Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines

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Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc.(collectively referred to as the "Company"), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.

When Services Are Eligible for Coverage
Coverage for eligible medical treatments or procedures, drugs, devices or biological products may be provided only if:

- *Benefits are available in the member's contract/certificate, and*
- *Medical necessity criteria and guidelines are met.*

Based on review of available data, the Company may consider a one-time genotypic or phenotypic analysis of the enzyme thiopurine methyltransferase (TPMT) in patients beginning therapy with azathioprine (AZA), mercaptopurine (6-MP) or thioguanine (6-TG) or in patients on thiopurine therapy with abnormal complete blood count (CBC) results that do not respond to dose reduction to be **eligible for coverage.**

When Services Are Considered Investigational
Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

Based on review of available data, the Company considers genotypic and/or phenotypic analysis of the enzyme thiopurine methyltransferase (TPMT) in all other situations to be **investigational.***

Based on review of available data, the Company considers 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) to be **investigational.***

Background/Overview

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 (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 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 (ie, have a variant on 1 chromosome), while those with low TPMT activity are homozygous for *TPMT* variants (ie, 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 (ie, 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.

FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)
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), PreventionGenetics (TPMT Deficiency via the TPMT Gene; Marshfield, WI), Genelex (TPMT; Seattle, WA), and Fulgent Genetics (TPMT; Temple City, CA).

Centers for Medicare and Medicaid Services (CMS)
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.

Rationale/Source

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 (ie, 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.

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The questions addressed in this evidence review are: (1) Is there evidence that testing for *TMPT* 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 *TMPT* 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 *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 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. 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. 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; 3 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 (eg, 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. This 2015 analysis updated a 2010 meta-analysis by Dong et al, and findings for both were similar. 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 was 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 (eg, 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. 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.

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). 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). 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/8×10^8 was the optimal therapeutic 6-TGN cutoff. A 2012 Dhaliwal studied 70 patients with autoimmune hepatitis who were in remission. Levels of 6-TGN were significantly higher in patients who maintained remission compared with those who did not (mean, 237 pmol/8×10^8 vs 177 pmol/8×10^8; p=0.025). According to receiver operating curve analysis, a cutoff of 220 pmol/8×10^8 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. 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. 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 X10^8 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 (eg, 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 while the remaining RCT 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). 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%; 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%).

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. 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. 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 *TPTM* 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 for 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, Gearry 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 *TMPT* 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%; 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 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-mercaptoprine 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.

