<table>
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<th>Date</th>
<th>Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines</th>
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<tr>
<td>Original Policy Date: December 7, 2006</td>
<td>Effective Date: May 1, 2018</td>
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<td>Section: 2.0 Medicine</td>
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**Policy Statement**

One-time genotypic or phenotypic analysis of the thiopurine methyltransferase (TPMT) enzyme may be considered **medically necessary in either** of the following situations:

- Patients beginning therapy with azathioprine (AZA), mercaptopurine (6-MP), or thioguanine (6-TG)
- Patients on thiopurine therapy with abnormal complete blood count (CBC) results that do not respond to dose reduction

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

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

**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 complete blood count results. Dosage reduction is recommended in patients with reduced TPMT activity. Alternative 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.

**Coding**

Effective January 1, 2018 there is a new specific CPT code for the analysis of common variants of the thiopurine methyltransferase (TPMT) gene:

- **81335**: TPMT (thiopurine S-methyltransferase) (e.g., drug metabolism), gene analysis, common variants (e.g., *2, *3)

The analysis of common variants of the thiopurine methyltransferase (TPMT) gene may also be reported with the following CPT code:

- **81401**: Molecular pathology procedure, Level 2 (e.g., 2-10 single nucleotide polymorphisms [SNPs], 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
There are no specific CPT codes for metabolite markers of azathioprine (AZA), mercaptopurine (6-MP), or thioguanine.

Genotypic, phenotypic, and metabolite markers are specialized laboratory tests typically performed in reference laboratories.

**Description**

The thiopurine 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 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.

**Related Policies**

- N/A

**Benefit Application**

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. 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.

Some state or federal mandates [e.g., Federal Employee Program (FEP)] prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

**Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Several thiopurine genotype, phenotype, and metabolite tests are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments 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 in San Diego, 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; Madison, NJ), ARUP Laboratories (TPMT DNA; Salt Lake City, UT), and Specialty Laboratories (TPMT GenoTypR®; Valencia, CA), Prevention Genetics (TPMT Deficiency via the TPMT Gene; Marshfield, WI), Genelex (TPMT; Seattle, WA), and Fulgent Genetics (TPMT; Temple City, CA).
Rationale

Background
Thiopurines
Thiopurines or purine analogues are immunomodulators. They include azathioprine (Imuran), mercaptopurine (6-MP; Purinethol), and thioguanine (6-TG; Tabloid). Thiopurines are used to treat malignancies, rheumatic diseases, dermatologic conditions, and irritable bowel disease, and are used in solid organ transplantation. They are considered an effective immunosuppressive treatment of irritable bowel disease, particularly in patients with corticosteroid-resistant disease. However, 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 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 (i.e., bone marrow suppression).

This variation in TPMT activity has been related to 3 distinct TPMT variants and has permitted the development of TPMT genotyping based on 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 (i.e., have a variant on 1 chromosome), while those with low TPMT activity are homozygous for TPMT variants (i.e., a variant is found on both chromosomes). Genetic analysis has been explored as a technique to identify patients at risk for myelotoxicity; those 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 and can also be informative. 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.

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 (i.e., 6-TGN, 6-MMPR) to predict response and complications, with the ultimate aim of tailoring drug therapy to each individual patient.

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 the initial dose and also evaluate any ongoing dosing.
Literature Review
The most recent literature update was performed through September 11, 2017 (see Appendix Table 1 for genetic testing categories).

Validation of the clinical use of any genetic test focuses on 3 main principles: (1) analytic validity, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent; (2) clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility (i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes). These factors are discussed next, both for pharmacogenomics and metabolite markers. The following is a summary of the key literature to date.

Genotype, Phenotype, And Metabolite Marker Testing for Patients Treated with Thiopurines
Clinical Context and Test Purpose
The purpose of testing for thiopurine methyltransferase (TPMT) function or metabolite marker 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) Is there evidence that testing for TPMT function or metabolite marker has clinical validity? and (2) Does patient management change in a way that potentially improves outcomes as a result of testing for TPMT function or metabolite marker?

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

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

Interventions
Commercial testing for metabolite marker or TPMT function is available from multiple labs and companies that include TPMT function testing as part of a panel test.

