Medical Policy

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Title: Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines

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Populations	Interventions	Comparators	Outcomes
Individuals: • Who are treated with thiopurines	Interventions of interest are: • Thiopurine methyltransferase genotype analysis	Comparators of interest are: • Standard management without genotyping analysis	Relevant outcomes include: • Symptoms • Morbid events • Change in disease status
Individuals: • Who are treated with thiopurines	Interventions of interest are: • Thiopurine methyltransferase phenotype analysis	Comparators of interest are: • Standard management without phenotype analysis	Relevant outcomes include: • Symptoms • Morbid events • Change in disease status
Individuals: • Who are treated with thiopurines	Interventions of interest are: • Azathioprine and/or 6-mercaptoprine metabolites analysis	Comparators of interest are: • Standard management without metabolite analysis	Relevant outcomes include: • Symptoms • Morbid events • Change in disease status

DESCRIPTION

The thiopurine class of drugs—which include azathioprine (a pro-drug for mercaptopurine), mercaptopurine, and thioguanine—are used to treat a variety of diseases; however, it is recommended that the use of thiopurines be limited due to a high rate of drug toxicity. Mercaptopurine and thioguanine are directly metabolized by the thiopurine S-methyltransferase (TPMT) enzyme. Susceptibility to drug toxicity is linked to the level of activity of TPMT activity. The variation in TPMT activity has been related to three distinct TPMT variants. Pharmacogenomic analysis of TPMT status is proposed to identify patients at risk of thiopurine drug toxicity and adjust medication doses accordingly, measurement of metabolite markers has also been proposed.

Objective

The objective of this evidence review is to evaluate whether genotypic or phenotypic analysis of thiopurine methyltransferase (TPMT) function or metabolite marker analysis improves the net health outcome in patients treated with thiopurines.

Background

THIOPURINES

Thiopurines or purine analogues are immunomodulators. They include azathioprine (AZA; Imuran), mercaptopurine (6-MP; Purinethol), and thioguanine (6-TG; Tabloid). Thiopurines are used to treat malignancies, rheumatic diseases, dermatologic conditions, inflammatory bowel disease, particularly in patients with corticosteroid-resistant disease. However, the use of thiopurines is limited by both its long onset of action (3-4 months) and drug toxicities, which include hepatotoxicity, bone marrow suppression, pancreatitis, and allergic reactions.

Pharmacogenomics

Thiopurines are converted to 6-MP in vivo, where it is subsequently metabolized to 2 active metabolites: either 6-thioguanine nucleotides (6-TGN) by the inosine-5′-monophosphate dehydrogenase (IMPDH) enzyme; or to 6-methyl-mercaptopurine ribonucleotides (6-MMPR) by the thiopurine methyltransferase (TPMT) enzyme. TPMT also converts 6-MP into an inactive metabolite, 6-methyl-mercaptopurine. 6-TGNs are considered cytotoxic and thus are associated with bone marrow suppression, while the 6-MMPR is associated with hepatotoxicity. In population studies, the activity of the TPMT enzyme has been shown to be trimodal, with 90% of subjects having high activity, 10% intermediate activity, and 0.3% with low or no activity. In patients with intermediate-to-low activity, the metabolism of 6-MP is shunted toward the IMPDH pathway with greater accumulation of 6-TGN; these patients are considered at risk for myelotoxicity (ie, bone marrow suppression).

This variation in TPMT activity has been related to 3 distinct TPMT variants and has permitted the development of TPMT genotyping using a polymerase chain reaction. For example, patients with high TPMT activity are found to have 2 normal (wild-type) TPMT

alleles; those with intermediate activity are heterozygous (ie, have a variant on 1 chromosome), while those with low TPMT activity are homozygous for TPMT variants (ie, have a variant on both chromosomes). Genetic analysis has been explored as a technique to identify patients at risk for myelotoxicity. Patients with high TPMT activity may be treated with standard doses of thiopurines, patients with intermediate TPMT activity may be initially treated with lower doses of thiopurines, while those with low TPMT activity may not be good candidates for thiopurine therapy.

TPMT activity can also be measured by phenotypic testing. Phenotypic testing determines the level of thiopurine nucleotides or TPMT activity in erythrocytes. Caution must be taken with phenotyping, because some coadministered drugs can influence the measurement of TPMT activity in blood, and recent blood transfusions will misrepresent a patient's actual TPMT activity.

Prospective TPMT genotyping or phenotyping may help identify patients at increased risk of developing severe, life-threatening myelotoxicity.

The genotypic analysis of the TPMT gene is based on well-established polymerase chain reaction technology to detect 3 distinct variants. Currently, 3 alleles (TPMT*2, TPMT*3A, TPMT*3C) account for about 95% of subjects with reduced TPMT enzyme activity. Subjects homozygous for these alleles are TPMT-deficient and those heterozygous for these alleles have variable TPMT (low or intermediate) activity. A study by Hindorf and Appell (2012) addressed the concordance between TPMT genotyping and phenotyping.1, The investigators evaluated data from 7195 unselected and consecutive TPMT genotype and phenotype tests. The genotyping tests examined the 3 most common TPMT variants, previously noted. TPMT genotyping identified 6454 (89.7%) as TPMT wild-type, 704 (9.8%) as TPMT heterozygous, and 37 (0.005%) as TPMT homozygous. The overall agreement between genotyping and phenotyping was 95%. Genotyping alone would have misclassified 3 (8%) of 37 homozygous patients as heterozygous; these 3 subjects were found to have uncommon variants. All three had low TPMT activity. The phenotype test would have misclassified 4 (11%) of 37 of homozygous patients because they had test results above the cutoff level for low TPMT activity (<2.5 U/mL red blood cells).

