



## Azathioprine Therapy and *TPMT* and *NUDT15* Genotype

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### Introduction

Azathioprine (brand names Imuran, Azasan) is an immunosuppressant that belongs to the drug class of thiopurines. It is used with other drugs to prevent kidney transplant rejection and to manage autoimmune and inflammatory conditions such as systemic lupus erythematosus, inflammatory bowel disease, systemic vasculitis, and rheumatoid arthritis.

Azathioprine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. The active metabolites are metabolized and inactivated by the enzyme thiopurine methyltransferase (TPMT) and the enzyme nudix hydrolase 15 (NUDT15). Thus, individuals with reduced activity of either enzyme are exposed to higher levels of thioguanine and have a higher risk of toxicity side effects, including severe bone marrow suppression (myelosuppression).

The FDA-approved drug label states that testing for TPMT and NUDT15 deficiency should be considered in individuals who experience severe bone marrow toxicities or repeated episodes of myelosuppression. The FDA recommends considering an alternative therapy for individuals who are known to have homozygous TPMT or NUDT15 deficiency, or both, and to reduce dosages for individuals who have a no function allele, cautioning that a more substantial dose reduction may be required for individuals who are either TPMT or NUDT15 poor metabolizers (Table 1) (1).

Dosing recommendations for thioguanine based on *TPMT* and *NUDT15* genotype have also been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC, Table 2, Table 3) and the Dutch Pharmacogenetics Working Group (DPWG). Both the CPIC and DPWG guidelines recommend specific dose reductions for individuals who have low or deficient enzyme activity, including starting dose and more information on how and when to adjust the dose e.g., the time allowed to reach steady state after each dose adjustment (2-4).

**Table 1.** FDA Drug Label Dosage and Administration of Azathioprine (2020)

Enzyme	Dosage and administration
TPMT	<ul style="list-style-type: none"> <li>Individuals with thiopurine S-methyl transferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency may be at an increased risk of severe and life-threatening myelotoxicity if receiving conventional doses of azathioprine.</li> <li>Death associated with pancytopenia has been reported in individuals with absent TPMT activity receiving azathioprine.</li> <li>In individuals with severe myelosuppression, consider evaluation for TPMT and NUDT15 deficiency.</li> <li>Consider alternative therapy in individuals with homozygous* TPMT or NUDT15 deficiency and reduced dosages in individuals with heterozygous deficiency.</li> </ul>
NUDT15	

\*This also applies to compound heterozygous TPMT or NUDT15 deficiency, as multiple no function alleles exist. See Tables 4 and 5. This FDA table is adapted (1).

**Table 2.** CPIC Recommended Dosing of Azathioprine by TPMT Phenotype (2018 Update)

Phenotype	Implications for azathioprine phenotypic measures	Dosing recommendations for azathioprine	Classification of recommendations <sup>b</sup>
TPMT normal metabolizer	Lower concentrations of TGN metabolites, higher MeTIMP, this is the “normal” pattern. Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with the normal starting dose <sup>a</sup> (e.g., 2–3 mg/kg/day) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.	Strong
TPMT intermediate metabolizer OR TPMT possible intermediate metabolizer	Moderate to high concentrations of TGN metabolites; low concentrations of MeTIMP. Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with reduced starting doses (30–80% of normal dose) if normal starting dose <sup>a</sup> is 2–3 mg/kg/day (e.g., 0.6–2.4 mg/kg/day), and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after $\geq 40$ –60 mg/m <sup>2</sup> /day (e.g., 20–48 mg/m <sup>2</sup> /day) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment.	Strong
TPMT poor metabolizer	Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease; no MeTIMP metabolites. Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignancy, start with drastically reduced doses (reduce daily dose <sup>a</sup> by 10-fold and dose 3 times weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment.	Strong

MeTIMP, metabolites of thiopurine methyltransferase; TGN, thioguanine nucleotides; TPMT, thiopurine methyltransferase.

<sup>a</sup>Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, a dose reduction might not be recommended for intermediate metabolizers.

<sup>b</sup>Rating scheme described in Supplemental Material (2).

This CPIC table is adapted from (2).