Comparators
The comparator of interest is standard management without TPMT function or metabolite marker testing.

Outcomes
Specific outcomes of interest are listed in Table 1.

Table 1. Outcomes of Interest for Individuals Undergoing Thiopurine Methyltransferase Function Testing

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Details</th>
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<tbody>
<tr>
<td>Symptoms</td>
<td>Reduce or eliminate steroid use, which can result in substantial morbidity due to side effects</td>
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<tr>
<td>Morbid events</td>
<td>Reduce or eliminate the incidence of toxicity associated with thiopurines such as bone marrow toxicity, hepatotoxicity, pancreatitis, or other adverse drug reactions (e.g., gastric intolerance, skin reactions) that may lead to clinically important improvements in health outcomes.</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Details</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>Change in disease status</td>
<td>Crohn’s Disease Activity Scale</td>
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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 potential loss of therapeutic benefit from undertreatment or adverse events from overtreatment or possibly from an alternative treatment other than thiopurines.

**Timing**

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

**Setting**

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

**Analytic Validity**

**Genotype and Phenotype Testing**

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 2012 study from Sweden addressed the concordance between TPMT genotyping and phenotyping. The investigators evaluated data from 7195 unselected and consecutive TPMT genotype and phenotype tests. The genotype tests examined the 3 most common TPMT variants, previously noted. TPMT genotyping identified 89% as TPMT wild-type, 704 (10%) as TPMT heterozygous, and 37 (0.5%) 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 mutations. 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 Marker Testing**

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.

**Section Summary: Analytic Validity**

TPMT genotypic analysis via polymerase chain reaction technology is expected to have high performance. Concordance between genotypic and phenotypic analysis for TPMT activity is high in at least one analysis. The analytic validity of metabolite marker analysis using high-performance liquid chromatography technology is optimal.

**Clinical Validity**

**Genotype and Phenotype Testing**

Several systematic reviews of studies on the diagnostic performance of TPMT genotyping have been published. Among the most recent was a 2011 review by Booth et al sponsored by the
Agency for Healthcare Research and Quality.\textsuperscript{2} Nineteen studies on test performance were identified; most were cross-sectional or prospective observational studies and approximately 70\% included patients with IBD. Among the 1735 total patients, 184 were heterozygous, and 16 were homozygous for variant alleles, a small subsample of subjects with variant alleles. Pooled analysis of data from 19 studies found a sensitivity of 79.9\% (95\% confidence interval [CI], 74.8\% to 84.6\%) for correctly identifying subjects with subnormal (intermediate or low) enzymatic activity. The specificity of the wild-type genotype for correctly identifying subjects with normal or high enzymatic activity approached 100\%. Seventeen studies addressed the association between TPMT status and thiopurine toxicity. The studies included 2211 patients, 357 of whom had intermediate and 74 had low enzymatic activity. In a pooled analysis of 3 studies (92 patients, 10 events), there were greater odds of myelotoxicity with low TPMT enzymatic activity than intermediate activity (pooled odds ratio [OR], 14.5; 95\% CI, 2.78 to 76.0). Similarly, in a pooled analysis of 3 studies (403 patients, 29 events), there were greater odds of myelotoxicity with low TPMT enzymatic activity than with normal levels (pooled OR=19.1; 95\% CI, 4.6 to 80.2). It is worth noting that the CIs were wide due to few events and small sample sizes.

Another systematic review published in 2011, by Donnan et al, identified 17 studies that reported the performance characteristics of TPMT genotyping tests (12 studies) and phenotyping (6 studies) compared with a reference standard.\textsuperscript{3} No true criterion standard was available. The enzymatic test was used as the reference standard in 9 studies, and the remainder used a genotyping test; studies compared 2 methods of genotyping. All studies used a method of genotyping as either the investigational test or the reference standard; the tests varied somewhat in the number and type of variants they were designed to detect. Sixteen of 17 studies either reported sensitivity and specificity, or reported sufficient data for these measures to be calculated. Only 3 studies considered confounding factors (e.g., concurrent medications, blood transfusions) in their exclusion criteria. Reviewers did not pool study findings. In the included studies, sensitivity of enzymatic tests ranged from 92\% to 100\% and the specificity ranged from 86\% to 98\%. The sensitivity of the genotype tests ranged from 55\% to 100\% and the specificity from 94\% to 100\%. In general, the enzymatic tests had a high sensitivity and a low positive predictive value when genotype tests were used as the reference standard. Genotype tests showed a lower sensitivity and a high positive predictive value when enzymatic tests were used as the criterion standard. The inconsistent use of a reference standard complicated interpretation of the findings.