Metabolite Markers

Monitoring of thiopurine therapy has been based on clinical assessment of response in addition to monitoring blood cell counts, liver function, and pancreatic function tests. However, there has been interest in monitoring intracellular levels of thiopurine metabolites (ie, 6-TGN and 6-MMRP) to predict response and complications, with the ultimate aim of tailoring drug therapy to each individual patient.

Metabolite markers have been assessed using high-performance liquid chromatography technology. It would be optimal to assess metabolite markers in peripheral leukocytes because they reflect the status of bone marrow precursors. However, it is technically easier to measure metabolites in red blood cells than in leukocytes.

While genotyping and phenotyping of TPMT would only be performed once, metabolite markers might be tested multiple times during the course of the disease to aid in determining initial dose and to evaluate ongoing dosing.

Regulatory Status
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Several thiopurine genotype, phenotype, and metabolite tests are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Prometheus®, a commercial laboratory, offers thiopurine genotype, phenotype, and metabolite testing for those on thiopurine therapy. The tests are referred to as Prometheus® TPMT Genetics, Prometheus® TMPT enzyme, and Prometheus® thiopurine metabolites, respectively. Other laboratories that offer TPMT genotyping include: Quest Diagnostics (TPMT Genotype); ARUP Laboratories (TPMT DNA); Specialty Laboratories (TPMT GenoTypR™); PreventionGenetics (TPMT Deficiency via the TPMT Gene); Genelex (TPMT); Fulgent Genetics (TPMT); and LabCorp (TPMT enzyme activity and genotyping).

POLICY

A. One-time genotypic or phenotypic analysis of the thiopurine methyltransferase (TPMT) enzyme may be considered **medically necessary** in patients:
   1. beginning therapy with azathioprine (AZA), mercaptopurine (6-MP) or thioguanine (6-TG)
      OR
   2. on thiopurine therapy with abnormal complete blood count (CBC) results that do not respond to dose reduction

B. Genotypic and/or phenotypic analysis of the TPMT enzyme is considered **experimental / investigational** in all other situations.

C. Analysis of the metabolite markers of azathioprine (AZA) and mercaptopurine (6-MP), including 6-methyl-mercaptopurine ribonucleotides (6-MMRP) and 6-thioguanine nucleotides (6-TGN), is considered **experimental / investigational**.

Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Policy Guidelines

Thiopurine methyltransferase (TPMT) testing cannot substitute for complete blood count (CBC) monitoring in patients receiving thiopurines. Early drug discontinuation may be considered in patients with abnormal CBC results. Dosage reduction is recommended in patients with reduced TPMT activity. Alternate therapies may need to be considered for patients who have low or absent TPMT activity (homozygous for nonfunctional alleles). Accurate phenotyping results are not possible in patients who received recent blood transfusions. TPMT genotyping and phenotyping would only need to be performed once.

Genetic Counseling

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

RATIONALE

This evidence has been updated with searches of the MEDLINE database. The most recent literature update was performed through September 9, 2018.

The primary goal of pharmacogenomics testing and personalized medicine is to achieve better clinical outcomes in compared with the standard of care. Drug response varies greatly between individuals, and genetic factors are known to play a role. However, in most cases, the genetic variation only explains a modest portion of the variance in the individual response because clinical outcomes are also affected by a wide variety of factors including alternate pathways of metabolism and patient- and disease-related factors that may affect absorption, distribution, and elimination of the drug. Therefore, assessment of clinical utility cannot be made by a chain of evidence from clinical validity data alone. In such cases, evidence evaluation requires studies that directly demonstrate that the pharmacogenomic test alters clinical outcomes; it is not sufficient to demonstrate that the test predicts a disorder or a phenotype.

Evidence reviews assess the clinical evidence to determine whether the use of technology improves the net health outcome. Broadly defined, health outcomes are length of life, quality of life, and ability to function—including benefits and harms. Every clinical condition has specific outcomes that are important to patients and to managing the course of that condition. Validated outcome measures are necessary to ascertain whether a condition improves or worsens; and whether the magnitude of that change is clinically significant. The net health outcome is a balance of benefits and harms.

To assess whether the evidence is sufficient to draw conclusions about the net health outcome of a technology, 2 domains are examined: the relevance and the quality and credibility. To be relevant, studies must represent one or more intended clinical use of the technology in the intended population and compare an effective and appropriate alternative at a comparable intensity. For some conditions, the alternative will be supportive care or surveillance. The quality and credibility of the evidence depend on study design and conduct, minimizing bias and confounding that can generate incorrect findings. The randomized controlled trial (RCT) is preferred to assess efficacy; however, in some circumstances, nonrandomized studies may be adequate. RCTs are rarely large enough or long enough to capture less common adverse events and long-term effects. Other types of studies can be used for these purposes and to assess generalizability to broader clinical populations and settings of clinical practice.

Testing to direct treatment with thiopurines
Clinical Context and Test Purpose
The purpose of testing for thiopurine methyltransferase (*TPMT*) genotype and phenotype or metabolite markers in patients treated with thiopurines is:

- to identify individuals likely or unlikely to be at high risk of adverse drug reactions (ADRs) from thiopurines; or
- to optimize dose selection or frequency by identifying individuals who are likely to require higher or lower doses of a drug.

