain reaction	TPMT*1, *2, *3A, *3B, *3C	Leukopenia

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Jae Hak, 2008 ⁶⁵	Asia Outpatient specialty clinic Case-control	Inclusion: Diagnosis of IBD; current or past thiopurine treatment for at least 6 months	AZA (NR (NR)), 6-MP (NR (NR)) NR	High performance liquid chromatography	TPMT*1, *2, *3A, *3B, *3C	Leukopenia
Joji, 2003 ⁶⁶	Europe NR Prospective cohort	NR	AZA (NR (NR)) 5-ASA	Polymerase chain reaction	TPMT*1, *2, *3A, *3B, *3C	Leukopenia, myelotoxicity
Jun, 2005 ⁶⁷	Asia NR Cross-sectional	Inclusion: Diagnosis of SLE; Korean	AZA (22.1 mg/day (NR)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C, *3D, *6	Hepatitis, leukopenia
Lopez, 2006 ⁶⁸	NR NR Case-control	NR	AZA (NR (NR)) NR	NR	TPMT*2, *3A, *3B, *3C, *3D	Pancreatitis
Marinaki, 2004 ⁶⁹	Europe Outpatient specialty clinic Case-control	Cases: Diagnosis of IBD; Caucasian; history of AZA-related adverse events. Controls: Caucasian; past AZA treatment for at least 3 months without adverse events.	AZA (Cases 1.81 (0.39-2.59) Controls 1.92 (0.91-3.26)) 5-ASA, steroids, others	Polymerase chain reaction	TPMT*2, *3A, *3C	Hepatitis, neutropenia, pancreatitis

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Okada, 2005 ³⁷	Asia NR Cross-sectional	Inclusion: Diagnosis of SLE	AZA (NR (NR)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Leukopenia
Palmieri, 2007 ⁷⁰	Europe NR Prospective cohort	Inclusion: Diagnosis of IBD; 21-58 years; current AZA or 6-MP treatment. Exclusion: Past AZA or 6-MP treatment of less than 6 months; no history of adverse events.	AZA (NR (2-2.5)), 6-MP (NR (1-1.25)) 5-ASA	Polymerase chain reaction	TPMT*3A, *3B, *3C	Hepatitis, leukopenia, pancreatitis
Reuther, 2003 ⁷¹	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of Crohn's disease; current AZA maintenance treatment; no history of adverse effects	AZA (1.57 (0.58-2.24)) 5-ASA, steroids	Polymerase chain reaction	TPMT*3A, *3B, *3C	Hepatitis, leukopenia
Schmeling, 2007 ⁷²	NR NR Cross-sectional	Inclusion: Diagnosis of SLE; current AZA treatment; pediatric	AZA (NR (NR)) NR	NR	TPMT*2, *3A, *3B, *3C	Hepatitis, leukopenia

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Seddik, 2003 ⁷³	NR NR Prospective cohort	NR	AZA (NR (100-200mg/day)), 6-MP (NR (50-100mg/day)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Leukopenia
Snow, 1995 ³⁹	North America NR Cross-sectional	NR	AZA (NR (0.4-2.6)) NR	NR	NR	Hepatitis, leukopenia
Stassen, 2009 ⁴⁰	NR NR Chart review	NR	AZA (NR (NR)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Anemia, leukopenia, thrombocytopenia
Stocco, 2007 ⁷⁴	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; current AZA treatment	AZA (2 (1-5)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Any infection, hepatitis, myelotoxicity, pancreatitis

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Stocco, 2005 ⁴¹	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; past thiopurine treatment for at least 3 months or having experienced an adverse effect during thiopurine treatment	AZA (2.0 (1-4) (median)); 6-MP (NR (NR)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Hepatitis, myelotoxicity, pancreatitis
Stocco, 2004 ⁴²	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; current AZA treatment	AZA (2 (1-3) (median)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Hepatitis, leukopenia, pancreatitis, thrombocytopenia
Tamori, 2007 ⁷⁵	Asia NR Cross-sectional	Inclusion: Diagnosis of AIH; Japanese	AZA (50 (NR)) NR	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Myelotoxicity
Tani, 2009 ⁷⁶	Europe NR Cross-sectional	Included: Diagnosis of rheumatic disease; current AZA treatment; Italian; Caucasian	AZA (1.42 (0.5-2)) 5-ASA, steroids, others	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Any infection, leukopenia, thrombocytopenia, withdrawal due to adverse events

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
van Dieren, 2005 ⁷⁷	NR NR Prospective cohort	Inclusion: Diagnosis of IBD; first AZA treatment between January 2003 and November 2004	AZA (NR (NR)) NR	Polymerase chain reaction	NR	Hepatitis, leukopenia, thrombocytopenia
von Ahsen, 2005 ⁴³	Europe NR Randomized controlled trial	Inclusion: Diagnosis of active Crohn's disease; >18 years of age; prednisone treatment >300 mg during last 4 weeks or relapse within 6 months after steroid pulse therapy. Exclusion: History of cancer; preexisting renal or hepatic disease; pregnant; breast feeding.	AZA (2.5 for first two weeks; subsequently two randomized groups: one remaining on 2.5, the other with 6-TGN concentration dose adjustment) 5-ASA, others	Polymerase chain reaction	TPMT*2, *3A, *3B, *3C	Hepatitis, myelotoxicity, pancreatitis
Winter, 2007 ⁴⁴	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; current or past treatment with thiopurine drugs	AZA (1.6 (NR) (median)) 5-ASA	Polymerase chain reaction	TPMT*2, *3A, *3B, * 3C	Hepatitis, leukopenia, pancreatitis,

Table C-21. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 1 (continued)