References

1. Blue Cross and Blue Shield Association, Medical Policy Reference Manual, "Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines, 2.04.19, 11:2017.
2. Hindorf U, Appell ML. Genotyping should be considered the primary choice for pre-treatment evaluation of thiopurine methyltransferase function. *J Crohns Colitis.* Jul 2012;6(6):655-659. PMID 22398041
3. Booth RA, Ansari MT, Loit E, et al. Assessment of thiopurine S-methyltransferase activity in patients prescribed thiopurines: a systematic review. *Ann Intern Med.* Jun 21 2011;154(12):814-823, W-295-818. PMID 21690596
4. Donnan JR, Ungar WJ, Mathews M, et al. Systematic review of thiopurine methyltransferase genotype and enzymatic testing strategies. *Ther Drug Monit.* Apr 2011;33(2):192-199. PMID 21240057
5. Liu YP, Wu HY, Yang X, et al. Association between thiopurine S-methyltransferase polymorphisms and thiopurine-induced adverse drug reactions in patients with inflammatory bowel disease: a meta-analysis. *PLoS One.* Mar 2015;10(3):e0121745. PMID 25799415
6. Dong XW, Zheng Q, Zhu MM, et al. Thiopurine S-methyltransferase polymorphisms and thiopurine toxicity in treatment of inflammatory bowel disease. *World J Gastroenterol.* Jul 7 2010;16(25):3187-3195. PMID 20593505
7. Roy LM, Zur RM, Uleryk E, et al. Thiopurine S-methyltransferase testing for averting drug toxicity in patients receiving thiopurines: a systematic review. *Pharmacogenomics.* Apr 2016;17(6):633-656. PMID 27020704
8. Zur RM, Roy LM, Ito S, et al. Thiopurine S-methyltransferase testing for averting drug toxicity: a meta-analysis of diagnostic test accuracy. *Pharmacogenomics J.* Aug 2016;16(4):305-311. PMID 27217052
9. Liang JJ, Geske JR, Boilson BA, et al. TPMT genetic variants are associated with increased rejection with azathioprine use in heart transplantation. *Pharmacogenet Genomics.* Dec 2013;23(12):658-665. PMID 24121523
10. Konidari A, Anagnostopoulos A, Bonnett LJ, et al. Thiopurine monitoring in children with inflammatory bowel disease: a systematic review. *Br J Clin Pharmacol.* Sep 2014;78(3):467-476. PMID 24592889
11. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology.* Apr 2000;118(4):705-713. PMID 10734022
12. Gilissen LP, Wong DR, Engels LG, et al. Therapeutic drug monitoring of thiopurine metabolites in adult thiopurine tolerant IBD patients on maintenance therapy. *J Crohns Colitis.* Jul 2012;6(6):698-707. PMID 22398098
13. Dhaliwal HK, Anderson R, Thornhill EL, et al. Clinical significance of azathioprine metabolites for the maintenance of remission in autoimmune hepatitis. *Hepatology.* Oct 2012;56(4):1401-1408. PMID 22488741
14. Kopylov U, Amre D, Theoret Y, et al. Thiopurine metabolite ratios for monitoring therapy in pediatric Crohn disease. *J Pediatr Gastroenterol Nutr.* Oct 2014;59(4):511-515. PMID 24918978
15. Wong DR, Coenen MJ, Derijks LJ, et al. Early prediction of thiopurine-induced hepatotoxicity in inflammatory bowel disease. *Aliment Pharmacol Ther.* Feb 2017;45(3):391-402. PMID 27943397
16. 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 inflammatory bowel disease. *Gastroenterology.* Oct 2015;149(4):907-917 e907. PMID 26072396
17. Newman WG, Payne K, Tricker K, et al. A pragmatic randomized controlled trial of thiopurine methyltransferase genotyping prior to azathioprine treatment: the TARGET study. *Pharmacogenomics.* Jun 2011;12(6):815-826. PMID 21692613
18. Sayani FA, Prosser C, Bailey RJ, et al. Thiopurine methyltransferase enzyme activity determination before treatment of inflammatory bowel disease with azathioprine: effect on cost and adverse events. *Can J Gastroenterol.* Mar 2005;19(3):147-151. PMID 15776134
19. Gisbert JP, Luna M, Mate J, et al. Choice of azathioprine or 6-mercaptopurine dose based on thiopurine methyltransferase (TPMT) activity to avoid myelosuppression. A prospective study. *Hepatogastroenterology.* May-Jun 2006;53(69):399-404. PMID 16795981
20. Gardiner SJ, Gearry RB, Begg EJ, et al. Thiopurine dose in intermediate and normal metabolizers of thiopurine methyltransferase may differ three-fold. *Clin Gastroenterol Hepatol.* Jun 2008;6(6):654-660; quiz 604. PMID 18467186
21. Gisbert JP, Nino P, Rodrigo L, et al. Thiopurine methyltransferase (TPMT) activity and adverse effects of azathioprine in inflammatory bowel disease: long-term follow-up study of 394 patients. *Am J Gastroenterol.* Dec 2006;101(12):2769-2776. PMID 17026564
22. Kennedy NA, Asser TL, Mountifield RE, et al. Thiopurine metabolite measurement leads to changes in management of inflammatory bowel disease. *Intern Med J.* Mar 2013;43(3):278-286. PMID 22946880
23. Morales A, Salguti S, Miao CL, et al. Relationship between 6-mercaptopurine dose and 6-thioguanine nucleotide levels in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* Apr 2007;13(4):380-385. PMID 17206711