The questions addressed in this evidence review are: (1) Does genotypic and phenotypic analysis of *TPMT* gene variants improve the net health outcomes in patients who are treated with thiopurines? and (2) Does metabolite marker analysis of TPMT improve the net health outcomes in patients who are treated with thiopurines?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is patients treated with thiopurines. Thiopurines are administered for a wide variety of clinical conditions such as malignancies, rheumatic diseases, dermatologic conditions, inflammatory bowel disease (IBD), and those undergoing solid organ transplants.

Interventions
Commercial testing for *TPMT* genotype and phenotype or metabolite marker (TPMT function) is available from multiple labs and companies.

The genotypic analysis of the *TPMT* gene is based on polymerase chain reaction technology to detect 3 distinct variants. Currently, 3 alleles (*TPMT*2*, *TPMT*3A*, *TPMT*3C*) account for about 95% of subjects with reduced TPMT enzyme activity. Subjects homozygous for these alleles are TPMT-deficient and those heterozygous for these alleles have variable TPMT (low or intermediate) activity.

Metabolite markers are measured from red blood cell samples using high-performance liquid chromatography. It would be optimal to assess metabolite markers in peripheral leukocytes because they reflect the status of bone marrow precursors; however, it is technically easier to measure metabolites in red blood cells than in leukocytes.

Comparators
The following practice is currently being used to treat malignancies, rheumatic diseases, dermatologic conditions, IBD, and those undergoing solid organ transplants: standard management without *TMPT* function or metabolite marker testing.

Outcomes
Specific outcomes of interest are listed in Table 1.

Table 1. Outcomes of Interest for Individuals Undergoing *TPMT* Function Testing

Outcomes	Details
Symptoms	
Morbid events	• Reduce or eliminate steroid use, which can result in substantial morbidity due to side effects • Reduce or eliminate the incidence of toxicity associated with thiopurines such as bone marrow toxicity, hepatotoxicity, pancreatitis, or other adverse drug reactions (eg, gastric intolerance, skin reactions) that may avoid potential downstream morbidity and hospitalization due to adverse events.
Change in disease status	Crohn's Disease Activity Scale

The potential beneficial outcomes of primary interest would be avoidance or minimization of toxicity associated with thiopurine administration.

The potential harmful outcomes are those resulting from a false test result. False-positive or false-negative test results can lead to under- or overtreatment with thiopurines including the potential loss of therapeutic benefit from undertreatment or adverse events from overtreatment or possibly from an alternative treatment other than thiopurines.

Timing
Testing is typically done prior to initiation of therapy with thiopurines but may also be done during treatment with thiopurines.

Setting
Thiopurines are used in a wide variety of clinical conditions and therefore may be prescribed by a wide variety of specialists such as rheumatologists, gastroenterologists, oncologists, dermatologists, and transplant surgeons (or team). Most patients are likely to be tested in an outpatient setting.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Genotype and Phenotype Testing

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Several systematic reviews of studies on the diagnostic performance of *TPMT* genotyping have been published.[2,3,4,5,6,7,] Most reviews have provided ranges of diagnostic performance measures, while two[2,7,] also conducted meta-analyses. The most recent meta-analysis (Zur et al [2016])[7,] included 27 studies and reported pooled genotyping sensitivity and specificity rates of 90% (95% credible interval, 79% to 99%) and 100%, respectively, and phenotyping sensitivity and specificity rates of 76% (95% credible interval, 58% to 87%) and 99% (95% credible interval, 96% to 100%), respectively. Limitations to the evidence included small numbers of homozygous patients and the inability to conduct subgroup analyses by ethnicity. The incidence of *TPMT* variants differs by ethnicity, which may affect sensitivity and specificity estimates.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

The use of pharmacogenomic testing creates the possibility of tailoring a drug regimen for each patient, with the ultimate goal of attaining disease remission and eliminating steroid therapy. The preferred study design would compare patient management (eg, drug choice) and health outcomes in patients managed with and without testing.

Randomized Controlled Trials

Three RCTs have compared *TPMT* testing with no testing and empirical weight-based thiopurine dosing. Genotype testing was used in 2 trials[8,9,] while the remaining RCT[10,] tested for phenotype enzymatic activity. In both RCTs using genotype testing, patients with a normal enzyme and genotype started full-dose thiopurine, while those with intermediate enzymatic activity or heterozygous genotype had a 50% dose reduction. Those with low or absent enzyme activity or homozygous genotype were not given thiopurine or were given a reduced dose at 0% to 10% of the initiation dose. These 3 RCTs are discussed below.

Coenen et al (2015) published results of the TOPIC trial, which randomized 761 patients with IBD across 30 centers to empirical weight-based thiopurine dosing (n=378) or genotype-guided dosing (n=405).[8,] The trial did not meet its primary end point of showing a statistically significant reduction in hematologic ADR among the group that received genotype-guided thiopurines dosing compared with empirical weight-based dosing. After 20 weeks, the percentage of patients with hematologic ADRs was 7.4% for genotype-based dosing and 7.9% for empirical weight-based thiopurine dosing, with a relative risk of 0.93 (95% confidence interval [CI], 0.57 to 1.52). However, among *TPMT* carriers, only 1 (2.6%) of 39 patients developed a hematologic ADR compared with 8 (22.9%) of 35 patients in the control group (relative risk, 0.11; 95% CI, 0.01 to

0.85). While the results of this secondary analysis were statistically significant, the event rate was low with a wide CI indicating imprecise estimates. Further, there was no statistically significant difference in clinical outcome between the groups in an intention-to-treat analysis at 20 weeks after treatment initiation (p=0.18 for Crohn's Disease Activity Scale score; p=0.14 for ulcerative colitis). In summary, 200 patients would have to be genotyped to avoid 1 episode of a hematologic ADR (7.4% vs 7.9%; ie, 0.5% risk difference). The number needed to treat to avoid 1 episode of a hematologic ADR would be 5 for at-risk individuals (risk difference in patients with a genetic variant, 20.3; 2.6% vs 22.9%).