Note, CPIC have also published recommendations for thiopurine dosing when the status of both TPMT and NUDT15 is known. Please see (2).

**Table 3.** CPIC Recommended Dosing of Azathioprine by *NUDT15* Phenotype (2018 Update)

Phenotype	Implications for azathioprine phenotypic measures	Dosing recommendations for azathioprine	Classification of recommendations <sup>b</sup>
NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with the normal starting dose <sup>a</sup> (e.g., 2–3 mg/kg/day) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.	Strong
NUDT15 intermediate metabolizer OR NUDT15 possible intermediate metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with reduced starting doses (30–80% of normal dose) if normal starting dose <sup>a</sup> is 2–3 mg/kg/day (e.g., 0.6–2.4 mg/kg/day), and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment.	Strong
NUDT15 poor metabolizer	Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses <sup>a</sup> (reduce daily dose by 10-fold) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment.	Strong

<sup>a</sup> Normal starting doses vary by race/ethnicity and treatment regimens. If the standard dose is below the normal recommended dose, dose reduction might not be recommended for intermediate metabolizers.

<sup>b</sup> Rating scheme described in Supplemental Material.

This CPIC table is adapted from (2).

Note, CPIC have also published recommendations for thiopurine dosing when the status of both thiopurine methyltransferase (*TPMT*) and nudix hydrolase 15 (*NUDT15*) is known. Please see (2).

## Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine).

All 3 agents have similar effects but are typically used for different indications. Thioguanine is most commonly used to treat myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

There is increasing evidence that DNA testing for *NUDT15* and *TPMT* before initiating thiopurine therapy would be clinically useful. In Europeans and Africans, inherited *TPMT* deficiency is the primary genetic cause of thiopurine intolerance, whereas for Asians, risk alleles in *NUDT15* explains most thiopurine-related myelosuppression (5, 6).

## Drug: Azathioprine

Azathioprine is an immunosuppressive agent used with other drugs to prevent rejection of kidney transplants. It is also used to manage autoimmune and other inflammatory conditions including active rheumatoid arthritis, (1) systemic lupus erythematosus, vasculitis, and inflammatory bowel disease (IBD).

Azathioprine is a slow-acting drug and for IBD, it typically takes at least 3 months of therapy before a therapeutic effect is observed. Therefore, azathioprine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses. Because the discontinuation of azathioprine is associated with a high rate of relapse of IBD, azathioprine is usually continued long-term if there are no adverse effects (7-9).

The use of azathioprine or the related drug mercaptopurine has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (10, 11).

The increased risk of malignancy led to the following boxed label on the FDA-approved drug label for azathioprine:

**Malignancy:** Patients receiving immunosuppressants, including azathioprine, are at increased risk of developing lymphoma and other malignancies, particularly of the skin. Physicians should inform patients of the risk of malignancy with azathioprine. As usual for patients with increased risk for skin cancer, exposure to sunlight and ultraviolet light should be limited by wearing protective clothing and using a sunscreen with a high protection factor (1).

Like all thiopurines, azathioprine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Azathioprine is first metabolized to mercaptopurine (6-MP), which is then bioactivated via hypoxanthine phosphoribosyltransferase. This is followed by a series of reactions to form TGNs, which are the major active metabolites. The cytotoxicity of azathioprine is due, in part, to the incorporation of TGNs into DNA.

Inactivation of 6-MP occurs via 2 major pathways: via methylation, which is catalyzed by TPMT, and via oxidation, which is catalyzed by xanthine oxidase (XO). In cells that have negligible XO levels (e.g., red blood cells), the TPMT activity is inversely correlated with TGN levels. And in individuals who take medicines that inhibit XO (e.g., allopurinol, used to manage gout), the level of azathioprine and its active metabolites may increase to a toxic level.

The NUDT15 enzyme also impacts TGN levels -- this enzyme is involved in the conversion of the active metabolites (TGNs) to inactive phosphorylated metabolites (1).

One of the most frequent adverse reactions to azathioprine is myelosuppression, which can occur in any individual and can typically be reversed by decreasing the dose of azathioprine. However, this risk increases in individuals who have reduced or absent TPMT and/or NUDT15 activity. (1).

