Title: Pharmacogenomic and Metabolite Markers for Patients Treated with Thiopurines

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DESCRIPTION
The use of thiopurines, medications for treating inflammatory bowel disease (IBD) and other conditions, is limited by a high rate of drug toxicity. Susceptibility to drug toxicity has been linked to the level of activity of the enzyme thiopurine methyltransferase (TPMT), which converts thiopurines into metabolites. This variation in TPMT activity has been related to 3 distinct TPMT mutations. Pharmacogenomic analysis of TPMT status is proposed to identify patients at risk of thiopurine drug toxicity and adjust medication doses accordingly. Measurement of metabolite markers has also been proposed.

Objective
The objective of this evidence review is to evaluate the technical performance, diagnostic performance, and whether testing is associated with improved patient outcomes for genotypic or phenotypic analysis of thiopurine methyltransferase (TPMT) function in patients treated with thiopurines.

Background
Thiopurines or purine analogues are immunomodulators. They include azathioprine (AZA; Imuran), mercaptopurine (6-MP; Purinethol), and thioguanine (6-TG; Tabloid). Thiopurines are used to treat malignancies, rheumatic diseases, dermatologic conditions, inflammatory bowel disease (IBD) and are used in solid organ transplantation. They are considered an effective immunosuppressive treatment of IBD, 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 mercaptopurine (6-MP) in vivo, where it is subsequently metabolized to 2 active metabolites; either 6-thioguanine nucleotides (6-TGN) by the enzyme IMPDH, or to 6-methyl-mercaptopurine ribonucleotides (6-MMRP) by the enzyme thiopurine methyltransferase (TPMT). TPMT also converts 6-MP to an inactive metabolite, 6-methyl-mercaptopurine (6-MMP). 6-TGNs are considered cytotoxic and thus are associated with bone marrow suppression, while 6-MMRP 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 to be at risk for myelotoxicity (ie, bone marrow suppression).
This variation in TPMT activity has been related to 3 distinct TPMT mutations 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) alleles for TPMT; those with intermediate activity are heterozygous (ie, have a mutation on 1 chromosome), while those with low TPMT activity are homozygous for TPMT mutations (ie, a mutation 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 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 who may be 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 and 6-MMRP) 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 initial dose and to evaluate ongoing dosing.

**Regulatory Status**

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

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

**POLICY**

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

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

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

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

**Policy Guidelines**

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

**Genetic Counseling**

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

**RATIONALE**
This evidence has been updated with searches of the MEDLINE database. The most recent literature review was performed through November 3, 2016 (see Appendix Table 1 for genetic testing categories). Following is a summary of the key literature to date.

There are 3 steps in the technology assessment process: evaluation of technical performance, evaluation of ability to accurately diagnose a clinical condition compared with the criterion standard, and determination of whether use of the test results in an improved patient outcome. These factors are discussed next, both for pharmacogenomics and metabolite markers.

**Technical Performance**

**Pharmacogenomics**
The genotypic analysis of the thiopurine methyltransferase (*TPMT*) gene is based on well-established polymerase chain reaction (PCR) technology to detect 3 distinct mutations. 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 2011 study from Sweden addressed the concordance between *TPMT* genotyping and phenotyping.1 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 3 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 [RBCs]).

**Metabolite Markers**
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 RBCs than in leukocytes.

**Section Summary: Technical Performance**
*TPMT* genotypic analysis via PCR technology is expected to have high performance. Concordance between genotypic and phenotypic analysis for *TPMT* activity is high in at least one analysis.

**Diagnostic Performance**

**Pharmacogenomics**
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.2 Nineteen studies on test performance were identified; most were cross-sectional or prospective observational studies and approximately 70%
included patients with inflammatory bowel disease (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 confidence intervals 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.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; 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 polymorphisms 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 (PPV) when genotype tests were used as the reference standard. Genotype tests showed a lower sensitivity and a high PPV 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 polymorphisms and adverse drug reactions (ADRs) in patients with IBD taking thiopurine drugs.4 This study updated a 2010 meta-analysis by Dong et al, and findings of the 2 analyses were similar.5 The Liu review included studies that compared TPMT polymorphism frequencies in patients who did and did not experience ADRs. The investigators 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 of 476 patients with (14.1%) and 57 (4.8%) of 1192 patients without an ADR were TPMT heterozygous or homozygous. The pooled odds ratio 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 \textit{TPMT} genotype or phenotype tests and a reference standard, such that it was possible to determine sensitivity, specificity, PPV, 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 \textit{TPMT} genotyping to detect a heterozygous or homozygous \textit{TPMT} mutation 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 \textit{TPMT} genotyping.

