# *Laboratory and Genetic Testing for use of Thiopurines*

**Effective**: April 1, 2019

**Next Review:** January 2020
**Last Review:** February 2019

## IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

## DESCRIPTION

Thiopurines or purine analogues are immunomodulators. They are used to treat malignancies, rheumatic diseases, dermatologic conditions, and irritable bowel disease, and are used in solid organ transplantation. The tests addressed in this policy are used to help identify patients at increased risk of developing severe, life-threatening myelotoxicity from thiopurines and to aid in determining the initial dose and evaluate any ongoing dosing.

## MEDICAL POLICY CRITERIA

I.   Genotypic or phenotypic analysis of the thiopurine methyltransferase (TPMT) enzyme may be considered **medically necessary** in patients prior to beginning thiopurine therapy (i.e. azathioprine, mercaptopurine, or thioguanine) OR in patients on thiopurine therapy when there is clinical documentation of abnormal complete blood count results that do not respond to dose reduction.

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

III. Genetic testing for *NUDT15* may be considered **medically necessary** in patients prior to beginning thiopurine therapy (i.e. azathioprine, mercaptopurine, or thioguanine) OR

in patients on thiopurine therapy when there is clinical documentation of abnormal complete blood count results that do not respond to dose reduction.

IV.   Genetic testing for *NUDT15* is considered **investigational** in all other situations.

V.   Analysis of the metabolite markers azathioprine and mercaptopurine, including 6-methyl-mercaptopurine ribonucleotides and 6-thioguanine nucleotides, is considered **investigational**.

*NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.*

## POLICY GUIDELINES

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1.   Name of the genetic test(s) or panel test
2.   Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3.   The exact gene(s) and/or mutation(s) being tested
4.   Relevant billing codes
5.   Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6.   Medical records related to this genetic test:
     o   History and physical exam including any relevant diagnoses related to the genetic testing
     o   Conventional testing and outcomes
     o   Conservative treatments, if any

Thiopurine methyltransferase (TPMT) testing cannot substitute for complete blood count 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.

## CROSS REFERENCES

None

## BACKGROUND

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.

## 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 two 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 three 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 two normal (wild-type) TPMT alleles; those with intermediate activity are heterozygous (i.e., have a variant on one 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.

The *NUDT15* gene encodes a Nudix hydrolase, a family of enzymes that catalyze the hydrolysis of nucleoside diphosphates. NUDT15 has been proposed to participate in the catabolism of thiopurines and act as a negative regulator of thiopurine activation and toxicity.

Correlations have been shown between *NUDT15* variants and thiopurine toxicity. Thus genetic analysis has been examined as a method to identify those at risk of thiopurine-induced toxicity.

**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.

## 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® TPMT 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 Quest Diagnostics (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).

## EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

### ANALYTIC VALIDITY

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

### CLINICAL VALIDITY

**TPMT Genotype and Phenotype Testing**

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

*NUDT15* **Genotyping**

Several systematic reviews of studies on the diagnostic performance of *NUDT15* genotyping have been published.

Liu (2018) conducted meta-analyses to evaluate the association between the *NUDT15* R139C (c.415C>T; rs116855232) variant and thiopurine-induced leukopenia in the Asian population.[8] A total of 14 studies (with 918 patients and 2,341 controls), addressing inflammatory bowel disease (IBD) or acute lymphoblastic leukemia, met the inclusion criteria. Study quality was evaluated with the Newcastle-Ottawa quality assessment scale (NOS) criteria. All included studies were rated greater than or equal to six, indicating good quality. Significant heterogeneity was identified for the dominant and heterozygote but not the recessive and homozygote comparisons. When assessed with a funnel plot and Egger's test, no evidence of publication bias was found. Results of the meta-analysis indicated that the *NUDT15* R139C variant was associated with thiopurine-induced leukopenia in all models evaluated (dominant model OR=9.04, 95% CI 6.05-13.50, p<0.001; recessive model OR=24.26, 95% CI 11.38-51.71, p<0.001; CT vs TT model OR =7.60, 95% CI 4.97-11.61, p<0.001; CC vs TT model OR =38.47, 95% CI 17.78-83.24, P<0.001).