Genetic testing to determine *TPMT* and *NUDT15* genotype does not replace the need for regular complete blood count monitoring. One study reported that in individuals with IBD receiving thiopurine therapy, *TPMT* polymorphisms were associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions such as liver damage and pancreatitis. Furthermore, *TPMT* and *NUDT15* variants do not fully explain all cases of bone marrow suppression in individuals taking azathioprine. Therefore, regular blood tests to monitor for side effects are still needed during therapy (12, 13).

## Gene: *TPMT*

The *TPMT* gene encodes thiopurine S-methyltransferase, which is historically classified as a phase II metabolism enzyme. Importantly, *TPMT* is one of the main enzymes involved in the metabolism of thiopurines, such as azathioprine.

The *TPMT* gene is highly polymorphic, with over 40 reported variant star (\*) alleles (14-17). The *TPMT\*1* allele is associated with normal enzyme activity (wild type).

The *TPMT\*1* is considered the wild-type allele when no variants are detected, and is associated with normal enzyme activity and the “normal metabolizer” phenotype. Individuals who are normal metabolizers are more likely to have a typical response to azathioprine and a low risk of myelosuppression; however, all individuals receiving azathioprine require close monitoring. (18-21).

Most individuals are *TPMT* normal metabolizers (~86–97%). Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (18, 19, 22):

- *TPMT\*2* (c.238G>C)
- *TPMT\*3A* (c.460G>A and c.719A>G in *cis*)
- *TPMT\*3C* (c.719A>G)

Individuals who are *TPMT* poor metabolizers (~ 0.3% of individuals of European or African ancestry) have 2 non-functional *TPMT* alleles (Table 4). When treated with standard doses of azathioprine, these individuals will universally experience life-threatening bone marrow suppression because of high levels of TGNs (1).

Individuals who are *TPMT* intermediate metabolizers (approximately 3–14% of the general population) are heterozygous with one no function *TPMT* allele. These individuals may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs and are at an increased risk of moderate to severe bone marrow suppression. However, some of these individuals, approximately 40–70%, can tolerate the full dose of azathioprine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as methylmercaptapurine nucleotides, than homozygous-deficient individuals (18, 19).

**Table 4.** Assignment of likely *TPMT* Phenotype based on Genotype (CPIC, 2018)

Likely phenotype <sup>a</sup>	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*8, *3A/*7
Poor metabolizer	An individual with 2 no function alleles	*3A/*3A, *2/*3A, *3A/*3C, *3C/*4, *2/*3C, *3A/*4
Indeterminate	An individual with 2 uncertain/unknown function alleles OR one normal function allele plus one uncertain allele function allele	*6/*8 *1/*8

*TPMT*, thiopurine methyltransferase; *NUDT15*, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15

<sup>a</sup> See *TPMT* and *NUDT15* Frequency Table and Diplotype-Phenotype Table (3) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.

This CPIC table is adapted from (2).

The frequency of *TPMT* variant alleles vary among different ethnic populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT*\*3A (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (14).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*\*3C (~2%), although *TPMT*\*8 may be more common in African populations than previously thought (~2%). In general, *TPMT*\*2 occurs much less commonly, and *TPMT*\*3B occurs rarely (14, 23).

## Gene: *NUDT15*

The *NUDT15* gene encodes an enzyme that belongs to the nudix hydrolase superfamily. Members of this superfamily catalyze the hydrolysis of nucleoside diphosphates, which are created as a result of oxidative damage (e.g., from treatment with drugs such as thiopurines).

Nudix hydrolase 15 is directly involved in the metabolism of thiopurines, as it catalyzes the conversion of active metabolites (TdGTP) to less toxic metabolites (TdGMP) and in doing so, prevents the incorporation of toxic metabolites into DNA (24).

In individuals with reduced or absent *NUDT15* activity (intermediate or poor metabolizers, Table 5), the reduction in *NUDT15*-mediated degradation of TdGTP results in more TdGTP available for incorporation into DNA, leading to increased DNA damage and cell death. These individuals subsequently have increased sensitivity to thiopurines at standard doses, including an increased risk of severe myelosuppression (25).