Newman et al (2011), reported results of the TARGET trial, which randomized 333 IBD patients to genotype-guided dosing or to empirical weight-based thiopurine dosing.[9,] Data were available for 322 (97%) of 333 patients at 4 months. The trial did not meet its primary end point of showing a statistically significant reduction in the proportion of patients stopping azathioprine treatment due to any ADR in genotype-guided dosing arm compared with empirical weight-based dosing. The respective proportion of patients in both arms who stopped taking azathioprine because of an ADR was 29% (47/163) and 28% (44/159; p=0.74), respectively. The trial included few patients with non-wild-type gene variants (7 heterozygous patients in the genotyping group; 2 heterozygous patients, 1 homozygous patient in the nongenotyping group) and therefore was underpowered to detect a difference of the impact of *TPMT* genotyping.

Observational Studies

Several prospective studies have examined variations in the efficacy of medication by patient *TPMT* status. In a study that involved 131 patients with IBD, Gisbert et al (2006) reported that the choice of azathioprine or mercaptopurine dose, based on red blood cells TPMT activity, did not prevent myelotoxicity; no patients in this study exhibited low activity.[11,]

In a study from New Zealand, Gardiner et al (2008) noted that initial target doses to attain therapeutic levels in patients with IBD ranged from 1 to 3 mg/kg/d in intermediate (heterozygous) and normal (wild-type) metabolizers.[12,] This conclusion was based on analysis of 52 patients with IBD who were started on azathioprine or mercaptopurine and followed for 9 months while 6-thioguanine nucleotide (6-TGN) levels and clinical status were evaluated. This study suggested that knowledge of TPMT activity could assist with initial dosing.

In a study from Europe that included 394 patients with IBD, Gisbert et al (2006) found the probability of myelotoxicity was 14.3% in the TPMT intermediate group compared with 3.5% in groups with high (wild-type) activity.[13,] Authors concluded that determining TPMT activity before initiating treatment with azathioprine could minimize the risk of myelotoxicity.

Section Summary: Genotype and Phenotype Testing

Several systematic reviews have evaluated the diagnostic performance of *TPMT* genotyping and phenotyping. The most recent meta-analysis reported genotyping sensitivity and specificity rates of 90% and 100%, respectively, and phenotyping sensitivity and specificity rates of 76% and 99%, respectively. Three RCTs (total N=1145 patients) compared *TPMT* genotype and phenotype testing with no testing and empirical weight-based thiopurine dosing. In these trials, only 0.17% (n=2) were homozygous. Genotype testing was used in two trials while the other used the phenotype enzymatic activity testing. Of the 3 RCTs, only the TOPIC trial (N=761) was adequately powered. Hematologic adverse events and treatment discontinuation were used as surrogate outcomes for the benefits of *TPMT* testing. There were no significant differences in

either outcome based on *TPMT* testing and treatment discontinuation. Additionally, there was also no significant difference in clinical remission rates in these groups based on *TPMT* testing in the largest RCT. However, secondary analysis of individuals who were intermediate enzymatic activity (a heterozygous genotype) or low enzymatic activity (a homozygous genotype) showed that *TPMT* testing to guide dosing was associated with an 89% risk reduction of hematologic adverse events. In conclusion, although the risk of harm from not testing a *TPMT* level before initiating therapy is minimal (indicated by a large number needed to treat), in most cases there is considerable risk of harm (indicated by a small number needed to harm) in the 0.3% patients who are homozygous genotype or have low or absent TPMT enzymatic activity.

Metabolite Marker Testing

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

One systematic review evaluating the diagnostic accuracy of metabolite testing has been identified. The review focused on the association between metabolite levels and disease remission or adverse events. In a literature search through January 2013, Konidari et al (2014) identified 15 studies (total N=1026 children with IBD), none of the studies were RCTs.[14,] Reviewers did not pool findings. Metabolite testing among the studies was inconsistent in terms of predicting clinical outcomes and assessing toxicity.

Several studies have considered the optimal therapeutic cutoff level of metabolites[15,16,17,] and the use of metabolite levels vs ratios of metabolite levels[18,] as predictors of clinical outcomes. Two studies suggested that 235 pmol/8×10^8 is the optimal therapeutic 6-TGN cutoff[15,16,] and another study[17,] suggested a cutoff of 220 pmol/8×10^8 between patients who did and did not stay in remission. Kopylov et al (2014) found that 6-methyl-mercaptopurine (6-MMP)/6-TGN ratios performed better than 6-TGN levels for predicting relapse in pediatric patients with Crohn disease.[18,]

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

The use of thiopurine metabolite testing creates the possibility of tailoring a drug regimen for each patient, with the ultimate goal of attaining disease remission and eliminating steroid therapy. The preferred study design would compare patient management (eg, drug choice) and health outcomes in patients managed with and without testing.