Study	Study region Study setting Study design	Inclusion and exclusion criteria	Thiopurine treatment dose (mean (range) in mg/kg/day unless otherwise noted) Concomitant treatments	Genotyping method	SNPs genotyped	Outcomes assessed
Zelinkova, 2006 ⁷⁸	Europe Outpatient specialty clinic Cross-sectional	Inclusion: Diagnosis of IBD; past AZA treatment; reliable data available on AZA use and related side effects	AZA (132 (50-250) mg/day) 5-ASA, steroids	Polymerase chain reaction	TPMT*1, *2, *3A, *3B, *3C	Hepatitis, leukopenia
Newman, 2010 ⁴⁵	Europe Outpatient specialty clinic Prospective Cohort	Nursing and pregnant women and those likely to experience adverse events were excluded	AZA (on average approx. 1mg/kg/day)	Polymerase chain reaction	TPMT *2, *3A, *3B, *3C	Mortality, WDAE, SAE, hepatitis, pancreatitis, and neutropenia
Kolorz 2009 ⁷⁹	Europe NR Cross-sectional	NR	AZA, Dosage NR	Polymerase chain reaction	TPMT *2, *3A, *3B, *3C	Leukopenia

Abbreviations: 5-ASA = 5-aminosalicylates; 6-MP = 6-mercaptopurine; AIH = autoimmune hepatitis; AZA = azathioprine; HIV = human immunodeficiency virus; IBD = inflammatory bowel disease; NR = not reported; NSAID = Non-steroidal anti-inflammatory drug; SLE = systemic lupus erythematosus; SNP = single nucleotide polymorphism; TPMT = thiopurine methyltransferase; WDAE = withdrawal due to adverse events; SAE = serious adverse events;

Table C-22. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 2

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Duration of observation on treatment mean (range) in months unless otherwise noted
Ansari, 2002 ²⁹	IBD NR	44	46	106	6 (1–108)
Czaja, 2006 ⁴⁸	AIH Adults	44 (SD 2)	71	86	26 (1-180)
Firooz, 2008 ⁴⁹	Pemphigus vulgaris NR	40.8	73.1	138	NR
Gisbert, 2006 ⁵⁰	IBD Adults	43 (SD: 14)	50	394	38.3 (95% CI: 10.3-66.3)
Hindorf, 2006 ³²	IBD Adults	28 (18-42) (median)	47.2	364	18 (6-36) (median)
Kader, 2000 ⁵¹	IBD Mixed	13.7 (6-21)	63.6	22	7.5 (1-24)
Lennard, 1989 ⁵²	Autoimmune disorders Adults	Controls: 52 (20-78) Cases: 55 (38-63)	42.9	21	Controls: 12 Cases: <3
Okada, 2005 ³⁷	SLE Mixed	SLE: 39.8 (14- 76) Healthy volunteers 25.4 (21-57)	NR	18	NR
Schedel, 2006 ⁵³	SLE, RA, IBD, AIH and others Adults	NR	NR	96	NR
Shah, 2008 ⁵⁴	IBD NR	NR	NR	131	19 (6-96)

Table C-22. KQ 3c: Association between TPMT genotype and thiopurine toxicity – patient and study characteristics, part 2 (continued)

Study	Underlying disease(s) Age group	Age (years) mean (range) (unless otherwise noted)	Females (%)	Number analyzed	Duration of observation on treatment mean (range) in months unless otherwise noted
Snow, 1995 ³⁹	Autoimmune dermatologic conditions Adults	NR (26-88)	38.5	26	NR
Stassen, 2009 ⁴⁰	Anti-neutrophil cytoplasmic antibody associated vasculitis NR	NR	NR	108	47
Stocco, 2005 ⁴¹	IBD Mixed	14.2 (0.8-38.8) (median)	51.4	28	19.6 (0.5–85.0)
Stolk, 1998 ⁵⁵	RA Adults	56.2 (33-74)	75	32	6
von Ahsen, 2005 ⁴³	IBD Adults	36 (SD: 11.6)	56.3	71	6
Winter, 2007 ⁴⁴	IBD NR	45	51.4	130	Adverse effects: 1.4 (median) No adverse effects: 30 (median)
Newman, 2010 ⁴⁵	Majority IBD patients	43	50.6	166	4
Kolorz 2009 ⁷⁹	IBD	35	41	87	NR

Abbreviations: AIH = autoimmune hepatitis; CI = confidence interval; IBD = inflammatory bowel disease; NR = not reported; RA = rheumatoid arthritis; SD = standard deviation; SLE = systemic lupus erythematosus; TPMT = thiopurine methyltransferase.

Table C-23. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 1

Study	Comparable genotype groups	Participants enrolled without prior knowledge of genotype	Unbiased outcome and genotype assessment	Clear genotyping method description	Reliable genotyping method	Reported ambiguous results due to genotyping error	HWE tested
Ansari, 2008 ²⁸	Unclear	Yes	Yes	No	Unclear	No	Yes
Bezier, 2008 ⁵⁶	Unclear	Yes	Unclear	Yes	Yes	No	No
Black, 1998 ⁵⁷	Unclear	Yes	Yes	Yes	Yes	Unclear	No
Corominas, 2003 ⁵⁸	Unclear	Yes	Unclear	Yes	Unclear	Unclear	No
De Ridder, 2006 ⁵⁹	Unclear	Yes	Unclear	No	No	No	No
Derijks, 2004 ⁶⁰	Unclear	Yes	Unclear	Yes	Yes	Yes	No
Dubinsky, 2000 ⁶¹	Unclear	Yes	Yes	Yes	Unclear	No	No
Gearry, 2004 ⁶²	Unclear	Yes	Unclear	Yes	Unclear	No	No
Hibi, 2003 ⁶³	Unclear	Yes	Unclear	Yes	Yes	No	No
Hindorf, 2006 ³²	Unclear	Unclear	Unclear	Unclear	Unclear	No	No
Ishioka, 1999 ⁶⁴	Unclear	Unclear	Unclear	Yes	No	No	No
Jae Hak, 2008 ⁶⁵	Unclear	Unclear	Unclear	No	Unclear	No	No