A 2018 systematic review by Cargnin estimate diagnostic accuracy of *NUDT15* variants for detection of thiopurine-induced leukopenia.[9] A total of 16 studies (3538 thiopurine-treated patients) met inclusion criteria assessing the rs116855232 (16 studies), rs186364861 (six studies) and rs554405994 (five studies) variants of *NUDT15*. Diagnostic odds ratios (DOR) were calculated. The rs116855232 DOR (8.44, 95% CI: 5.46-13.03) was found to be higher than the rs554405994 DOR (4.336, 95% CI 2.924-6.429) or the rs186364861 DOR (2.742, 95% CI 1.453-5.175). Subgroup analyses showed a significant DOR for early- but not late-onset leukopenia (early rs186364861: 4.04, 95% CI 1.78-9.20; rs554405994: 2.94, 95% CI 1.74-4.95 vs late rs186364861: 1.52, 95% CI 0.52-4.43; rs554405994: 2.02, 95% CI 0.93-4.40).

In 2017, Zhang performed a systematic review and meta-analysis on the association of *NUDT15* c.415C>T allele and thiopurine-induced leukocytopenia in Asians.[10] Studies published through July 10, 2016 were searched and seven studies including 1138 patients met inclusion criteria. Six of the studies were cohort studies and one was a case control study. The quality of all studies was found to be high according to the NOS. When assessed with a funnel plot and Egger's test, no evidence of publication bias was found. A random-effects model meta-analysis indicated that the presence of the T allele was significantly associated with high

incidences of leukocytopenia. The risk ratio of developing leukopenia was 3.79 for CT + TT versus CC, 3.41 for CT verus CC, and 6.54 for TT versus CC.

Yin (2017) conducted meta-analyses to analyze the relationship between the *NUDT15* c.415C>T allele and two outcomes: thiopurine myelotoxicity susceptibility and thiopurine intolerance dose.[11] Data from two cohorts were assessed for the two outcomes. These cohorts included patients with ALL and those with IBD. ALL and IBD patients were separated in the second analysis because thiopurine dosage used in ALL patients was significantly higher than that used in IBD patients. No publication bias was detected in either meta-analysis. The first meta-analysis aimed to determine the relationship between the *NUDT15* variant and thiopurine-induced myelotoxicity. Six of the seven studies from the Zhang systematic review above, plus one additional study, were analyzed. The *NUDT15* c.415C>T allele was found to contribute 7.86-fold higher risk for developing leukopenia with 91.74% specificity and 43.19% sensitivity. Specificity for early leukopenia was 84.59%.

The second analysis aimed to determine the association between the *NUDT15* c.415C>T variant and thiopurine intolerance dose. 2745 patients from 13 cohorts were assessed. There was high heterogeneity between studies. Patients with CT and TT genotypes required 28% lower mean thiopurine dose compared to patients with CC genotypes, a difference that was statistically significant.

**Metabolite Marker Testing**

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

One systematic review evaluating the diagnostic accuracy of metabolite testing has been identified. The review focused on the association between metabolite levels and disease remission or adverse events. In a literature search through January 2013, Konidari (2014) identified 15 studies (total N=1026 children with IBD), none of the studies were RCTs.[12] Reviewers did not pool findings. Metabolite testing among the studies was inconsistent in terms of predicting clinical outcomes and assessing toxicity.