Randomized Controlled Trials

Sayani et al (2005) reported results of a small RCT (N=29) in which IBD patients were randomized to the *TPMT* assay testing (n=15) or no assay testing (n=14) prior to azathioprine

dosing.[10,] All 14 patients who received *TPMT* assay were found to have normal *TPMT* levels and therefore commenced azathioprine at 2.5 mg/kg/d while the individuals in the control arm underwent an upward dose-titration protocol to a target dose of 2.5 mg/kg/d. While the trial was small and did not report power calculations, results showed that 53% (8/15) in the no assay group and 57% (8/14) in the *TPMT* assay group, withdrew as a result of azathioprine-induced adverse events.

Friedman et al (2018) conducted a multicenter RCT in which 73 patients with clinically active or steroid-dependent IBD were randomized to 2 different doses of adjunctive allopurinol with thiopurine (azathioprine or mercaptopurine) therapy.[19,] The purpose of the trial was to compare the efficacy of the 2 different doses of allopurinol (50 mg or 100 mg), as the thiopurine dose was modified based on metabolite testing at 4, 12, and 18 weeks. The modifications in dosing were aimed at achieving a therapeutic level of more than 260 pmol/8×10^8 red blood cells. The primary outcome was the proportion of patients in steroid-free clinical remission at 24 weeks. Tables 2 and 3 summarize the trial characteristics and results. Adverse events did not differ between the 2 groups.

Table 2. Summary of Randomized Controlled Trial Characteristics

Study	Country	Sites	Dates	Participants	Interventions	
					Active	Comparator
Sasyani (2005)[10,]	Canada	1	2002-2003	Patients with Crohn disease or ulcerative colitis, starting azathioprine	TPMT assay (n=14)	No TPMT assay (n=15)
Friedman et al (2018)[19,]	Australia	NR	2011-2014	Patients with Crohn disease or ulcerative colitis, taking thiopurine for at least 8 wk and are thiopurine shunters[a]	Allopurinol 50 mg (n=37)	Allopurinol 100 mg (n=36)

NR: not reported.
[a] Defines as patients who preferentially metabolize thiopurines to produce high levels of 6-methyl-mercaptopurine and low levels of 6-thioguanine nucleotide.

Table 3. Summary of Randomized Controlled Trial Results

Study	**Number (%) Withdrawing**	**Reason for Withdrawal (n)**			
Sasyani (2005)[10,]					
TPMT Assay	8 (57)	Nausea, vomiting, fatigue, cramps, headache (5); pancreatitis (1); no therapeutic effect (2)			
No TPMT Assay	8 (53)	Nausea, vomiting, fatigue, cramps, headache (5); leukopenia (1); elevated liver enzymes (2)			
	Achieving Steroid-Free Remission, %	**6-TGN Concentration pmol/8×10^8**	**p**	**6-MMP Concentration pmol/8×10^8**	**p**
Friedman et al (2018)[19,]					
Whole cohort (N=61)	53	NR		NR	
Allopurinol 50 mg group (n=34)	54	382		1735	
Allopurinol 100 mg group (n=27)	53	420	*NS*	435	0.03

NR: not reported; 6MMP: 6-methyl-mercaptopurine; 6-TGN: 6-thioguanine nucleotide.

The purpose of the gaps tables (see Tables 4 and 5) is to display notable gaps identified in each study. This information is synthesized as a summary of the body of evidence following each table and provides the conclusions on the sufficiency of evidence supporting the position statement. The gaps stated in these tables are specific to the current review and do not reflect a comprehensive assessment.

Table 4. Relevance Gaps

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Sasyani (2005)[10,]					
Friedman et al (2018)[19,]		3. Different doses of allopurinol is not the intervention of interest			

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 5. Study Design and Conduct Gaps

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Sasyani (2005)[10,]					3. 57% and 53% withdrawal from each arm of study	
Friedman et al (2018)[19,]					3. 25% drop out rate in 1 arm of study	

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Observational Studies

Garritsen et al (2018) measured thiopurine metabolite levels in patients with atopic dermatitis and/or chronic dermatitis during maintenance (n=32) and dose escalation (n=8).[20,] The patient population included both high and intermediate activity genotypes and 6-TGN metabolite levels varied widely, from 42 to 696 pmol/8×10^8 red blood cells. Interpretation of results is limited due to the small sample size and the heterogeneity in patient genotypes and drug doses.

Meijer et al (2017) retrospectively reviewed the charts of 24 patients with 6-MMP-induced leukocytopenia.[21,] The authors reported that patients' symptoms resolved on altering the treatment regimens. However, due to the retrospective nature of the study, the altering of treatment regimens cannot be attributed directly to metabolite testing.

Wong et al (2017) reported on the result of a post hoc analysis of the TOPIC trial to address the predictive value of 6-methyl-mercaptopurine ribonucleotide concentrations 1 week after treatment initiation for development of hepatotoxicity during the first 20 weeks of treatment.[22,] They reported that, in more than 80% of patients, hepatotoxicity could be explained by elevated 6-methyl-mercaptopurine ribonucleotide concentrations and the independent risk factors of age, sex, and body mass index, allowing personalized thiopurine treatment in IBD to prevent early failure. Placing 174 patients on a stable thiopurine dose showed that those exceeding the 6-methyl-mercaptopurine ribonucleotide threshold of 3615 pmol/8×0^8 erythrocytes were more likely to have hepatotoxicity (odds ratio, 3.8; 95% CI, 1.8 to 8.0).

Goldberg et al (2016) retrospectively reviewed medical records of patients (N=169) with IBD who were treated with thiopurines for at least 4 weeks.[23,] Metabolite levels of 6-TGN showed 52% were subtherapeutic, 34% were therapeutic, and 14% were supratherapeutic. Among patients who experienced active disease despite therapy, 86% were managed differently following metabolite testing. Clinical outcomes following the management changes were not reported.