Table C-23. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 1 (continued)

Study	Comparable genotype groups	Participants enrolled without prior knowledge of genotype	Unbiased outcome and genotype assessment	Clear genotyping method description	Reliable genotyping method	Reported ambiguous results due to genotyping error	HWE tested
Joji, 2003 ⁶⁶	Unclear	Yes	Unclear	Yes	Unclear	No	No
Jun, 2005 ⁶⁷	Unclear	Yes	Unclear	Yes	Unclear	No	No
Lopez, 2006 ⁶⁸	Unclear	Unclear	Unclear	No	No	No	No
Marinaki, 2004 ⁶⁹	Unclear	Yes	Unclear	No	No	No	No
Okada, 2005 ³⁷	Unclear	Yes	Unclear	Yes	Yes	No	No
Palmieri, 2007 ⁷⁰	Yes	Yes	Unclear	Yes	Unclear	No	Yes
Reuther, 2003 ⁷¹	Unclear	Yes	Unclear	No	No	Yes	No
Schmeling, 2007 ⁷²	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Seddik, 2003 ⁷³	Unclear	Unclear	Unclear	No	Unclear	No	No
Snow, 1995 ³⁹	Unclear	Unclear	Unclear	No	Unclear	No	No
Stassen, 2009 ⁴⁰	Unclear	Yes	Unclear	Yes	Unclear	No	No
Stocco, 2007 ⁷⁴	Unclear	Yes	Unclear	Yes	No	No	No
Stocco, 2005 ⁴¹	Unclear	Yes	Unclear	No	Unclear	No	No

Table C-23. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 1 (continued)

Study	Comparable genotype groups	Participants enrolled without prior knowledge of genotype	Unbiased outcome and genotype assessment	Clear genotyping method description	Reliable genotyping method	Reported ambiguous results due to genotyping error	HWE tested
Stocco, 2004 ⁴²	Unclear	Yes	Yes	Yes	Unclear	Unclear	No
Tamori, 2007 ⁷⁵	Unclear	Yes	Unclear	Yes	Unclear	Unclear	No
Tani, 2009 ⁷⁶	Unclear	Yes	Unclear	Yes	No	No	No
van Dieren, 2005 ⁷⁷	Unclear	Yes	Unclear	No	Unclear	No	No
von Ahsen, 2005 ⁴³	Unclear	Yes	Unclear	Yes	Yes	No	No
Winter, 2007 ⁴⁴	Unclear	Yes	Unclear	Yes	Unclear	No	No
Zelinkova, 2006 ⁷⁸	Unclear	Yes	Unclear	Yes	Unclear	No	No
Newman, 2010 ⁴⁵	Unclear	Yes	Yes	Yes	Unclear	No	No
Kolorz 2009 ⁷⁹	Unclear	Yes	Unclear	Yes	Unclear	No	No