Several studies have considered the optimal therapeutic cutoff level of metabolites[13-15] and the use of metabolite levels vs ratios of metabolite levels[16] as predictors of clinical outcomes. Two studies suggested that 235 pmol/$8{\times}10^8$ is the optimal therapeutic 6-TGN cutoff[13,14] and another study[15] suggested a cutoff of 220 pmol/$8{\times}10^8$ between patients who did and did not stay in remission. Kopylov (2014) found that 6-methyl-mercaptopurine (6-MMP)/6-TGN ratios performed better than 6-TGN levels for predicting relapse in pediatric patients with Crohn disease.[16]

**Section Summary: Clinical Validity**

Several systematic reviews have evaluated the diagnostic performance of TPMT genotyping and phenotyping. The most recent meta-analysis reported genotyping sensitivity and specificity rates of 90% and 100%, respectively, and phenotyping sensitivity and specificity rates of 76% and 99%, respectively.  The evidence is limited by relatively small numbers of events and wide CIs.

Several systematic reviews have evaluated the clinical validity of *NUDT15* genetic testing. One reported 91.74% specificity and 43.19% sensitivity for thiopurine myelotoxicity susceptibility, and 84.59% specificity for early leukopenia.

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.

### TPMT Genotype and Phenotype Testing

Three RCTs have compared TPMT testing with no testing and empirical weight-based thiopurine dosing. Genotype testing was used in two studies[17,18] while the remaining RCTs 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 three RCTs are discussed below.

In 2015, Coenen 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).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 one episode of a hematologic ADR (7.4% vs 7.9%; i.e., 0.5% risk difference). The number needed to treat to avoid one episode of a hematologic ADR would be five for at-risk individuals (risk difference in patients with a genetic variant, 20.3; 2.6% vs 22.9%).

In 2011, Newman reported on the results of the TARGET trial, which randomized 333 IBD patients to genotype-guided dosing for empirical weight-based thiopurine dosing.[18] Data were available for 322 (97%) of 333 patients at four 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 (seven heterozygous patients in the genotyping group; two heterozygous patients, one homozygous patient in the nongenotyping group) and therefore was underpowered to detect a difference of the impact of TPMT genotyping.

Sayani (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.[19] 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.[20] In a 2008 study from New Zealand, Gardiner 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.[21] This conclusion was based on analysis of 52 patients with IBD who were started on AZA or 6-MP and followed for nine 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 found the probability of myelotoxicity was 14.3% in the TPMT intermediate group compared with 3.5% in groups with high (wild-type) activity.[22] Authors concluded that determining TPMT activity before initiating treatment with AZA could minimize the risk of myelotoxicity.

### *NUDT15* Genotyping

Yi (2017) analyzed the outcomes of *NUDT15* genotype-based thiopurine dose adjustments in 258 Korean children with acute lymphoblastic leukemia.[23] Variants identified were c.[36_37insGGAGTC; c.415C>T], c.415C>T, c.416G>A, c.52G>A, and c.36_37insGGAGTC. Patients were classified as having normal (wild-type; n = 190), intermediate (heterozygous variant; n = 61), or low (homozygous or compound heterozygous variant; n = 7) *NUDT15* activity. Patients with *TPMT* variants were excluded from the analysis. The low- intermediate- and normal-activity groups were administered 7.5, 24.4, and 31.1 mg/m2/day 6-mercaptopurine, respectively. Therapy interruption was 169, 30, and 16 days for the low-intermediate- and normal-activity groups, respectively, with the low-activity group experiencing significantly longer therapy interruption than the other groups, longer duration of leukopenia (low-activity group 131 days, intermediate-activity group 92 days, normal-activity group 59 days, $p < 0.01$), and lowest blood cell counts.

### Metabolite Marker Testing

Friedman (2018) conducted a multicenter RCT in which 73 patients with clinically active or steroid-dependent IBD were randomized to two different doses of adjunctive allopurinol with thiopurine (azathioprine or mercaptopurine) therapy.[24] The purpose of the trial was to compare the efficacy of the two different doses of allopurinol (50 mg or 100 mg), as the thiopurine dose was modified based on metabolite testing at 4, 12, and 18 weeks. The modifications in dosing were aimed at achieving a therapeutic level of more than 260 pmol/8×$10^8$ red blood cells. The primary outcome was the proportion of patients in steroid-free clinical remission at 24 weeks. Of the 34 patients in the 50 mg group that completed the study, 54% achieved steroid-free remission. This was not significantly different from the 100 mg group, of which 53% of the 27 patients completing the study achieved steroid-free remission. The 6-TGN concentration did not significantly differ between groups and adverse events did

not differ between the two groups. Limitations of this study include a high loss of patients to follow-up.