A 2015 meta-analysis by Liu et al evaluated the relation between TPMT variants and adverse drug reactions (ADRs) in patients with IBD taking thiopurine drugs.\textsuperscript{4} This 2015 analysis updated a 2010 meta-analysis by Dong et al, and findings for both were similar.\textsuperscript{5} The Liu review included studies that compared TPMT variant frequencies in patients who did and did not experience ADRs. Reviewers initially screened 353 articles, and 14 studies (total N=2276 IBD patients) were ultimately found to meet eligibility criteria. In a meta-analysis of data from 10 studies, 67 (14.1\%) of 476 patients with and 57 (4.8\%) of 1192 patients without an ADR were TPMT heterozygous or homozygous. The pooled odds were 3.36 (95\% CI, 1.82 to 6.19), and the difference between groups was statistically significant. In analyses of specific adverse reactions, there were statistically significant associations between the presence of TPMT alleles and bone marrow toxicity, but not hepatotoxicity, pancreatitis, or other ADRs (e.g., gastric intolerance, skin reactions). The number of events in some analyses was relatively small, and these studies may have been underpowered to detect differences between groups. For example, 2 (3.3\%) of 62 IBD patients with pancreatitis were TPMT heterozygous or homozygous compared with 116 (7.7\%) of 1500 patients without pancreatitis (OR=0.97; 95\% CI, 0.38 to 2.48).

In 2016, Roy et al reported on the association between TPMT genotype or phenotype tests and a reference standard, such that it was possible to determine sensitivity, specificity, positive predictive value, negative predictive value, or concordance, in patients receiving thiopurines.\textsuperscript{6} Sixty-six studies were included and appraised for quality. Based on data from 25 studies reporting on test performance on genotyping, the calculated sensitivity for TPMT genotyping to detect a heterozygous or homozygous TPMT variant ranged from 13.4\% to 100.0\%, while the specificity
ranged from 90.9% to 100.0%. A smaller 2016 systematic review by Zur et al reported higher sensitivities and specificities for TPMT genotyping.\(^7\)

No systematic reviews of studies on TPMT genotyping or phenotyping tests in patients undergoing solid organ transplantation were identified. One study identified addressed this population and provided support for genotype analysis. In 2013, Liang et al published data on 93 heart transplant patients treated with azathioprine (AZA).\(^8\) Eighty-three patients had the wild-type genotype, and 10 were heterozygous for variants. The TMPT activity level was significantly lower in the heterozygous subjects (13.1 U/mL) than in subjects with the wild-type genotype (21 U/mL red blood cells; \(p<0.001\)). Moreover, there was a significantly higher rate of severe rejection in heterozygous subjects (7/10 [70%]) than in subjects with a wild-type genotype (12/83 [15%]; \(p<0.001\)). In addition, heterozygous subjects developed severe rejection earlier than wild-type subjects, at a median of 29 days vs 36 days (\(p=0.046\)). There were no statistically significant associations between TMPT genotype and the development of hepatotoxicity or leukopenia.

**Metabolite Marker Testing**

Studies on the diagnostic accuracy of metabolite testing have focused on assessing the association between metabolite levels and disease remission or ADRs. One systematic review was identified; it focused on studies conducted in the pediatric population. In a literature search through January 2013, Konidari et al (2014) identified 15 studies (total \(N=1026\) children with IBD).\(^9\) There were 9 retrospective, 6 prospective case series, and no randomized controlled trials (RCTs). Reviewers did not pool findings. Among studies that evaluated the association between metabolite markers and clinical remission, five found significantly higher rates of remission with higher levels of 6-thioguanine nucleotides (6-TGN), and six did not find significant differences in 6-TGN levels between responders and nonresponders. Moreover, 5 studies found significant associations between 6-methyl-mercaptopurine ribonucleotides (6-MMPR) levels and hepatotoxicity, while three did not.