Abbreviations: HWE = Hardy-Weinberg equilibrium; TPMT = thiopurine methyltransferase.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Ansari, 2008²⁸	No	No	Yes	Yes	No	No	Applicable	Fair - Methods to control confounding not described. Genotyping method unclear.
Bezier, 2008⁵⁶	No	No	Yes	Yes	No	No	Unclear – Uncertain representativeness of patients with autoimmune bullous diseases. Included only hospitalized severe cases.	Fair – HWE not reported. Compliance not clear. Unclear similarity of groups. Unclear if blinded outcome or genotype assessment.
Black, 1998⁵⁷	No	Yes	Yes	Yes	No	Unclear	Unclear - Uncertain representativeness of patients with several rheumatic diseases.	Fair – HWE not reported.
Corominas, 2003⁵⁸	Unclear	No	Yes	Yes	Unclear	Unclear	Applicable	Fair - HWE not reported. Methods to control confounding not described. Unclear if blinded outcome or genotype assessment.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
De Ridder, 2006 ⁵⁹	No	No	Yes	No	No	No	Unclear - Uncertain representativeness of pediatric patients with IBD.	Fair - Avoidance of selection bias is unclear. Unclear if blinded outcome or genotype assessment. Inadequate description of genotyping method. Compliance not clear.
Derijks, 2004 ⁶⁰	No	No	Yes	No	No	Unclear	Unclear - Uncertain representativeness of patients with IBD. Strict eligibility criteria.	Fair – Potential selection bias. Unclear if blinded outcome or genotype assessment.
Dubinsky, 2000 ⁶¹	No	No	Yes	Yes	No	Yes	Not applicable – Not representative of children with IBD. Excluded children with leukopenia and increased hepatic or pancreatic enzymes before treatment initiation.	Fair – HWE not reported. Unclear similarity of groups. Unclear reliability of genotyping method.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Gearry, 2004 ⁶²	No	No	Yes	No	No	No	Unclear - Uncertain representativeness of patients with IBD.	Fair – Avoidance of selection bias is unclear. Unclear similarity of groups. Unclear if blinded outcome or genotype assessment.
Hibi, 2003 ⁶³	No	No	Yes	Yes	No	No	Unclear - Uncertain representativeness of patients with IBD.	Fair - Potential selection bias. Unclear if blinded outcome or genotype assessment.
Hindorf, 2006 ³²	Unclear	No	Unclear	Yes	Unclear	No	Unclear - Unclear representativeness of patients with IBD population. Included only patients with history of TPMT metabolite or enzymatic assay testing.	Fair - Inadequate description of genotyping method. Unclear reliability of genotyping method. Unclear similarity of groups. Methods to control confounding not described.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Ishioka, 1999 ⁶⁴	No	No	Yes	Yes	No	No	Unclear – Unclear representativeness of Japanese patients with rheumatic diseases.	Fair - HWE not reported. Unclear similarity of groups. Unclear if blinded outcome or genotype assessment. Unclear reliability of genotyping method.
Jae Hak, 2008 ⁶⁵	No	No	Unclear	No	Unclear	No	Unclear - Uncertain representativeness of patients with IBD.	Fair - HWE not reported. Unclear if blinded outcome or genotype assessment. Unclear similarity of groups.
Joji, 2003 ⁶⁶	No	No	Yes	No	No	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Fair - HWE not reported. Unclear if blinded outcome or genotype assessment. Unclear similarity of groups.
Jun, 2005 ⁶⁷	No	No	Yes	No	No	No	Unclear - Unclear representativeness of Korean patients with SLE.	Fair – Avoidance of selection bias is unclear. Unclear if blinded outcome or genotype assessment.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Lopez, 2006 ⁶⁸	No	No	Unclear	No	Unclear	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Poor - Poor reporting of quality items (only abstract available)
Marinaki, 2004 ⁶⁹	Yes	No	Yes	No	No	No	Unclear - Uncertain representativeness of patients with IBD.	Fair - Avoidance of selection bias is unclear. Unclear if blinded outcome or genotype assessment. Inadequate description of genotyping method.
Okada, 2005 ³⁷	No	No	Yes	No	Unclear	Unclear	Unclear - Uncertain representativeness of Japanese patients with SLE.	Fair - Avoidance of selection bias is unclear. Small sample size. Unclear if blinded outcome or genotype assessment.
Palmieri, 2007 ⁷⁰	No	No	Yes	No	No	No	Applicable	Fair - Compliance not clear. Unclear if blinded outcome or genotype assessment. Unclear reliability of genotyping method.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Reuther, 2003 ⁷¹	No	No	Yes	Yes	No	No	Unclear - Uncertain representativeness of patients with Crohn's disease.	Fair - Avoidance of selection bias is unclear. Inadequate description of genotyping method.
Schmeling, 2007 ⁷²	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Poor - Poor reporting of risk of bias items (only abstract available)
Seddik, 2003 ⁷³	No	No	Unclear	No	Unclear	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Poor - Poor reporting of risk of bias items (only abstract available)
Snow, 1995 ³⁹	No	No	Unclear	Yes	No	Unclear	Unclear - Uncertain representativeness of patients with autoimmune dermatologic conditions.	Poor - Unclear if blinded outcome or genotype assessment. Inadequate description of genotyping method. Unclear reliability of genotyping method. HWE not reported.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Stassen, 2009 ⁴⁰	No	Unclear	Yes	No	Unclear	No	Unclear – Unclear representativeness of patients with anti-neutrophil cytoplasmic antibody associated vasculitis	Fair - HWE not reported. Unclear if blinded outcome or genotype assessment. Unclear similarity of groups. Unclear if genotype was determined prospectively or retrospectively.
Stocco, 2007 ⁷⁴	No	No	Yes	No	No	No	Unclear - Uncertain representativeness of patients with IBD.	Fair – Unclear if blinded outcome or genotype assessment. Inadequate description of genotyping method. Unclear reliability of genotyping method. HWE not reported. Incomplete outcomes data.
Stocco, 2005 ⁴¹	No	No	Yes	No	Unclear	No	Unclear - Uncertain representativeness of patients with IBD.	Fair – Potential selection bias.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Stocco, 2004 ⁴²	Unclear	Unclear	Yes	Yes	Unclear	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Fair - HWE not reported. Compliance not clear. Avoidance of selection bias not clear.
Tamori, 2007 ⁷⁵	No	Unclear	Yes	Unclear	Unclear	No	Unclear – Unclear representativeness of Japanese patients with autoimmune hepatitis. Small sample size.	Fair - HWE not reported. Unclear if blinded outcome or genotype assessment.
Tani, 2009 ⁷⁶	No	No	Yes	Yes	No	No	Unclear – Unclear representativeness of patients with rheumatic diseases. Included only those in a tertiary care setting.	Fair - HWE not reported. Unclear if blinded outcome or genotype assessment. Unclear similarity of groups. Unclear if genotype was determined prospectively or retrospectively.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
van Dieren, 2005 ⁷⁷	No	Unclear	Unclear	No	Unclear	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Fair - Poor reporting of risk of bias items (only abstract available). Unclear if blinded outcome or genotype assessment. Inadequate description of genotyping method. Unclear reliability of genotyping method. Avoidance of selection bias is not clear.
von Ahsen, 2005 ⁴³	No	Unclear	Unclear	Yes	Unclear	Yes	Unclear - Uncertain representativeness of patients with IBD. Excluded those likely to experience adverse event.	Fair – Avoidance of selection bias is unclear. Unclear if blinded outcome or genotype assessment. Potential outcome reporting bias.
Winter, 2007 ⁴⁴	No	No	Yes	Yes	No	No	Unclear - Uncertain representativeness of patients with IBD. Included only those who had previously received thiopurines.	Fair - Unclear similarity of groups. Methods to control confounding not described.

Table C-24. KQ 3c: Association between TPMT genotype and thiopurine toxicity – risk of bias assessment, part 2 (continued)

Study	Assessed gene-gene interaction	Assessed compliance with thiopurine treatment (and corrected differences as required)	Consistent DNA source for all patients	Reported loss to followup	Potential for survival bias	Potential for financial conflict of interest	Applicability	Summary risk of bias assessment
Zelinkova, 2006 ⁷⁸	No	No	Yes	No	Unclear	Unclear	Unclear - Uncertain representativeness of patients with IBD.	Fair - Unclear if blinded outcome or genotype assessment. Avoidance of selection bias is unclear.
Newman, 2010 ⁴⁵	No	Yes	Yes	Yes	Unclear	No	Unclear -- excluded those likely to experience adverse event and no description provided of those who did not agree to participate	Good. The main study was an RCT.
Kolorz 2009 ⁷⁹	No	No	Yes	No	Unclear	No	Unclear -- little information is provided regarding sample selection and those who were included versus not	Fair – unclear blinded assessment of outcomes and comparability of groups

Abbreviations: DNA = deoxyribonucleic acid; HWE = Hardy-Weinberg equilibrium; IBD = inflammatory bowel disease; SLE = systemic lupus erythematosus; TPMT = thiopurine methyltransferase.