Garritsen (2018) measured thiopurine metabolite levels in patients with atopic dermatitis and/or chronic dermatitis during maintenance (n=32) and dose escalation (n=8).[25] The patient population included both high and intermediate activity genotypes and 6-TGN metabolite levels varied widely, from 42 to 696 pmol/$8\times10^8$ red blood cells. Interpretation of results is limited due to the small sample size and the heterogeneity in patient genotypes and drug doses.

Meijer (2017) retrospectively reviewed the charts of 24 patients with 6-MMP-induced leukocytopenia.[26] The authors reported that patients' symptoms resolved on altering the treatment regimens. However, due to the retrospective nature of the study, the altering of treatment regimens cannot be attributed directly to metabolite testing.

Wong (2017) reported on the result of a post hoc analysis of the TOPIC trial to address the predictive value of 6-MMP ribonucleotide concentrations one week after treatment initiation for development of hepatotoxicity during the first 20 weeks of treatment.[27] They reported that, in more than 80% of patients, hepatotoxicity could be explained by elevated 6-MMP ribonucleotide concentrations and the independent risk factors of age, sex, and body mass index, allowing personalized thiopurine treatment in IBD to prevent early failure. Placing 174 patients on a stable thiopurine dose showed that those exceeding the 6-MMP ribonucleotide threshold of 3615 pmol/$8\times0^8$ erythrocytes were more likely to have hepatotoxicity (odds ratio, 3.8; 95% CI, 1.8 to 8.0).

Goldberg (2016) retrospectively reviewed medical records of patients (N=169) with IBD who were treated with thiopurines for at least four weeks.[28] Metabolite levels of 6-TGN showed 52% were subtherapeutic, 34% were therapeutic, and 14% were supratherapeutic. Among patients who experienced active disease despite therapy, 86% were managed differently following metabolite testing. Clinical outcomes following the management changes were not reported.

In 2013, Kennedy retrospectively reviewed medical records of patients who had undergone metabolite testing in South Australia.[29] The analysis reported on 151 patients with IBD who had been taking a thiopurine for at least four weeks, underwent at least one 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.

Smith (2013) retrospectively reviewed medical records of 189 patients with IBD who had 6-TGN metabolite monitoring during thiopurine treatment.[30] When 6-TGN concentrations were

below the therapeutic range (n=47), 18 of the patients were given dose increases and two patients were given a combination of allopurinol with azathioprine. When 6-TGN concentrations were above the upper limit of the therapeutic range (n=55), 14 of the patients were given dose reductions. When nonresponders (n=53) were identified, 74% underwent treatment changes including dose increases, switching to a treatment combination of allopurinol and azathioprine or methotrexate, or surgery. Clinical outcomes related to the management changes were not reported.

Armstrong (2011) conducted a retrospective chart review of pediatric patients who had a poor clinical response to thiopurine medication for at least three months for the treatment of IBD (N=70).[31] Testing of 6-TGN found that 32% of values were within therapeutic levels. Management was changed based on metabolite measurements in 25 (36%) of the patients (lowering dose, increasing dose, or switching to methotrexate). Clinical outcomes following the management changes were not reported.

**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 two studies while one used the phenotype enzymatic activity. Of the three RCTs, only the TOPIC trial with a large sample (N=761) was adequately powered while the remaining two 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 the use of metabolite marker testing to manage patients who are treated with thiopurines is limited to two RCTs and a number of retrospective studies. One small RCT had over 50% withdrawal rate due to adverse effects of the treatment, limiting interpretation of results. Another RCT used metabolite testing to adjust thiopurine doses, the purpose of the trial was to compare two different allopurinol doses. Most of the retrospective studies have described changes in management following metabolite testing, but clinical outcomes following the management changes were not reported. Without a control group in these studies, outcomes cannot be compared for patients managed without metabolite testing. It is possible that, in the absence of metabolite testing, patients who were not seeing a benefit or who were experiencing adverse events would have had their treatments adjusted without having metabolite testing.