Kennedy et al (2013) retrospectively reviewed medical records of patients who had undergone metabolite testing in South Australia.[24,] The analysis reported on 151 patients with IBD who had been taking a thiopurine for at least 4 weeks and underwent at least 1 metabolite test. The 151 patients had a total of 157 tests. Eighty (51%) of 157 tests were done because of flare or lack of medication efficacy, 18 (12%) were for adverse events, and 54 (34%) tests were routine. Forty-four (55%) of the 80 patients who had a metabolite test due to flare or lack of efficacy had better outcomes after the test was performed. Outcomes also improved after testing for 5 (28%) of 18 patients with an adverse event to a thiopurine. For patients who had routine metabolite tests, 7 (13%) of 54 had better outcomes following testing. The rate of benefit was significantly higher in patients tested because of flare or lack of efficacy compared with those who underwent routine metabolite testing ($p<0.001$). Changes in patient management included medication dose adjustments, change in medication, and surgical treatment. The study lacked a control group, and thus, outcomes cannot be compared with patients managed without metabolite testing

Smith et al (2013) retrospectively reviewed medical records of 189 patients with IBD who had 6-TGN metabolite monitoring during thiopurine treatment.[25,] When 6-TGN concentrations were below the therapeutic range (n=47), 18 of the patients were given dose increases and 2 patients were given a combination of allopurinol with azathioprine. When 6-TGN concentrations were above the upper limit of the therapeutic range (n=55), 14 of the patients were given dose reductions. When nonresponders (n=53) were identified, 74% underwent treatment changes including dose increases, switching to a treatment combination of allopurinol and azathioprine or methotrexate, or surgery. Clinical outcomes related to the management changes were not reported.

Armstrong et al (2011) conducted a retrospective chart review of pediatric patients who had a poor clinical response to thiopurine medication for at least 3 months for the treatment of IBD (N=70).[26,] Testing of 6-TGN found that 32% of values were within therapeutic levels.

Management was changed based on metabolite measurements in 25 (36%) of the patients (lowering dose, increasing dose, or switching to methotrexate). Clinical outcomes following the management changes were not reported.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

The current evidence base is insufficient to construct a chain of evidence supporting the use of metabolite testing for this patient population.

Section Summary: Metabolite Marker Testing
One systematic review assessed the diagnostic accuracy of metabolite testing. The review did not pool results due to heterogeneity across studies. Results among the studies were inconsistent in terms of predicting clinical outcomes and assessing toxicity. The evidence for the use of metabolite marker testing to manage patients who are treated with thiopurines is limited to 2 RCTs and a number of retrospective studies. One small RCT had over 50% withdrawal rate due to adverse effects of the treatment, limiting interpretation of results. Another RCT used metabolite testing to adjust thiopurine doses, the purpose of the trial was to compare 2 different allopurinol doses. Most of the retrospective studies have described changes in management following metabolite testing, but clinical outcomes following the management changes were not reported. Without a control group in these studies, outcomes cannot be compared for patients managed without metabolite testing. It is possible that, in the absence of metabolite testing, patients who were not seeing a benefit or who were experiencing adverse events would have had their treatments adjusted without having metabolite testing.

Summary of Evidence
For individuals who are treated with thiopurines who receive *TPMT* genotype analysis or *TPMT* phenotype analysis, the evidence includes studies of diagnostic performance, systematic reviews, and RCTs. Relevant outcomes are symptoms, morbid events, and change in disease status. A large number of studies have assessed the diagnostic performance of *TPMT* genotyping and phenotyping tests. The most recent meta-analysis reported genotyping sensitivity and specificity of 90% and 100%, respectively, and a phenotyping sensitivity and specificity of 76% and 99%, respectively, for identifying patients with subnormal enzymatic activity. Three RCTs (total N=1145 patients) have compared *TPMT* genotype/phenotype testing with no testing and empirical weight-based thiopurine dosing. There were no significant differences in the incidence of hematologic adverse events, treatment discontinuation rates, or clinical remission rates. However, secondary analysis of a small number of individuals who had intermediate enzymatic activity (a heterozygous genotype) or a low enzymatic activity (a homozygous genotype) showed that *TPMT* testing to guide dosing was associated with statistically significant risk reduction in hematologic adverse events with a wide margin of error. In summary, 200 patients would have to be genotyped to avoid 1 episode of a hematologic adverse drug reaction (7.4% vs 7.9%; ie, 0.5% risk difference). The number needed to treat to avoid 1 episode of a hematologic adverse drug reaction would be 5 for at-risk individuals (risk difference in patients with a genetic variant, 20.3%; 2.6% vs 22.9%). In addition, a small, inadequately powered RCT, which assessed phenotype *TPMT* testing, found no difference in treatment discontinuation rates due to adverse drug reactions between the 2 arms. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are treated with thiopurines who receive azathioprine and/or 6-mercaptoprine metabolite analysis, the evidence includes a systematic review as well as prospective and retrospective studies. Relevant outcomes are symptoms, morbid events, and change in disease status. The systematic review, which assessed the diagnostic accuracy of metabolite testing, reported that the ability of the metabolite tests to predict clinical outcomes and toxicity was inconsistent across studies. There is insufficient evidence from prospective studies to determine whether knowledge of metabolite marker status will lead to improved outcomes (primarily improved disease control and/or less adverse drug events). Findings from studies evaluating the association between metabolite markers and clinical remission are mixed, and no prospective comparative trials have compared health outcomes in patients managed using metabolite markers with current approaches to care. The evidence is insufficient to determine the effects of the technology on health outcomes.