Several studies have considered the optimal therapeutic cutoff level of metabolites. A 2000 study by Dubinsky et al (\(N=92\) patients) and a 2012 study by Gilissen et al (\(N=100\) patients) both found that 235 pmol/8\(\times\)10\(^8\) was the optimal therapeutic 6-TGN cutoff.\(^10,11\) A 2012 Dhaliwal studied 70 patients with autoimmune hepatitis who were in remission.\(^12\) Levels of 6-TGN were significantly higher in patients who maintained remission compared with those who did not (mean, 237 pmol/8\(\times\)10\(^8\) vs 177 pmol/8\(\times\)10\(^8\); \(p=0.025\)). According to receiver operating curve analysis, a cutoff of 220 pmol/8\(\times\)10\(^8\) best discriminated between patients who did and did not stay in remission. A 2014 study by Kopylov et al 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.\(^13\) The study included 237 patients treated with a thiopurine for at least 3 months. A total of 7.7% were TPMT heterozygous; none was TPMT homozygous. Patients were followed for 18 months; mercaptopurine (6-MP) metabolite concentration levels were measured every 3 to 4 months, or at the time of a clinical relapse or adverse event. The investigators found that 6-MMP/6-TGN ratios between 4 and 24 were significantly protective against relapse; 6-TGN levels alone were not significantly associated with relapse rates.

Wong et al (2017) reported on the result of a post hoc analysis of the TOPIC trial to address the predictive value of 6-MMPR concentrations 1 week after treatment initiation for development of hepatotoxicity during the first 20 weeks of treatment.\(^14\) They reported that, in more than 80% of patients, hepatotoxicity could be explained by elevated 6-MMPR 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-MMPR threshold of 3615 pmol/8 \(\times\)10\(^8\) erythrocytes were more likely to have hepatotoxicity (OR=3.8; 95% CI, 1.8 to 8.0).

**Section Summary: Clinical Validity**

Systematic reviews of genotype and phenotype testing have shown a pooled sensitivity of about 80% and specificity near 100% for identifying patients with subnormal enzymatic activity. In
addition, studies have found a greater likelihood of ADRs with low TPMT activity. The evidence is limited by relatively small numbers of events and wide CIs.

The association between metabolite markers and adverse drug events was less consistent, although a post hoc analysis of a large RCT showed that metabolite markers could be used to predict the likelihood of hepatotoxicity with thiopurines.

**Clinical Utility**

The use of pharmacogenomics and 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 (e.g., drug choice) and health outcomes in patients managed with and without testing.

**Genotype and Phenotype Testing**

Three RCTs have compared TPMT testing with no testing and empirical weight-based thiopurine dosing. Genotype testing was used in 2 studies\(^\text{15-16}\) while the remaining RCT\(^\text{17}\) used the 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/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. The 3 RCTs are discussed below.

In 2015, Coenen et al published results of the TOPIC trial, which randomized 761 patients with IBD across 30 centers to receive empirical weight-based thiopurine dosing (n=378) or genotype-guided dosing (n=405).\(^\text{15}\) The trial did not meet the 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% vs 7.9% in the genotype-based dosing vs empirical weight-based thiopurine dosing, with a relative risk of 0.93 (95% 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% i.e., 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%).

In 2011, Newman et al, reported on the results of the TARGET trial, which randomized 333 IBD patients to genotype-guided dosing for empirical weight-based thiopurine dosing.\(^\text{16}\) Data were available for 322 (97%) of 333 patients at 4 months. The trial did not meet the primary endpoint of showing a statistically significant reduction in the proportion of patients stopping AZA 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 AZA 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.

Sayani et al (2005) reported on the results of a small RCT (N=29) in which IBD patients were randomized to the TPMT assay (n=15) or no assay (n=14) prior to AZA dosing.\(^\text{17}\) All 14 patients who received TPMT assay were found to have normal TPMT levels and therefore commenced AZA 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) and 57% (8/14) in the no assay and TPMT assay groups, respectively, withdrew as a result of AZA-induced adverse events.