No systematic reviews of studies on \textit{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 mutations. 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 RBCs; \textit{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\%]; \textit{p}<0.001). In addition, heterozygous subjects developed severe rejection earlier than wild-type subjects, at a median of 29 days versus 36 days (\textit{p}=0.046). There were not statistically significant associations between \textit{TMPT} genotype and the development of hepatotoxicity or leukopenia.

**Metabolite 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 4, 2013, Konidari et al identified 15 studies (total \textit{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, 5 found significantly higher rates of remission with higher levels of 6-thioguanine nucleotides (6-TGN), and 6 studies did not find significant differences in 6-TGN levels between responders and nonresponders. Moreover, 5 studies found significant associations between 6-methyl-mercaptopurine ribonucleotides levels and hepatotoxicity, while 3 studies did not.

Several studies have considered the optimal therapeutic cutoff level of metabolites. A 2000 study by Dubinsky et al (\textit{N}=92 patients) and a 2012 study by Glissen et al (\textit{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, \textit{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 \textit{TPMT} heterozygous; none was \textit{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.

### Section Summary: Diagnostic Performance

Systematic reviews show 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 adverse drug reactions with low TPMT activity. The evidence is limited by relatively small numbers of events and wide confidence intervals. The association between metabolite markers and adverse drug events is less consistent.

### Improvement in Health Outcomes

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.

### Pharmacogenomics

In 2015, Coenen et al published results of the TOPIC trial, which randomized 761 patients with IBD across 30 centers to receive standard treatment or pretreatment screening for 1 of 3 common \textit{TPMT} genotype variants, followed by reduced thiopurine (AZA or 6-MP) treatment doses if patients were found to be heterozygous or homozygous carriers.\textsuperscript{14} For the trial’s primary outcome, hematologic ADRs, there were no significant differences in rates over the 20-week study period between the intervention group (n=405) and the control group (n=378) (7.4% vs 7.9%; relative risk [RR], 0.93; 95% CI, 0.57 to 1.52). However, a significantly smaller proportion of \textit{TPMT} carriers in the intervention (testing) group developed hematologic ADRs (2.6%) than those in the control group (22.9%; RR=0.11; 95% CI, 0.01 to 0.85).

Another controlled trial, known as TARGET, randomized 333 patients to receive \textit{TPMT} genotyping or usual care (no genotyping) before AZA therapy.\textsuperscript{15} Study eligibility included age 16 years or older with a diagnosis of IBD. In the testing arm, results were generated within 1 week, and the study clinician informed. Clinicians were advised to recommend the following: maintenance dose of AZA (ie, 1.5-3 mg/kg/d) for patients with wild-type \textit{TPMT}, low-dose AZA (ie, 25-50 mg/d) titrated to a maintenance dose for patients with heterozygous \textit{TPMT} variant alleles, and an alternative therapy (no AZA) for patients homozygous for \textit{TPMT} variant alleles. All final treatment decisions were at the discretion of the individual provider (ie, this was a pragmatic RCT). Genotyping was also done on samples from patients in the control group, but results were not made available until the end of the study.

Data were available for 322 (97%) of 333 patients at 4 months. The primary trial end point was stopping AZA at any ADR in the first 4 months of treatment. At 4 months, 91 (28%) of 322 patients had stopped taking AZA because of an ADR, 47 (29%) of 163 in the genotyping group and 44 (28%) of 159 in the nongenotyping group. The difference between groups was not statistically significant (p=0.74). In the genotyping arm, the average starting dose of AZA was significantly lower in \textit{TPMT} heterozygote than wild-type patients (p=0.008), suggesting that clinicians followed dosing recommendations. However, at 4 months, the mean dose was similar.
across both arms (1.68 mg/kg/d, p=0.25), and there was no difference in dose between patients heterozygous or wild type for TPMT variant alleles (p=0.99). Moreover, at 4 months, there was no significant difference between groups in the level of clinical symptoms. For example, mean Harvey-Bradshaw Index score was 4.5 in each group (p=0.80) (54 patients in the genotyping group and 56 patients in the nongenotyping group were included in this analysis). It is important to note that this study included few patients with non-wild-type gene variants (7 heterozygous patients in the genotyping group; 2 heterozygous patients and 1 homozygous patient in the nongenotyping group). Thus, the study was underpowered to evaluate the impact of TPMT genotyping on patients with variant alleles.