**SUMMARY OF EVIDENCE**

For individuals who are treated with thiopurines who receive TPMT genotype or 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 one 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 one episode of a hematologic adverse drug reaction would be five 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 two 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.

## PRACTICE GUIDELINE SUMMARY

### NATIONAL COMPREHENSIVE CANCER NETWORK

National Comprehensive Cancer Network (v.1.2018) guidelines on acute lymphoblastic leukemia state :[32]

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

### 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.[33] 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."

## AMERICAN GASTROENTEROLOGICAL ASSOCIATION INSTITUTE

Recommendations from a 2017 American Gastroenterological Association Institute guidelines on therapeutic drug monitoring in IBD are summarized in Table 1.[34]

**Table 1. Evidence-Based Clinical Guidelines on Therapeutic Drug Monitoring in IBD**

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

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

## SUMMARY

There is enough research to show that TPMT genotype or phenotype analysis prior to thiopurine therapy improves health outcomes. In addition, there is enough research to show that TPMT genotype or phenotype analysis in patients on thiopurine therapy with abnormal complete blood count results that do not respond to dose reduction improves health outcomes. These improved outcomes include reduced adverse events due to drug toxicity. Clinical guidelines based on research recommend genotype or phenotype analysis prior to thiopurine therapy and in patients on thiopurine therapy with abnormal complete blood count results that do not respond to dose reduction. Therefore, TPMT genotype or phenotype analysis may be considered medically necessary prior to thiopurine therapy and in patients on thiopurine therapy when there is clinical documentation of abnormal complete blood count results that do not respond to dose reduction when policy criteria are met.

There is not enough research to show that TPMT genotype or phenotype analysis improves health outcomes in people who do not meet the criteria. Therefore, genotypic and/or phenotypic analysis of the TPMT enzyme is considered investigational in all other situations.

There is not enough research to show that analysis of the metabolite markers azathioprine and mercaptopurine improves health outcomes. Therefore, analysis of the metabolite markers azathioprine and mercaptopurine, including 6-methyl-mercaptopurine ribonucleotides and 6-thioguanine nucleotides, is considered investigational.

There is enough research to show that genetic testing of *NUDT15* prior to thiopurine therapy improves health outcomes. In addition, there is enough research to show that *NUDT15* in patients on thiopurine therapy with abnormal complete blood count results that do not respond to dose reduction improves health outcomes. These improved outcomes include reduced adverse events due to drug toxicity. Therefore, genetic testing of *NUDT15* may be considered medically necessary prior to thiopurine therapy and in patients on thiopurine therapy when there is clinical documentation of abnormal complete blood count results that do not respond to dose reduction when policy criteria are met.

There is not enough research to show that genetic testing of *NUDT15* improves health outcomes in people who do not meet the criteria. Therefore, genetic testing of *NUDT15* is considered investigational in all other situations.

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## CODES

| Codes | Number | Description |
|-------|--------|-------------|
| CPT | 0034U | TPMT (thiopurine S-methyltransferase), NUDT15 (nudix hydroxylase 15)(eg, thiopurine metabolism), gene analysis, common variants (ie, TPMT *2, *3A, *3B, *3C, *4, *5, *6, *8, *12; NUDT15 *3, *4, *5) |

| Codes | Number | Description |
|---|---|---|
| | 81306 | NUDT15 (nudix hydrolase 15) (eg, drug metabolism) gene analysis, common variant(s) (eg, *2, *3, *4, *5, *6) |
| | 81335 | TPMT (thiopurine S-methyltransferase) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3) |
| | 81401 | Molecular Pathology Procedure Level 2 |
| HCPCS | None | |

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