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Ansari, 2008 ²⁸	TPMT *3A, *3B, *3C	WDAE	66	122	NR	NR	15	4		6.93 (2.21, 21.74)	
		NA									
		Hepatitis	8	180	NR	NR	0	19		0.54 (0.03, 9.8)	
		Pancreatitis	8	180	NR	NR	0	19		0.54 (0.03, 9.8)	
		Leukopenia	2	186	NR	NR	5	14		33.21 (5.9, 186.88)	
Hindorf, 2006 ³²	TPMT *2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8, *10, *14, *15	WBC < 3500/mm3 or neutrophils < 1500/mm3									
		Hepatitis	15	25	4	2	0	6	3.33 (0.54, 20.45)	0.13 (0.01, 2.41)	23.4 (0.89, 613.02)
		All types of hepatotoxic reactions									
		Pancreatitis	13	27	2	4	0	6	1.04 (0.17, 6.42)	0.16 (0.01, 2.99)	7.22 (0.28, 189.2)
		NR									

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Myelotoxicity Included all types of haematological toxicity (anaemia, leucopenia, neutropenia, thrombocytopenia)	22	18	3	3	3	3	0.82 (0.15, 4.56)	0.82 (0.15, 4.56)	1 (0.1, 9.61)
Winter, 2007 ⁴⁴	TPMT *2, *3A, *3B, *3C	Hepatitis	8	111	0	0	1	10		1.39 (0.16, 12.24)	
		deranged liver function tests									
		Pancreatitis	1	118	0	0	0	11		3.43 (0.13, 89.24)	
		NR									
		Leukopenia WBC < 3000/mm ³	9	110	0	0	1	10		1.22 (0.14, 10.65)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
De Ridder, 2006 ⁵⁹	TPMT*2, *3A, *3B, *3C	Hepatitis Hepatotoxicity by serum alanine transaminase levels greater than twice the upper normal limit (50 IU/L) and resolution after withdrawal of AZA	0	67	0	3	0	2			
		Pancreatitis Severe abdominal pain and hyperamylasemia and resolution after withdrawal of AZA	4	63	0	3	0	2	2.02 (0.09, 45.36)	2.82 (0.12, 68.1)	
		Leukopenia Leucocyte count < 2.5x10 ⁹ cells	1	66	0	3	1	1	6.33 (0.22, 185.33)	66 (2.2, 1984.33)	0.14 (0, 5.95)
Black, 1998 ⁵⁷	TPMT *3A, TPMT*2	Leukopenia Low leukocyte count	0	61	0	0	5	1		451 (16.35, 12442.54)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Hepatitis	6	55	0	0	1	5		1.83 (0.18, 18.41)	
		Abnormal liver function test									
Ishioka, 1999 ⁶⁴	TPMT*1, *2, *3A, *3B, *3C	Leukopenia WBC<4000/mm ³ & 75% before administration	4	29	0	0	3	0		45.89 (2.02, 1044.27)	
Derijks, 2004 ⁶⁰	TPMT *2, *3A, *3B, *3C	Hepatitis	1	19	0	1	0	4	4.33 (0.12, 159.53)	1.44 (0.05, 41.62)	
		Hepatotoxicity - ALT>80 U/L, AST>80 U/L, bilirubins > 40 µmol/L									
		Pancreatitis	3	17	0	1	0	4	1.67 (0.06, 49.95)	0.56 (0.02, 12.82)	
		Pancreatitis - amylase > 220 U/L, lipase > 120 U/L (elevations>2times normal upper limit)									

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Leukopenia Myelotoxicity (leukocyte count $< 4.0 \times 10^9/L$, platelet count $< 100 \times 10^9/L$)	1	19	1	0	2	2	39 (1.06, 1435.74)	19 (1.15, 314.99)	3 (0.08, 115.35)
Gearry, 2004 ⁶²	TPMT*1, *2, *3A, 3C	Hepatitis Hepatitis: elevation of serum liver transaminases greater than twice the upper limit of normal	18	115	0	1	0	13	2.08 (0.08, 53.04)	0.23 (0.01, 4.06)	
		Pancreatitis Pancreatitis: severe abdominal pain associated with an elevation of serum amylase greater than three times the upper limit of normal	5	128	0	1	2	11	7.79 (0.28, 213.81)	4.65 (0.81, 26.83)	1.53 (0.05, 49.8)