Similar to *TPMT*, the *NUDT15* gene is polymorphic, as the [PharmVar Consortium](#) currently has catalogued 21 variant alleles. However, most variants are rare, and the clinical significance of many *NUDT15* star (\*) alleles is currently unclear.

The first *NUDT15* variant associated with thiopurine toxicity is p.R139C (rs116855232), which is present in both the *NUDT15*\*2 and *NUDT15*\*3 haplotypes. This amino acid change results in an unstable protein with almost no enzymatic activity. (25)

The FDA drug label for thioguanine cites one study of 1028 children with acute lymphoblastic leukemia, wherein the tolerated maintenance dose of the related drug, mercaptopurine, varied greatly, depending on the degree of deficiency in *TPMT*, or *NUDT15*, or both. Individuals who were heterozygous deficient for only one gene tolerated between 50–90% of the planned dosage. However, the tolerated dosage dropped to 30–50% of the planned dosage for individuals who were heterozygous deficient for both *TPMT* and *NUDT15*. Individuals who had bi-allelic deficiency of either *TPMT* or *NUDT15* only tolerated 5–10% of the planned mercaptopurine dosage. (24, 26)

Deficiency of *NUDT15* is rare among individuals with European or African ancestry (found in less than 1%); however, *NUDT15* deficiency is more common among individuals with East Asian ancestry (e.g, Korea, China, Japan, Vietnam) (~ 2%) (2, 27, 28).

**Table 5.** Assignment of likely *NUDT15* Phenotype based on Genotype (CPIC, 2018)

Likely phenotype <sup>a</sup>	Genotype	Examples of diplotype
Normal metabolizer	An individual with 2 normal function alleles	*1/*1
Intermediate metabolizer	An individual with one normal function allele PLUS one no function allele	*1/*2, *1/*3
Possible intermediate metabolizer	An individual with one uncertain/unknown function allele PLUS one no function allele	*2/*5, *3/*6
Poor metabolizer	An individual with 2 no function alleles	*2/*2, *2/*3, *3/*3



Table 5. continued from previous page.

Likely phenotype <sup>a</sup>	Genotype	Examples of diplotype
Indeterminate	An individual with 2 uncertain function alleles OR one normal function allele plus one uncertain function allele	*1/*4, *1/*5 *4/*5, *5/*6

TPMT, thiopurine methyltransferase; NUDT15, Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 15

<sup>a</sup> See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table (3) for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.

This CPIC table is adapted from (2).

## Linking Gene Variation with Treatment Response

Genetic variation in the *TPMT* and *NUDT15* genes strongly influences the safety of thiopurine therapy, specifically, influencing the risk of treatment-related bone marrow suppression (29).

Thiopurine methyltransferase deficiency is the primary genetic cause of thiopurine intolerance in Europeans and Africans, and *NUDT15* deficiency is a more common cause in Asians and Hispanics.

The clinical impact of variant *NUDT15* alleles was discovered more recently than for *TPMT*, and there is less evidence available to guide dose adjustments. However, there currently is one clinical trial in progress that addresses azathioprine dosing guided by the status of both *TPMT* and *NUDT15* expression, for the treatment of IBD (5, 30-32).

Currently, *TPMT* and *NUDT15* testing is not required by the FDA before starting treatment with any thiopurine (azathioprine, mercaptopurine, or thioguanine); however, both genes were listed in the recently published FDA Association tables as pharmacogenetics associations with data supporting therapeutic management recommendations (33). Consequently, routine genotyping for *TPMT* and *NUDT15* polymorphisms has not been universally adopted (34).

## Genetic Testing

The NIH Genetic Testing Registry, [GTR](#), displays genetic tests that are currently available for the [azathioprine](#) drug response, and the genes [TPMT](#) and [NUDT15](#). The genes may be tested separately, or together, as part of a test panel that evaluates the drug response to thiopurines.

As with many commercial tests, only the most common variants are usually tested for (e.g., for *TPMT*, the \*2, \*3A, and \*3C allele, which accounts for more than 90% of known inactivating alleles). This means that rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (18, 19, 35-38).