SUPPLEMENTAL INFORMATION

Practice Guidelines and Position Statements

National Comprehensive Cancer Network

National Comprehensive Cancer Network (v.1.2018) guidelines on acute lymphoblastic leukemia state:

- "For patients receiving 6-MP [mercaptopurine], consider testing for TPMT [thiopurine methyltransferase] gene polymorphisms, particularly in patients who develop severe neutropenia after starting 6-MP."
- "Determination of patient TPMT genotype using genomic DNA is recommended to optimize 6-MP dosing, especially in patients who experience myelosuppression at standard doses."
- "Quantification of 6-MP metabolites can be very useful in determining whether the lack of myelosuppression is due to non-compliance or hypermetabolism."[27,]

North American Society for Pediatric Gastroenterology, Hepatology and Nutrition

The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (2013) on inflammatory bowel disease (IBD) published consensus recommendations on the role of the TMPT enzyme and thiopurine metabolite testing in pediatric IBD.[28,] Recommendations (high and moderate) included:

1. "TPMT testing is recommended before initiation of TPs [thiopurines] to identify individuals who are homozygous recessive or have extremely low TPMT activity…
2. Individuals who are homozygous recessive or have extremely low TPMT activity should avoid use of TPs because of concerns for significant leucopenia.
3. …All individuals on TPs should have routine monitoring of CBC [complete blood cell] and WBC [white blood cell] counts to evaluate for leucopenia regardless of TPMT testing results.
4. Metabolite testing can be used to determine adherence to TP therapy.
5. Metabolite testing can be used to guide dosing increases or modifications in patients with active disease….
6. Routine and repeat metabolite testing has little or no role in patients who are doing well and taking an acceptable dose of a TP."

British Association of Dermatologists

The guidelines from the British Association of Dermatologists (2011) addressed the safe and effective prescribing of azathioprine for the management of autoimmune and inflammatory skin diseases.[29,] The guidelines included the following recommendations on the analysis of TMPT activity and azathioprine toxicity:

- "There is strong evidence that baseline testing predicts severe neutropenia in patients with absent TMPT activity.
- There is good evidence that intermediate TMPT activity is associated with myelotoxicity in patients using conventional azathioprine doses.
- TMPT testing only identifies … haematological toxicity, hence the continued need for regular monitoring of blood counts irrespective of TMPT status."
- The guidelines also provided recommendations on azathioprine dosing:
- "Patients with normal TPMT activity are at low risk of profound neutropenia and may be prescribed standard doses of azathioprine … (Strength of recommendation: A; level of evidence: 1+).
- Patients with intermediate (heterozygous) range TPMT activity … have an increased risk of neutropenia and should receive lower doses of azathioprine maintenance dose … (Strength of recommendation: C; level of evidence: 2+).
- Patients with … [low] TPMT activity … are at very high risk of profound neutropenia and should in general not be prescribed azathioprine (Strength of recommendation: A; level of evidence: 1+)."

American Gastroenterological Association Institute

Recommendations from the American Gastroenterological Association Institute (2017) guidelines on therapeutic drug monitoring in IBD are summarized in Table 6.[30,31,]

Table 6. Evidence-Based Clinical Guidelines on Therapeutic Drug Monitoring in IBD

Recommendation	SOR	QOE
In adults with IBD being started on thiopurines, AGA suggests routine *TPMT* testing (enzymatic activity or genotype) to guide thiopurine dosing	Conditional	Low
In adults treated with thiopurines with active IBD or adverse effects thought to be due to thiopurine toxicity, AGA suggests reactive thiopurine metabolite monitoring to guide treatment changes	Conditional	Very low
In adults with quiescent IBD treated with thiopurines, AGA suggests against routine thiopurine metabolite monitoring	Conditional	Very low

AGA: American Gastroenterological Association; IBD: inflammatory bowel disease; QOE: quality of evidence; SOR: strength of recommendation; *TPMT*: thiopurine methyltransferase.

U.S. Preventive Services Task Force Recommendations

Not applicable.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 7.

Table 7. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT02929706	Effectiveness of Thiopurine Dose Optimization by NUDT 15 R139C on Reducing Thiopurine-Induced Leucopenia in Inflammatory Bowel Disease	400	Aug 2018 (ongoing)
NCT03093818	PREemptive Pharmacogenomic Testing for Preventing Adverse Drug REactions (PREPARE)	6892	May 2020
Unpublished			
NCT02297126	A Prospective Trial to Assess Cost and Clinical Outcomes of a Clinical Pharmacogenomic Program (INGenious)	4465	May 2018

NCT: national clinical trial.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

81335	TPMT (thriopurine S-methyltransferase) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3)
81479	Unlisted molecular pathology procedure
0034U	TPMT (thiopurine S-methyltransferase), NUDT15 (nudix hydroxylase 15)(eg, thiopurine metabolism) gene analysis, common variants (ie, TPMT *2, *3A, *3B, *3C, *4, *5, *6, *8, *12; NUDT15 *3, *4, *5) [Mayo Clinic]

- The analysis of common variants of the thiopurine methyltransferase (TPMT) gene would be reported with CPT code 81335.
- There are no specific CPT codes for metabolite markers of azathioprine (AZA), mercaptopurine (6-MP), or thioguanine.