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Leukopenia WBC < 3000/mm ³ or neutrophils < 2000/mm ³	2	131	1	0	1	12	157.8 (5.08, 4904.53)	5.46 (0.46, 64.67)	25 (0.67, 934.5)
Joji, 2003 ⁶⁶	TPMT *1, *2, *3A, *3B, *3C	Leukopenia	12	13	0	0	2	0		5.4 (0.24, 123.81)	
		WBC<3000/mm ³									
		Myelotoxicity	1	24	0	0	0	2		3.27 (0.1, 103.43)	
		Pancytopenia									
Hibi, 2003 ⁶³	TPMT *2, 3B, *3A, *3C	Leukopenia WBC < 3000/mm ³	5	69	1	0	6	1	37.91 (1.38, 1044.73)	82.8 (8.27, 828.71)	0.69 (0.02, 26.91)
Coromina S, 2003 ⁵⁸	TPMT *2, *3A, *3B, *3C, *7, *8	WDAE NA	3	32	0	0	3	2		16 (1.87, 136.7)	
Dubinsky, 2000 ⁶¹	TPMT *3A, *3B, *3C	Leukopenia WBC<4000/mm ³	12	72	0	0	1	7		0.86 (0.1, 7.6)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Bezier, 2008 ⁵⁶	TPMT*2, *3A, *3B, *3C	Any infection (including sepsis)	9	22	0	0	0	2		0.47 (0.02, 10.83)	
		Grade 1 (minor) to grade 4 (sepsis with hypotension)									
		WDAE	15	16	0	0	1	1		1.07 (0.06, 18.62)	
		NA									
		Leukopenia WBC <3.9 x 10 ⁹ /L	8	23	0	0	0	2		0.55 (0.02, 12.73)	
		Neutropenia WMC <1.9 x 10 ⁹ /L	4	27	0	0	0	2		1.22 (0.05, 29.86)	
Reuther, 2003 ⁷¹	TPMT *3A, *3B, *3C	Anemia <109 g/L	7	24	0	0	0	2		0.65 (0.03, 15.16)	
		Hepatitis Alanine transaminase levels twice the upper normal level (40 U/L)	0	62	0	0	0	4			

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Leukopenia WBC less than $3 \times 10^9/L$	0	62	0	0	0	4			
Stocco, 2005 ⁴¹	TPMT *2, *3A, *3B, *3C	Hepatitis ALT, GGT or alkaline phosphatase more than twice normal levels	6	59	0	0	0	5		0.83 (0.04, 16.82)	
		Pancreatitis Severe abdominal pain, accompanied by serum amylase level of greater than twice normal levels	4	61	0	0	0	5		1.24 (0.06, 26.21)	
		Myelotoxicity Leucopenia (WBC < $3000mm^3$) and/or thrombocytopenia (platelets < $100,000mm^3$)	7	58	0	0	0	5		0.71 (0.04, 14.15)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Zelinkova, 2006 ⁷⁸	TPMT *1, *2, *3A, *3B, *3C	Leukopenia WBC < 3x10 ⁹ /L resolving after discontinuation or dose reduction	8	230	1	0	3	20	81.35 (3.08, 2147.05)	4.31 (1.06, 17.55)	17.57 (0.59, 524.14)
		Hepatitis Hepatotoxicity - serum alanine transaminase levels greater than twice the upper normal limit (45 U/L) and resolution after withdrawal or dose reduction	9	229	0	0	2	22		2.31 (0.47, 11.38)	
Okada, 2005 ³⁷	TPMT *2, *3A, *3B, *3C	Leukopenia WBC < 2300/mm ³	2	14	0	0	1	1		7 (0.3, 162.21)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Stocco, 2007 ⁷⁴	TPMT *2, *3A, *3B, *3C	Pancreatitis	6	59	0	0	1	4		2.46 (0.24, 25.69)	
		Severe abdominal pain accompanied by serum amylase level more than twice the normal limit									
		Hepatitis	3	64	0	0	0	3		2.63 (0.11, 61.59)	
		NR									
		Leukopenia	2	65	0	0	0	3		3.74 (0.15, 93.79)	
Tamori, 2007 ⁷⁵	TPMT *2, *3A, *3B, *3C	NR									
		Thrombocytopenia	1	66	0	0	0	3		6.33 (0.22, 185.33)	
		NR									
		Myelotoxicity	1	7	1	0	0	0	15 (0.39, 576.73)		
		Thrombocytopenia, granulocytopenia and neutropenia									

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Marinaki, 2004 ⁶⁹	TPMT *2, *3A, *3C	Hepatitis Serum alanine transaminase levels greater than twice upper normal limit (50 IU/L) and resolution after withdrawal of AZA	3	110	0	0	1	16		2.29 (0.22, 23.39)	
		Pancreatitis Severe abdominal pain and serum amylase > 800 U/L	7	106	0	0	1	16		0.95 (0.11, 8.21)	
		Neutropenia Neutrophil count of <2x10 ⁹ cells	10	103	0	0	1	16		0.64 (0.08, 5.37)	
von Ahsen, 2005 ⁴³	TPMT *2, *3A, *3B, *3C	Hepatitis Aspartate or alanine aminotransferase 2 times the upper limit of reference interval	3	63	0	0	0	5		1.65 (0.08, 36.2)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygote s vs. non-carriers	Heterozygote s vs. non-carriers	Homozygote s vs. heterozygote s
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Pancreatitis Upper abdominal pain with pancreatic amylase or lipase 2 times the upper limit of the reference interval	1	65	0	0	1	4		16.25 (0.85, 310.48)	
		Myelotoxicity Myelosuppression - leukocyte counts < $2.5 \times 10^9/L$ or platelet counts < $100 \times 10^9/L$	0	66	0	0	0	5			
Jun, 2005 ⁶⁷	TPMT *2, *3A, *3B, *3C, 3D, *6	Hepatitis ≥ Two fold elevation of upper normal limit	1	86	0	0	0	7		3.84 (0.14, 102.83)	
		Leukopenia WBC < 4000 mm ³ and <75% before administration	16	71	0	0	1	6		0.74 (0.08, 6.58)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Stocco, 2004 ⁴²	TPMT *2, *3A, *3B, *3C	Any infection (including sepsis) Infection documented clinically or microbiologically	3	37	0	0	0	4		1.19 (0.05, 26.97)	
		Hepatitis ALT, GGT or alkaline phosphatase more than twice their normal levels	2	38	0	0	0	4		1.71 (0.07, 41.54)	
		Pancreatitis Severe abdominal pain, accompanied by a serum amylase level of greater than twice their normal levels	2	38	0	0	0	4		1.71 (0.07, 41.54)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
		Myelotoxicity WBC count < 3000 cells / mm ³ , and platelets < 100,000 / mm ³	13	27	0	0	0	4		0.23 (0.01, 4.52)	
Snow, 1995 ³⁹	NR	Hepatitis	1	20	0	0	0	5		1.24 (0.04, 34.93)	
		9 (Raised AST and ALT)									
		Leukopenia	1	20	0	0	2	3		13.33 (0.91, 196.38)	
Tani, 2009 ⁷⁶	TPMT*2, *3A, *3B, *3C	NR									
		WDAE	18	58	0	1	0	1	1.05 (0.04, 27)	1.05 (0.04, 27)	
		NA									
		Leukopenia <3000/mm ³	7	69	0	1	0	1	3.09 (0.12, 82.76)	3.09 (0.12, 82.76)	
		Thrombocytopenia <100000/ mm ³	1	75	0	1	0	1	16.78 (0.47, 605.23)	16.78 (0.47, 605.23)	
		Any infection (including sepsis)	4	72	0	1	0	1	5.37 (0.19, 151.44)	5.37 (0.19, 151.44)	
		NR									