It is important to note that for *TPMT*\*3A, 2 variants, c.460G>A and c.719A>G, are in *cis*. The variant, c.460G>A by itself is *TPMT*\*3B and c.719A>G by itself is *TPMT*\*3C. Most clinical laboratories are unable to phase the 2 variants. In most cases, especially if the individual is of European ancestry, the laboratory will assume the 2 variants are in *cis*, though the possibility of the variants being in *trans* cannot be ruled out.

Phenotype testing is also available for *TPMT*. Phenotype tests directly measure TPMT enzyme activity in red blood cells, but accurate phenotyping is not possible in individuals who have recently received blood transfusions (20). However, one study reported that *TPMT* genotyping was more reliable than phenotyping in identifying individuals at risk of adverse reactions from thiopurine treatment, and several studies reported that the *TPMT* genotype is a better indicator than TPMT activity for predicting TGN accumulation or treatment outcome (21, 39-41).

## Therapeutic Recommendations based on Genotype

This section contains excerpted<sup>1</sup> information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

### 2020 Statement from the US Food and Drug Administration (FDA):

Genetic polymorphisms influence TPMT and NUDT15 activity. Several published studies indicate that patients with reduced TPMT or NUDT15 activity receiving usual doses of 6-MP or azathioprine, accumulate excessive cellular concentrations of active 6-TGNs, and are at higher risk for severe myelosuppression. Because of the risk of toxicity, patients with TPMT or NUDT15 deficiency require alternative therapy or dose modification.

Approximately 0.3% (1:300) of patients of European or African ancestry have two loss-of-function alleles of the TPMT gene and have little or no TPMT activity (homozygous deficient or poor metabolizers), and approximately 10% of patients have one loss-of-function TPMT allele leading to intermediate TPMT activity (heterozygous deficient or intermediate metabolizers). The *TPMT\*2*, *TPMT\*3A*, and *TPMT\*3C* alleles account for about 95% of individuals with reduced levels of TPMT activity. NUDT15 deficiency is detected in <1% of patients of European or African ancestry. Among patients of East Asian ancestry (i.e., Chinese, Japanese, Vietnamese), 2% have two loss-of-function alleles of the *NUDT15* gene, and approximately 21% have one loss-of-function allele. The p.R139C variant of *NUDT15* (present on the \*2 and \*3 alleles) is the most commonly observed, but other less common loss-of-function *NUDT15* alleles have been observed.

[...]

Patients with thiopurine S-methyl transferase (TPMT) or nucleotide diphosphatase (NUDT15) deficiency may be at an increased risk of severe and life-threatening myelotoxicity if receiving conventional doses of azathioprine. Death associated with pancytopenia has been reported in patients with absent TPMT activity receiving azathioprine. In patients with severe myelosuppression, consider evaluation for TPMT and NUDT15 deficiency. Consider alternative therapy in patients with homozygous TPMT or NUDT15 deficiency and reduced dosages in patients with heterozygous deficiency.

[...]

TPMT and NUDT15 Testing: Consider genotyping or phenotyping patients for TPMT deficiency and genotyping for NUDT15 deficiency in patients with severe myelosuppression. TPMT and NUDT15 testing cannot substitute for complete blood count (CBC) monitoring in patients receiving azathioprine. Accurate phenotyping (red blood cell TPMT activity) results are not possible in patients who have received recent blood transfusions.

[...]

#### Patients with TPMT and/or NUDT15 Deficiency

Consider testing for TPMT and NUDT15 deficiency in patients who experience severe bone marrow toxicities. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction.

Homozygous deficiency in either TPMT or NUDT15 Because of the risk of increased toxicity, consider alternative therapies for patients who are known to have TPMT or NUDT15 deficiency.

<sup>1</sup> The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.



Heterozygous deficiency in TPMT and/or NUDT15 Because of the risk of increased toxicity, dosage reduction is recommended in patients known to have heterozygous deficiency of TPMT or NUDT15. Patients who are heterozygous for both TPMT and NUDT15 deficiency may require more substantial dosage reductions.