ICD-10 Diagnoses Codes (Effective October 1, 2015)

K50.00	Crohn's disease of small intestine without complications
K50.011	Crohn's disease of small intestine with rectal bleeding
K50.012	Crohn's disease of small intestine with intestinal obstruction
K50.013	Crohn's disease of small intestine with fistula
K50.014	Crohn's disease of small intestine with abscess
K50.018	Crohn's disease of small intestine with other complication
K50.10	Crohn's disease of large intestine without complications
K50.111	Crohn's disease of large intestine with rectal bleeding
K50.112	Crohn's disease of large intestine with intestinal obstruction
K50.113	Crohn's disease of large intestine with fistula
K50.114	Crohn's disease of large intestine with abscess
K50.118	Crohn's disease of large intestine with other complication
K50.80	Crohn's disease of both small and large intestine without complications

K50.811	Crohn's disease of both small and large intestine with rectal bleeding
K50.812	Crohn's disease of both small and large intestine with intestinal obstruction
K50.813	Crohn's disease of both small and large intestine with fistula
K50.814	Crohn's disease of both small and large intestine with abscess
K50.818	Crohn's disease of both small and large intestine with other complication
K51.00	Ulcerative (chronic) pancolitis without complications
K51.011	Ulcerative (chronic) pancolitis with rectal bleeding
K51.012	Ulcerative (chronic) pancolitis with intestinal obstruction
K51.013	Ulcerative (chronic) pancolitis with fistula
K51.014	Ulcerative (chronic) pancolitis with abscess
K51.018	Ulcerative (chronic) pancolitis with other complication
K51.019	Ulcerative (chronic) pancolitis with unspecified complications
K51.20	Ulcerative (chronic) proctitis without complications
K51.211	Ulcerative (chronic) proctitis with rectal bleeding
K51.212	Ulcerative (chronic) proctitis with intestinal obstruction
K51.213	Ulcerative (chronic) proctitis with fistula
K51.214	Ulcerative (chronic) proctitis with abscess
K51.218	Ulcerative (chronic) proctitis with other complication
K51.30	Ulcerative (chronic) rectosigmoiditis without complications
K51.311	Ulcerative (chronic) rectosigmoiditis with rectal bleeding
K51.312	Ulcerative (chronic) rectosigmoiditis with intestinal obstruction
K51.313	Ulcerative (chronic) rectosigmoiditis with fistula
K51.314	Ulcerative (chronic) rectosigmoiditis with abscess
K51.318	Ulcerative (chronic) rectosigmoiditis with other complication
K51.80	Other ulcerative colitis without complications
K51.811	Other ulcerative colitis with rectal bleeding
K51.812	Other ulcerative colitis with intestinal obstruction
K51.813	Other ulcerative colitis with fistula
K51.814	Other ulcerative colitis with abscess
K51.818	Other ulcerative colitis with other complication

REVISIONS	
11-29-2010	Policy added to the bcbsks.com web site.
07-19-2011	Updated Description section
	In Policy section: ▪ Added the word "enzyme" to read, "One-time genotypic or phenotypic analysis of the enzyme TPMT (thiopurine methyltransferase) may be considered medically necessary in patients:"
	Updated Rationale section
	Updated References
02-14-2012	In Coding section: ▪ Added CPT code: 81401 (effective 01-01-2012) ▪ Added the following notation: "81401 should be used for genotypic analysis of the TPMT gene effective 01-01-2012." ▪ Revised the following notation by removing the words "genotypic or" to read, "There are no specific CPT codes for phenotypic analysis of the TPMT gene or for metabolite markers of azathioprine, mercaptopurine (6-MP) or thioguanine."

REVISIONS	
08-13-2012	Description section updated
	In Policy section: ▪ Added in B. the abbreviation "(AZA)" to read, "B. Analysis of the metabolite markers of azathioprine (AZA) and…"
	Rationale section updated
	In Coding section: ▪ Updated nomenclature in CPT Code 81401.
	References updated
01-15-2013	In Coding section: ▪ Added CPT code: 81479 (effective 01-01-2013) ▪ Updated coding instructions to remove reference to 83891, 83896, 83898, and 83912 which are no longer effective as of 12-31-2012.
02-28-2014	Description section updated
	Rationale section updated
	In Coding section: ▪ Removed ICD-9 Diagnoses Codes: 555.9, 556.4, 556.5, 556.9 ▪ ICD-10 Diagnoses Codes added
	References updated
11-05-2015	Description section updated
	In policy section: ▪ Added "Genotypic and/or phenotypic analysis of the enzyme TPMT is considered experimental / investigational in all other situations." This addition did not change the policy from its original intent, but is more clear that any situation not meeting the criteria is considered E/I.
	Rationale section updated
	References updated
	Added Appendix Table 1. Categories of Genetic Testing Addressed in Policy
05-10-2017	Description section updated
	In Policy section: ▪ Policy Guidelines updated to add information on Genetic Counseling.
	Rationale section updated
	In Coding section: ▪ Coding notations updated
	References updated
12-20-2017	Description section updated
	Rationale section updated
	In Coding section: ▪ Removed CPT Code: 81401 (Effective 12-31-2017) ▪ Added CPT Code: 81335 (Effective 01-01-2018)
	References updated
04-10-2019	Description section updated
	Rationale section updated
	In Coding section: ▪ Added PLA Code: 0034U (Effective 01-01-2018) ▪ Added ICD Codes: K50.10, K50.111, K50.112, K50.113, K50.114, K50.118, K50.80, K50.811, K50.812, K50.813, K50.814, K50.818, K51.80, K51.811, K51.812, K51.813, K51.814, K51.818
	References updated

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