24. Nguyen TV, Vu DH, Nguyen TM, et al. Relationship between azathioprine dosage and thiopurine metabolites in pediatric IBD patients: identification of covariables using multilevel analysis. *Ther Drug Monit.* Apr 2013;35(2):251-257. PMID 23503453
25. Gearry RB, Barclay ML, Roberts RL, et al. Thiopurine methyltransferase and 6-thioguanine nucleotide measurement: early experience of use in clinical practice. *Intern Med J.* Oct 2005;35(10):580-585. PMID 16207256
26. Armstrong L, Sharif JA, Galloway P, et al. Evaluating the use of metabolite measurement in children receiving treatment with a thiopurine. *Aliment Pharmacol Ther.* Nov 2011;34(9):1106-1114. PMID 21929546
27. National Comprehensive Cancer Network (NCCN). NCCN Clinical practice guidelines in oncology: Acute Lymphoblasic Leukemia. Version 3.2017 https://www.nccn.org/professionals/physician_gls/pdf/all.pdf. Accessed September 18, 2017.
28. Benkov K, Lu Y, Patel A, et al. Role of thiopurine metabolite testing and thiopurine methyltransferase determination in pediatric IBD. *J Pediatr Gastroenterol Nutr.* Mar 2013;56(3):333-340. PMID 23287804
29. Meggitt SJ, Anstey AV, Mohd Mustapa MF, et al. British Association of Dermatologists' guidelines for the safe and effective prescribing of azathioprine 2011. *Br J Dermatol.* Oct 2011;165(4):711-734. PMID 21950502
30. Feuerstein JD, Nguyen GC, Kupfer SS, et al. American Gastroenterological Association Institute guideline on therapeutic drug monitoring in inflammatory bowel disease. *Gastroenterology.* Sep 2017;153(3):827-834. PMID 28780013

Policy History

Original Effective Date: 04/15/2009
Current Effective Date: 11/21/2018

05/07/2009	Medical Director review
05/20/2009	Medical Policy Committee approval. New policy.
06/03/2010	Medical Policy Committee approval
06/16/2010	Medical Policy Implementation Committee approval. Policy title changed by taking out azathioprine (6-MP) and replacing it with “Thiopurines”. Policy statement changed to “a one-time genotypic or phenotypic analysis of the thiopurine methyltransferase (TPMT) gene in patients beginning therapy with azathioprine (AZA), mercaptopurine (6-MP) or thioguanine (6-TG) or in patients on thiopurine therapy with abnormal complete blood count (CBC) results that do not respond to dose reduction to be eligible for coverage.”
06/02/2011	Medical Policy Committee review
06/15/2011	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
06/14/2012	Medical Policy Committee review
06/20/2012	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
01/11/2013	Codes updated.
06/06/2013	Medical Policy Committee review
06/25/2013	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
06/05/2014	Medical Policy Committee review
06/18/2014	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
08/03/2015	Coding update: ICD10 Diagnosis code section added; ICD9 Procedure code section removed.
09/03/2015	Medical Policy Committee review
09/23/2015	Medical Policy Implementation Committee approval. Statement added that genotypic and/or phenotypic analysis of the enzyme TPMT is considered investigational in all other situations.
11/03/2016	Medical Policy Committee review
11/16/2016	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
01/01/2017	Coding update: Removing ICD-9 Diagnosis Codes
11/02/2017	Medical Policy Committee review
11/15/2017	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
04/01/2018	Coding update
11/08/2018	Medical Policy Committee review
11/21/2018	Medical Policy Implementation Committee approval. Coverage eligibility unchanged.

Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines

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Next Scheduled Review Date: 11/2019

Coding

The five character codes included in the Blue Cross Blue Shield of Louisiana Medical Policy Coverage Guidelines are obtained from Current Procedural Terminology (CPT®)‡, copyright 2017 by the American Medical Association (AMA). CPT is developed by the AMA as a listing of descriptive terms and five character identifying codes and modifiers for reporting medical services and procedures performed by physician.

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Codes used to identify services associated with this policy may include (but may not be limited to) the following:

Code Type	Code
CPT	81401 Code added eff 1/1/18: 0034U
HCPCS	No codes
ICD-10 Diagnosis	K50.00-K50.919 K50.90-K50.919 K51.00-K51.919 M05.00-M05.9 M06.00-M06.9 M08.00-M08.99 M12.00-M12.09

*****Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. Whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. Food and Drug Administration (FDA) and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or
B. Whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:
    1. Consultation with the Blue Cross and Blue Shield Association technology assessment program (TEC) or other nonaffiliated technology evaluation center(s);
    2. Credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community; or
    3. Reference to federal regulations.

**Medically Necessary (or "Medical Necessity") - Health care services, treatment, procedures, equipment, drugs, devices, items or supplies that a Provider, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury, disease or its symptoms, and that are:

A. In accordance with nationally accepted standards of medical practice;
B. Clinically appropriate, in terms of type, frequency, extent, level of care, site and duration, and considered effective for the patient's illness, injury or disease; and
C. Not primarily for the personal comfort or convenience of the patient, physician or other health care provider, and not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.

For these purposes, "nationally accepted standards of medical practice" means standards that are based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community, Physician Specialty Society recommendations and the views of Physicians practicing in relevant clinical areas and any other relevant factors.

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