**Please review the complete therapeutic recommendations that are located here: (1).**

## 2018 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC)

### TPMT recommendation

If starting doses are already high (e.g., 75 mg/m<sup>2</sup> of mercaptopurine), as is true in some ALL treatment regimens, lower than normal starting doses should be considered in TPMT intermediate metabolizers and markedly reduced doses (10-fold reduction) should be used in TPMT poor metabolizers. This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL. Even at these markedly reduced dosages, erythrocyte TGN concentrations in TPMT poor metabolizers remain well above those tolerated and achieved by the majority of patients (who are TPMT normal metabolizers).

In some nonmalignant conditions, alternative agents may be chosen for TPMT intermediate or poor metabolizers rather than reduced doses of thiopurines; if thiopurines are used, full starting doses are recommended for TPMT normal metabolizers, reduced doses (30–80% of target dose) in TPMT intermediate metabolizers, and substantially reduced doses (or use of an alternative agent) in TPMT poor metabolizers.

Some of the clinical data upon which dosing recommendations are based rely on measures of TPMT phenotype rather than genotype; however, because TPMT genotype is strongly linked to TPMT phenotype, these recommendations apply regardless of the method used to assess TPMT status.

### NUDT15 recommendation

Similar to TPMT, tolerated mercaptopurine dosage is also correlated with the number of nonfunctional alleles of the NUDT15 gene. In fact, the degree of thiopurine intolerance (e.g., for mercaptopurine) is largely comparable between carriers of TPMT vs. NUDT15 decreased function alleles, there remains a paucity of multi-ethnic studies examining both TPMT and NUDT15 variants.

Therefore, our NUDT15 recommendations parallel those for TPMT. For NUDT15 normal metabolizers (*NUDT15*\*1/\*1), starting doses do not need to be altered. For NUDT15 intermediate metabolizers (e.g., *NUDT15*\*1/\*3), reduced starting doses should be considered to minimize toxicity, particularly if the starting doses are high (e.g., 75 mg/m<sup>2</sup>/ day for mercaptopurine). For NUDT15 poor metabolizers (e.g., *NUDT15*\*3/\*3), substantially reduced doses (e.g., 10 mg/m<sup>2</sup>/ day of mercaptopurine) or the use of an alternative agent should be considered.

As for TPMT, there is substantial variability in the tolerated thiopurine dosages within NUDT15 intermediate metabolizers, with a minority of individuals who do not seem to require significant dose reduction. Therefore, genotype-guided prescribing recommendations apply primarily to starting doses; subsequent dosing adjustments should be made based on close monitoring of clinical myelosuppression (or disease-specific guidelines). In contrast, a full dose of mercaptopurine poses a severe risk of prolonged hematopoietic toxicity in NUDT15 poor metabolizers and pre-emptive dose reductions are strongly recommended.

The NUDT15 poor metabolizer phenotype is observed at a frequency of about 1 in every 50 patients of East Asian descent, which is more common than the TPMT poor metabolizer phenotype in Europeans, and, thus, genotyping *NUDT15* in the Asian populations may be of particular clinical importance. NUDT15 deficiency is also more prevalent in individuals of Hispanic ethnicity, particularly those with high levels of Native American genetic ancestry.

Please review the complete therapeutic recommendations, which include CPIC's recommended course of action if both TPMT and NUDT15 genotypes are known, located here: (2).

## 2019 Summary of recommendations from the Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

The Dutch Pharmacogenetics Working Group considers genotyping before starting azathioprine or 6-mercaptopurine to be essential for drug safety. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection.

### TPMT Intermediate Metabolizer

Grade 2 leukopenia occurs in 23% of these patients with normal therapy for immunosuppression. The genetic variation increases the quantity of the active metabolites of azathioprine and mercaptopurine.

Recommendation:

#### IMMUNOSUPPRESSION

- Start with 50% of the standard dose

Adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.

Dose adjustment is not required for doses lower than 1.5 mg/kg per day for azathioprine or 0.75 mg/kg per day for mercaptopurine.

#### LEUKEMIA

- Start with 50% of the standard mercaptopurine dose, or start with the standard dose and reduce to 50% if side effects necessitate a dose reduction

It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.

The initial dose should be adjusted based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also NUDT15 IM or NUDT15 PM.