Several prospective studies have examined variations in the efficacy of medication by patient TPMT status. For example, in a study that involved 131 patients with IBD, investigators from Europe did not find that the choice of AZA or 6-MP dose based on red blood cells TPMT activity prevented myelotoxicity; no patients in this study exhibited low activity. In a 2008 study from New Zealand, Gardiner et al 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. This conclusion was based on analysis of 52 patients with IBD who were started on AZA or 6-MP and followed for 9 months while 6-TGN levels and clinical status were evaluated. This study suggests that knowledge of TPMT activity can assist with initial dosing. In a 2006 study from Europe that included 394 patients with IBD, Gisbert et al found the probability of myelotoxicity was 14.3% in the TPMT intermediate group compared with 3.5% in groups with high (wild-type) activity. Authors concluded that determining TPMT activity before initiating treatment with AZA could minimize the risk of myelotoxicity.

Metabolite Marker Testing
In 2013, Kennedy et al retrospectively reviewed medical records of patients who had undergone metabolite testing in South Australia. The analysis reported on 151 patients with IBD who had been taking a thiopurine for at least 4 weeks, underwent at least 1 metabolite test, and were managed at a study site. 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 in 5 (28%) of 18 patients with an ADR 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. It is possible that, even in the absence of metabolite testing, patients who were not seeing a benefit or who were experiencing ADRs would have had their treatments adjusted, which could have improved outcomes.

Other relevant studies have examined the association between drug dose and the level of metabolite markers. In general, studies have reported that there is only weak correlation between metabolite levels and drug dose. One 2013 retrospective study, however, found a positive correlation between levels of 6-TGN and 6-MMP and weight-based AZA dose in children with IBD. In addition, studies have reported that levels obtained with testing are often outside of the therapeutic range. For example, Geary et al (2005) reported that 41% of values were within the therapeutic range and Armstrong et al (2011) found that 32% of values were within therapeutic levels.

Section Summary: Clinical Utility
Three RCTs (total N=1145 patients) were identified that compared TPMT genotype and phenotype testing with no testing and empirical weight-based thiopurine dosing. In these studies, only 0.17% (n=2) were homozygous. Genotype testing was used in 2 studies while one used the phenotype enzymatic activity. Of the 3 RCTs, only the TOPIC trial with a large sample (N=761) was adequately powered while the remaining 2 were underpowered. Hematologic adverse events and treatment discontinuation were used as surrogate outcomes for 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 in these groups based on TPMT testing in the largest RCT. However, secondary analysis of individual who were intermediate enzymatic activity/heterozygous genotype or homozygous
genotype/low enzymatic activity 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/absent TPMT enzymatic activity.

The evidence for metabolite marker testing is limited to retrospective studies that report a benefit of metabolite test in terms of better outcomes. However, due to lack of data from RCTs, outcomes cannot be compared with patients managed without 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 randomized controlled trials. 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. A meta-analysis found a pooled sensitivity of about 80% and specificity near 100% for identifying patients with subnormal enzymatic activity. Three randomized controlled trials (total N=1145 patients) compared TPMT genotype/phenotype testing with no testing and empirical weight-based thiopurine dosing. There was no significant difference in the incidence of hematologic adverse events, treatment discontinuation rates, or clinical remission. However, secondary analysis of a small number of individuals who had intermediate enzymatic activity/heterozygous genotype or homozygous genotype/low enzymatic activity 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%; i.e., 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 randomized controlled trial that 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-mercaptopurine metabolites 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. 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 for 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.3.2017) guidelines on acute lymphoblastic leukemia state that testing for thiopurine methyltransferase (TPMT) gene variants should be considered for patients receiving mercaptopurine (6-MP)—in particular, patients who develop severe neutropenia on 6-MP.26
North American Society for Pediatric Gastroenterology, Hepatology and Nutrition
In 2013, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition on inflammatory bowel disease (IBD) published consensus recommendations on the role of the TPMT enzyme and thiopurine metabolite testing in pediatric IBD. 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 2011 guidelines from the British Association of Dermatologists addressed the safe and effective prescribing of azathioprine for the management of autoimmune and inflammatory skin diseases. The guidelines included the following recommendations on analysis of TPMT activity and azathioprine toxicity:

- There is strong evidence that baseline testing predicts severe neutropenia in patients with absent TPMT activity.
- There is good evidence that intermediate TPMT activity is associated with myelotoxicity in patients using conventional azathioprine doses.
- TPMT testing only identifies... hematological toxicity, hence the continued need for regular monitoring of blood counts irrespective of TPMT status.