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Palmieri, 2007 ⁷⁰	TPMT *3A, *3B, *3C	Pancreatitis Upper abdominal pain with pancreatic amylase and lipase greater than twice the normal upper limit	16	377	0	2	0	27	4.58 (0.21, 99.18)	0.42 (0.02, 7.12)	
		Leukopenia WBC <3.0 x 10 ⁹ /L	17	376	2	0	4	23	107.57 (4.97, 2326.61)	3.85 (1.2, 12.37)	26.11 (1.06, 640.27)
		Hepatitis Serum alanine transaminase increase greater than twice the upper normal limit and resolution after withdrawal or dose reduction	11	382	0	2	1	26	6.65 (0.3, 146.57)	1.34 (0.17, 10.75)	3.53 (0.11, 111.68)
Jae Hak, 2008 ⁶⁵	TPMT *1, *2, *3A, *3B, *3C	Leukopenia WBC<3.0 x 10 ⁹ /L	113	166	0	0	5	2		3.67 (0.7, 19.26)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
Schmelin g, 2007 ⁷²	TPMT *2, *3A, *3B, *3C	Leukopenia WBC <3.0x 10 ⁹ /L	4	48	0	0	2	2		12 (1.32, 109.34)	
		Hepatitis LFT >100U/L	12	40	0	0	0	4		0.36 (0.02, 7.16)	
Lopez, 2006 ⁶⁸	TPMT*2, *3A, *3B, *3C, *3D	Pancreatitis NR	52	50	0	0	1	4		0.24 (0.03, 2.23)	
Seddik, 2003 ⁷³	TPMT *2, *3A, *3B, *3C	Leukopenia WBC <3.0 x 10 ⁹ /L	6	63	0	0	1	5		2.1 (0.21, 21.04)	
van Dieren, 2005 ⁷⁷	NR	Hepatitis NR	8	77	0	1	0	10	3.04 (0.11, 80.61)	0.43 (0.02, 8.08)	
		Leukopenia NR	6	79	1	0	2	8	36.69 (1.35, 993.86)	3.29 (0.57, 19.09)	10.2 (0.31, 336.95)
		Thrombocytopenia NR	0	85	1	0	0	10	513 (7.4, 35584.39)		63 (0.87, 4537.84)
Stassen, 2009 ⁴⁰	TPMT *2, *3A, *3B,	Leukopenia NR	34	67	0	0	3	4		1.48 (0.31, 6.98)	

Table C-25. KQ 3c: Association between TPMT genotype and thiopurine toxicity – summary data (continued)

First Author, year	TPMT alleles tested	Outcomes and definition	Non-carriers		Homozygotes		Heterozygotes		Homozygotes vs. non-carriers	Heterozygotes vs. non-carriers	Homozygotes vs. heterozygotes
			n with events	n without events	n with events	n without events	n with events	n without events	Odds Ratio (95% CI)		
	*3C	Thrombocytopenia	8	93	0	0	0	7		0.73 (0.04, 13.98)	
		NR									
		Anemia	30	70	0	0	3	4		1.75 (0.37, 8.30)	
		NR									
Newman, 2010 ⁴⁵	TPMT *2, *3A, *3B, *3C	Mortality	3	147	0	1	0	15	14.05 (0.48, 409.13)	1.36 (0.07, 27.55)	
		WDAE	0	150	0	1	0	15			
		SAE	7	143	1	0	0	15	57.4 (2.15, 1531.14)	0.62 (0.03, 11.33)	93 (1.31, 6606.26)
		Hepatitis	8	142	0	1	0	15	5.59 (0.21, 147.71)	0.54 (0.03, 9.83)	
		Pancreatitis	4	146	0	1	0	15	10.85 (0.39, 304.77)	1.05 (0.05, 20.43)	
		Neutropenia	0	150	1	0	0	15	903 (13.05, 62488.75)		93 (1.31, 6606.26)
Kolorz 2009 ⁷⁹	TPMT *2, *3A, *3B, *3C	Leukopenia	16	64	0	0	5	2		10 (1.77, 56.35)	

Abbreviations: ALT = Alanine transaminase; AZA = azathioprine; GGT = Gamma-glutamyl transferase; L = liter; LFT = liver function test; mm = millimetre; NR = not reported; TPMT = thiopurine methyltransferase; WBC = white blood cell count; U = Unit of enzymatic activity = nanomole of product per hour; WDAE = withdrawal due to adverse events; SAE = serious adverse event

Key Question 4: For patients with chronic autoimmune disease, costs of TPMT testing, and treating drug-associated complications.