### TPMT Poor Metabolizer

Grade 2 leukopenia and intolerance occurred in 98% of these patients with standard therapy. The gene variation increases the quantities of the active metabolites of azathioprine and mercaptopurine.

Recommendation:

- Choose an alternative or use 10% of the standard dose.

Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and effectiveness.

If the dose is decreased: advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur

### Background information:

Azathioprine is converted in the body to mercaptopurine. Mercaptopurine is an inactive pro-drug, which is converted to the active metabolites - thioguanine nucleotides - in the body.

Two catabolic routes reduce mercaptopurine bio-availability for thioguanine nucleotide formation. Thiopurine methyltransferase (TPMT) catalyses S-methylation of both mercaptopurine and the 6- mercaptopurine ribonucleotides formed in the metabolic pathway. In addition to this, mercaptopurine is oxidised to the inactive 6-thiouric acid by the enzyme xanthine oxidase (XO), which occurs primarily in the liver and intestines.

For more information about the TPMT phenotypes: see the general background information about TPMT on the KNMP Knowledge Bank or on [www.knmp.nl](http://www.knmp.nl) (search for TPMT).

### **NUDT15 Intermediate Metabolizer**

Grade  $\geq 2$  leukopenia occurs in 42% of these patients with standard immunosuppression therapy. The gene variation increases the concentration of the fully activated metabolite of azathioprine and mercaptopurine.

#### **IMMUNOSUPPRESSION**

- start with 50% of the standard dose

Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.

#### **LEUKEMIA**

- start at 50% of the standard mercaptopurine dose, or start with the standard dose and reduce to 50% if side effects necessitate a dose reduction

It is not known whether dose reduction in advance results in the same efficacy as dose reduction based on toxicity.

Adjustment of the initial dose should be performed based on toxicity (monitoring of the blood counts) and efficacy.

Note: more stringent dose reductions are necessary if the patient is also TPMT IM or TPMT PM.

### **NUDT15 Poor Metabolizer**

Grade  $\geq 2$  leukopenia occurs in 96% of these patients with standard therapy. The gene variation increases the concentration of the fully activated metabolite of azathioprine and mercaptopurine.

- avoid azathioprine and mercaptopurine
- if it is not possible to avoid azathioprine and mercaptopurine: use 10% of the standard dose and advise patients to seek medical attention when symptoms of myelosuppression (such as severe sore throat in combination with fever, regular nosebleeds and tendency to bruising) occur

Any adjustment of the initial dose should be guided by toxicity (monitoring of blood counts) and efficacy.

Background information:

NUDT15 reverses the last step in the formation of the active metabolite of mercaptopurine and its precursor azathioprine. It converts 6-thio-deoxyguanosine triphosphate (6-thio-dGTP), which is incorporated in DNA, to 6-thio-deoxyguanosine monophosphate (6-thio-dGMP). Lower metabolic activity of NUDT15 therefore leads to increased intracellular concentrations of the active metabolite 6- thio-dGTP. This increases the risk of side effects, such as myelosuppression.

For more information about TPMT and NUDT15 phenotypes: see the general background information in the KNMP Knowledge Bank or on [www.knmp.nl](http://www.knmp.nl) (search for TPMT or NUDT15).

**Please review the complete therapeutic recommendations that are located here:** (3, 4).

## Nomenclature for Selected *TPMT* and *NUDT15* Alleles

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
<i>TPMT</i> *2	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
<i>TPMT</i> *3A	This allele contains 2 variants in cis: c.460G>A and c.719A>G			
<i>TPMT</i> *3B	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
<i>TPMT</i> *3C	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345
<i>NUDT15</i> *3	p.R139C c.415C>T	NM_018283.4:c.415C>T	NP_060753.1:p.Arg139Cys	rs116855232

Note: the p.R139C variant of nudix hydrolase 15 (*NUDT15*) is present on the *NUDT15*\*2 and *NUDT15*\*3 alleles.

The [TPMT Nomenclature Committee](#) defines the nomenclature and numbering of novel thiopurine methyltransferase (*TPMT*) variants.

Nomenclature for *NUDT15* is available from the Pharmacogene Variation ([PharmVar](#)) Consortium.

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society ([HGVS](#))

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To view an earlier version of this summary, please see:

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