American Gastroenterological Association Institute
Recommendations from a 2017 American Gastroenterological Association Institute guidelines on therapeutic drug monitoring in IBD are summarized in Table 2.

Table 2. Evidence-Based Clinical Guidelines on Therapeutic Drug Monitoring in Inflammatory Bowel Disease

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>SOR</th>
<th>QOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>In adults with IBD being started on thiopurines, AGA suggests routine TPMT testing (enzymatic activity or genotype) to guide thiopurine dosing</td>
<td>Conditional</td>
<td>Low</td>
</tr>
<tr>
<td>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</td>
<td>Conditional</td>
<td>Very low</td>
</tr>
<tr>
<td>In adults with quiescent IBD treated with thiopurines, AGA suggests against routine thiopurine metabolite monitoring</td>
<td>Conditional</td>
<td>Very low</td>
</tr>
</tbody>
</table>


U.S. Preventive Services Task Force Recommendations
Not applicable.

Medicare National Coverage
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials
Some currently unpublished trials that might influence this review are listed in Table 3.
Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines

Table 3. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT02297126</td>
<td>A Prospective Trial to Assess Cost and Clinical Outcomes of a Clinical Pharmacogenomic Program (INGenious)</td>
<td>6000</td>
<td>Jun 2018</td>
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<tr>
<td>NCT03093818</td>
<td>PREemptive Pharmacogenomic Testing for Preventing Adverse Drug REactions (PREPARE)</td>
<td>8100</td>
<td>Dec 2019</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

References

15. Coenen MJ, de Jong DJ, van Marrewijk CJ, et al. Identification of patients with variants in TPMT and dose reduction reduces hematologic events during thiopurine treatment of


**Documentation for Clinical Review**

**Please provide the following documentation (if/when requested):**

- History and physical and/or consultation notes including:
  - Tests required
  - Purpose of testing
  - Treatment plan
• Laboratory report(s)

**Post Service**
• Results/reports of tests performed

### Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

**MN/IE**

The following services may be considered medically necessary in certain instances and investigational in others. Services may be considered medically necessary when policy criteria are met. Services may be considered investigational when the policy criteria are not met or when the code describes application of a product in the position statement that is investigational.

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>81335</td>
<td>TPMT (thiopurine S-methyltransferase) (e.g., drug metabolism), gene analysis, common variants (e.g., *2, *3) (Code effective 1/1/2018)</td>
</tr>
<tr>
<td></td>
<td>81401</td>
<td>Molecular pathology procedure, Level 2</td>
</tr>
</tbody>
</table>

| HCPCS | None |
| ICD-10 Procedure | None |

### Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Action</th>
<th>Reason</th>
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</thead>
<tbody>
<tr>
<td>12/07/2006</td>
<td>New Policy Adoption</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>01/07/2011</td>
<td>Policy title change from Pharmacogenomic and Metabolite Markers for Patients Treated with Azathioprine (6-MP) Policy revision with position change</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>02/22/2013</td>
<td>Coding update</td>
<td>Administrative Review</td>
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<tr>
<td>06/30/2015</td>
<td>Coding update</td>
<td>Administrative Review</td>
</tr>
<tr>
<td>02/01/2016</td>
<td>Policy title change from Pharmacogenomic and Metabolite Markers in Inflammatory Bowel Disease Policy revision without position change</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>02/01/2017</td>
<td>Policy revision without position change</td>
<td>Medical Policy Committee</td>
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<tr>
<td>02/01/2018</td>
<td>Policy revision without position change Coding update</td>
<td>Medical Policy Committee</td>
</tr>
<tr>
<td>05/01/2018</td>
<td>Coding update</td>
<td>Administrative Review</td>
</tr>
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</table>
Definitions of Decision Determinations

**Medically Necessary:** A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

**Investigational/Experimental:** A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

**Split Evaluation:** Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.