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 RBC TPMT activity prevented myelotoxicity; no patients in this study exhibited low activity.\(^{16}\) 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.\(^{17}\) This conclusion was based on analysis of 52 patients with IBD who were started on AZA or 6-MP and followed up 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 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.\(^{18}\) Authors concluded that determining TPMT activity before initiating treatment with AZA could minimize the risk of myelotoxicity.

**Metabolite Testing**

No prospective comparative trials identified compared use of metabolite markers with current approaches to care. In 2013, Kennedy et al retrospectively reviewed medical records of patients who had undergone metabolite testing in South Australia.\(^{19}\) 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 to 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.\(^{20}\) 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.\(^{21}\) In addition, studies have reported that levels obtained with testing are often outside...
of the therapeutic range. For example, Gearry et al reported that 41% of values were within the therapeutic range\textsuperscript{22} and Armstrong et al found that 32% of values were within therapeutic levels.\textsuperscript{23}

**Summary of Evidence**

For individuals who are treated with thiopurines who receive thiopurine methyltransferase (TPMT) pharmacogenomics analysis or *TPMT* phenotype analysis, the evidence includes studies of diagnostic performance, systematic reviews, and randomized controlled trials (RCTs). 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. In addition, studies have found a greater likelihood of adverse drug reactions with low TPMT activity. One RCT reporting on health outcomes was identified; this trial did not find a significant difference in outcomes for patients managed with and without *TPMT* genotype testing; it may have been underpowered. 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 metabolite markers will lead to improved outcomes (primarily improved disease control and/or less adverse drug effects). Findings of studies evaluating the association between metabolite markers and clinical remission are mixed, and no prospective comparative trials have compared health outcomes in patients managed with metabolite markers and with current approaches to care. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Practice Guidelines and Position Statements**

**National Comprehensive Cancer Network**

National Comprehensive Cancer Network (v.2.2016)\textsuperscript{24} guidelines on acute lymphoblastic leukemia state that testing for thiopurine methyltransferase (*TPMT*) gene polymorphisms should be considered for patients receiving mercaptopurine (6-MP), in particular patients who develop severe neutropenia on 6-MP.\textsuperscript{24}

**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 *TMPT* enzyme and thiopurine metabolite testing in pediatric IBD.\textsuperscript{25} 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 TMPT activity and azathioprine toxicity:

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

**National Academy of Clinical Biochemistry**
The 2010 guidelines from the National Academy of Clinical Biochemistry (NACB) stated: “thiopurine methyltransferase (TPMT) genotyping is recommended as a useful adjunct to a regimen for prescribing azathioprine.” This A-I recommendation indicated that NACB strongly recommended adoption. The recommendation was based on evidence with consistent results from well-designed and well-conducted studies in representative populations.

**American Gastroenterological Association**
A 2006 position statement from the American Gastroenterological Association on the treatment of IBD included the following recommendations:

- “Current FDA [U.S. Food and Drug Administration] recommendations suggest that individuals should have TPMT [thiopurine methyltransferase] genotype or phenotype assessed before initiation of therapy with AZA [azathioprine] or 6-MP [mercaptopurine] in an effort to detect individuals who have low enzyme activity (or who are homozygous deficient in TPMT) in an effort to avoid AZA or 6-MP therapy ... and thus avoid potential adverse events. (Grade B)
- Individuals who have intermediate or normal TPMT activity (wild type or heterozygotes) need measurement of frequent complete blood counts (as above) in addition to TPMT assessment because these individuals may still develop myelosuppression subsequent to use of AZA or 6-MP. (Grade B)”
- “Thiopurine metabolite monitoring in the treatment of patients with 6-MP or AZA is useful when attempting to determine medical noncompliance and may be helpful for optimizing dose and monitoring for toxicity. (Grade C)”

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

**Ongoing and Unpublished Clinical Trials**
A search of ClinicalTrials.gov in November 2016 did not identify any ongoing or unpublished trials that would likely influence this review.
CODING

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

CPT/HCPCS

81401  Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) -TPMT (thiopurine S-methyltransferase) (eg, drug metabolism), common variants (eg, *2, *3)

81479  Unlisted molecular pathology procedure

- The analysis of common variants of the thiopurine methyltransferase (TPMT) gene would be reported with CPT code 81401.
- There are no specific CPT codes for metabolite markers of azathioprine (AZA), mercaptopurine (6-MP), or thioguanine.