Table C-26. KQ 4: Costs of TPMT testing

Study	Currency	Year of costing data	Source of costing data	Type of test	Costing details	Cost reported in study	Converted to USD 2009*
Hagaman 2010 ⁸⁰	NZ	2007	Not reported	Phenotype	Not reported	\$300.00	\$320.98
Gurwitz 2009 ⁸¹	GBP	2006	Estimated from a RCT	Genotype	Cost of test per patient	\$30.00	\$61.67
Gurwitz 2009 ⁸¹	Euro	2006	UCB Pharma (Spain)	Phenotype	Cost of test per patient	\$40.00	\$58.57
Gurwitz 2009 ⁸¹	GBP	2006	Average from the Guy's Hospital and the London City Hospital	Phenotype	Cost of test per patient	\$29.00	\$59.61
Compagni 2008 ⁸²	Euro	2006	Survey of European labs	Genotype TPMT*1, *2, *3A, *3B, *3C	Pharmaco-genetic kits, cost per patient	\$33.00	\$46.25
Compagni 2008 ⁸²	Euro	2006	Survey of European labs	Genotype TPMT*2, *3A, *3B, *3C	Pharmaco-genetic kits, cost per patient	\$21.00	\$29.43
Compagni 2008 ⁸²	Euro	2006	Survey of European labs	Genotype	DNA extraction, PCR, technician time total costs per patient	\$20.00 to \$100.00	\$28.03 to \$140.14
Sayani 2005 ⁸³	CAD	2005	Alberta's provincial laboratory fees	Phenotype	Cost of test per patient	\$50.00	\$47.95
Priest 2006 ⁸⁴	USD	2004	Cost at local lab	Genotype	Cost of test per patient	\$78.00	\$94.48
Priest 2006 ⁸⁴	USD	2004	Cost at local lab	Phenotype	Cost of test per patient	\$57.75	\$46.36
Dubinsky 2005 ⁸⁵	USD	2004	Author's estimate based on literature search (private lab costs)	Genotype	Cost of test per patient	\$510.06	\$617.80
Winter 2004 ⁸⁶	GBP	2003	Cost at local lab	Genotype	Cost of test per patient	\$30.00	\$61.92
Oh 2004 ⁸⁷	USD	2002	Hanyang University Hospital,	Genotype	Cost of test per patient	\$100.00	\$131.51

Table C-26. KQ 4: Costs of TPMT testing

Study	Currency	Year of costing data	Source of costing data	Type of test	Costing details	Cost reported in study	Converted to USD 2009*
			Seoul				
Marra 2002⁸⁸	CAD	1999	Based on other PCR tests	Genotype	Cost of test per patient	\$100.00	\$100.88
Tavadia 2000⁸⁹	CAD	1999	Cost at local lab and other PCR tests	Genotype	Cost of test per patient	\$100.00	\$100.88

Note: * inflation and conversion rate.

Abbreviations: CAD = Canadian dollar; DNA = deoxyribonucleic acid; Euro = European Union dollar; GBP = British pound; PCR = polymerase chain reaction; RCT=randomized controlled trial; TPMT = thiopurine methyltransferase; USD = United States dollar.

Table C-27. KQ 4: Costs of treating azathioprine-associated complications

Study	Currency, year of data	Source of costing data	Costing item	Costing details	Cost reported in study	Converted to USD 2009*
Prakshar 1995⁹⁰	USD, 1995	Accounting data of 3 US hospitals	One-time cost of adverse drug events (low range)	Derived by assuming that 10% of patients developed side-effect that is resolved in 6 months	\$802.41	\$1366.82
Prakshar 1995⁹⁰	USD, 1995	Accounting data of 3 US hospitals	One-time cost of adverse drug events (high range)	Derived by assuming that 30% patients developed side-effect that is resolved in 6 months	\$2407.22	\$4100.45
Tavadia 2000⁸⁹	CAD, 1999	Sunnybrook and Women's College Health Centre	One-time cost of adverse drug events		\$7048.00	\$7110.02
Marra 2002⁸⁸	CAD, 1999	Canadian provincial guide to medical fees	One-time cost of adverse drug events	Derived by assuming 50% will require outpatient and 50% will require inpatient care	\$1734.50	\$1749.76
Winter 2004⁸⁶	GBP, 2003	Information and statistics division of Common Services Agency	One-time cost of adverse drug events	Derived by assuming 32% will develop leukopenia	\$1367.00	\$2821.66
Oh 2004⁸⁷	USD, 2002	Hanyang University Hospital, Seoul	One-time cost of adverse drug events	Average of 4 cases with AZA-induced neutropenia	\$2501.00	\$3289.13
Priest 2006⁸⁴	USD, 2004	Local public hospital in New Zealand	One-time cost of adverse drug events	Average costs of life-threatening, severe, and moderate leukopenia	\$5009.67	\$6067.82
Hagaman 2010⁸⁰	USD, 2007	DRG 420, Professional costs	One-time cost of adverse drug events	Average cost of complicated leukopenia and uncomplicated leukopenia	\$5279.50	\$5648.65
Hagaman 2010⁸⁰	USD, 2007	DRG 420, Professional costs	One-time cost of adverse drug events	Cost of complicated leukopenia leading to death	\$14666.00	\$15691.46

Note: * inflation and conversion rate.

Abbreviation: AZA = azathiopurine; CAD = Canadian dollar; GBP = British pound; USD = United States dollar.

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Appendix D. Excluded Studies

Level II Exclusions by reasons (for all 334 excluded records):

Exclude on record type (editorial, review, commentary, letter, news, report or case report), n=44:

17th IFCC-FESCC European Congress of Clinical Chemistry and Laboratory Medicine/60th National Congress of the Netherlands-Society-for-Clinical-Chemistry-and-Laboratory-Medicine (EUROMEDLAB 2007), Amsterdam, NETHERLANDS, June 03 -07, 2007. Clinical Chemistry and Laboratory Medicine 2007;45(Suppl. S):S5,S7,S9-S5,SS448.

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Appendix E. Additional Acknowledgements

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