ICD-9 Diagnoses Codes

555.0  Regional enteritis; small intestine
555.1  Regional enteritis; large intestine
555.2  Regional enteritis; small intestine with large intestine
556.0  Ulcerative (chronic) enterocolitis
556.1  Ulcerative (chronic) ileocolitis
556.2  Ulcerative (chronic) proctitis
556.3  Ulcerative (chronic) proctosigmoiditis
556.6  Universal ulcerative (chronic) colitis
556.8  Other ulcerative colitis

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

K50.00  Crohn's disease of small intestine without complications
K50.011 Crohn's disease of small intestine with rectal bleeding
K50.012 Crohn's disease of small intestine with intestinal obstruction
K50.013 Crohn's disease of small intestine with fistula
K50.014 Crohn's disease of small intestine with abscess
K50.018 Crohn's disease of small intestine with other complication
K50.10  Crohn's disease of large intestine without complications
K50.111 Crohn's disease of large intestine with rectal bleeding
K50.112 Crohn's disease of large intestine with intestinal obstruction
K50.113 Crohn's disease of large intestine with fistula
K50.114 Crohn's disease of large intestine with abscess
K50.118 Crohn's disease of large intestine with other complication
K50.80  Crohn's disease of both small and large intestine without complications
K50.811 Crohn's disease of both small and large intestine with rectal bleeding
K50.812 Crohn's disease of both small and large intestine with intestinal obstruction
K50.813 Crohn's disease of both small and large intestine with fistula
REVISIONS

11-29-2010 Policy added to the bcbsks.com web site.

07-19-2011 Updated Description section

In Policy section:

- Added the word “enzyme” to read, “One-time genotypic or phenotypic analysis of the
  enzyme TPMT (thiopurine methyltransferase) may be considered medically necessary in
  patients:”

Updated Rationale section

Updated References

02-14-2012 In Coding section:

- Added CPT code: 81401 (effective 01-01-2012)
- Added the following notation:
  “81401 should be used for genotypic analysis of the TPMT gene effective
  01-01-2012.”
- Revised the following notation by removing the words “genotypic or” to read,
  “There are no specific CPT codes for phenotypic analysis of the TPMT gene or for
  metabolite markers of azathioprine, mercaptopurine (6-MP) or thioguaine.”

08-13-2012 Description section updated

In Policy section:

- Added in B. the abbreviation “(AZA)” to read, “B. Analysis of the metabolite markers of
  azathioprine (AZA) and…”
Pharmacogenomic and Metabolite Markers for Patients Treatment with Thiopurines

<table>
<thead>
<tr>
<th>DATE</th>
<th>DESCRIPTION</th>
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</table>
| 01-15-2013   | Rationale section updated  
In Coding section:  
- Updated nomenclature in CPT Code 81401.  

References updated |
| 02-28-2014   | Description section updated  
Rationale section updated  
In Coding section:  
- Added CPT code: 81479 (effective 01-01-2013)  
- Updated coding instructions to remove reference to 83891, 83896, 83898, and 83912 which are no longer effective as of 12-31-2012.  

References updated |
| 11-05-2015   | Description section updated  
In policy section:  
- Added “Genotypic and/or phenotypic analysis of the enzyme TPMT is considered experimental / investigational in all other situations.” This addition did not change the policy from its original intent, but is more clear that any situation not meeting the criteria is considered E/I.  

Rationale section updated  
References updated  
Added Appendix Table 1. Categories of Genetic Testing Addressed in Policy |
| 05-10-2017   | Description section updated  
In Policy section:  
- Policy Guidelines updated to add information on Genetic Counseling.  

Rationale section updated  
In Coding section:  
- Coding notations updated  

References updated |

REFERENCES


**Appendix**

**Appendix Table 1. Categories of Genetic Testing**

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
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</thead>
<tbody>
<tr>
<td>1. Testing of an affected individual’s germline to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>1a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>1b. Prognostic</td>
<td></td>
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<tr>
<td>1c. Therapeutic</td>
<td>X</td>
</tr>
<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
<td></td>
</tr>
<tr>
<td>2a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>2b. Prognostic</td>
<td></td>
</tr>
<tr>
<td>2c. Therapeutic</td>
<td></td>
</tr>
<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
<td></td>
</tr>
<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
<td></td>
</tr>
<tr>
<td>5. Reproductive testing</td>
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</tr>
<tr>
<td>5a. Carrier testing: preconception</td>
<td></td>
</tr>
<tr>
<td>5b. Carrier testing: prenatal</td>
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</tr>
<tr>
<td>5c. In utero testing: aneuploidy</td>
<td></td>
</tr>
<tr>
<td>5d. In utero testing: mutations</td>
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</tr>
<tr>
<td>5e. In utero testing: other</td>
<td></td>
</tr>
<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
<td></td>
</tr>
</tbody>
</table>