Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on *TPMT* and *NUDT*15 Genotypes: 2018 Update

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Thiopurine methyltransferase (TPMT) activity exhibits a monogenic codominant inheritance and catabolizes thiopurines. *TPMT* variant alleles are associated with low enzyme activity and pronounced pharmacologic effects of thiopurines. Loss-of-function alleles in the *NUDT15* gene are common in Asians and Hispanics and reduce the degradation of active thiopurine nucleotide metabolites, also predisposing to myelosuppression. We provide recommendations for adjusting starting doses of azathioprine, mercaptopurine, and thioguanine based on *TPMT* and *NUDT15* genotypes (updates on www.cpicpgx.org).

This document is an update to the Clinical Implementation Consortium (CPIC) Guidelines for Thiopurine Methyltransferase Genotype and Thiopurine guideline updated last in April 2013. The guideline text, evidence table, and recommendations have been updated to reflect new evidence. Specifically, this guideline adds a recommendation for *NUDT15* genotype with minor changes to the *TPMT* recommendations. Although most of the dosing recommendations have been generated from clinical studies in just a few diseases, we have extrapolated recommended doses to all conditions, given the pharmacokinetic nature of the genotype/phenotype associations. CPIC guidelines are published and periodically updated on www.cpicpgx.org. Detailed guidelines for use of phenotypic tests (e.g., TPMT activity and thiopurine metabolite levels) as well as analyses of cost-effectiveness are beyond the scope of this document.

FOCUSED LITERATURE REVIEW

A systematic literature review focused on *TPMT* and *NUDT15* genotypes and thiopurine use was conducted (details in **Supplement**). Definitive reviews[1–4] were relied upon to summarize much of the earlier literature.

DRUGS: THIOPURINES

Background. Three thiopurines are used clinically: azathioprine (a prodrug for mercaptopurine), mercaptopurine, and thioguanine. Although all three medications share many of the same pharmacologic effects, mercaptopurine and azathioprine are generally used for nonmalignant immunologic disorders, mercaptopurine for lymphoid malignancies, and thioguanine for myeloid leukemias. Because azathioprine is a prodrug for mercaptopurine, the two drugs can be considered to have identical interactions with thiopurine methyltransferase (TPMT) and nudix (nucleoside diphosphate linked moiety X)-type motif 15 (NUDT15). Recommendations for individuals with variants in one or both of these genes will be addressed in detail in the following sections.

GENES: *TPMT* AND *NUDT15*

Background

TPMT. TPMT activity is inherited as a monogenic, autosomal codominant trait (**Figure S1**). Three *TPMT* single nucleotide polymorphisms (SNPs), which result in unstable proteins and enhanced TPMT protein degradation,[2,3] account for over 90% of low activity phenotypes and are the most common inactivating

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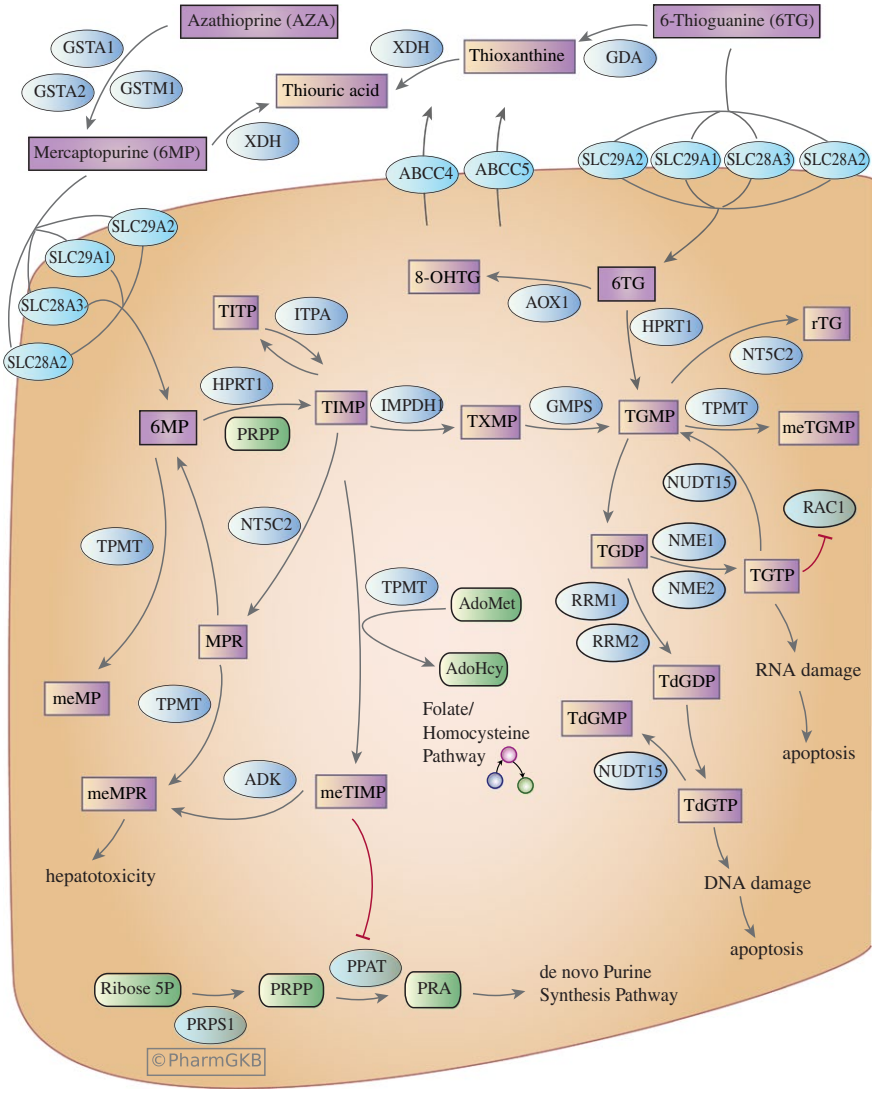

Figure 1 Metabolism of azathioprine, thioguanine, and mercaptopurine.[41] Permission has been given by PharmGKB and Stanford to use figure (https://www.pharmgkb.org/pathway/PA2040). Pathway images and data are available under a Creative Commons BY-SA 4.0 license.

alleles, and so genotyping tests including these three variants have a high likelihood of being informative for TPMT phenotype.[5,6] Complementary phenotype laboratory tests can be helpful adjuncts to genotyping tests (**Supplement**, Other Considerations).[7]

TPMT catabolizes mercaptopurine to an inactive methylmercaptopurine base, leaving less parent drug available for eventual anabolism to active thioguanine nucleotides (TGNs; **Figure 1**). The secondary metabolite of mercaptopurine, thioinosine monophosphate (TIMP), is also a substrate for TPMT, and methyl-TIMP (and its further phosphorylated metabolites, methylmercaptopurine nucleotides (MeMPN)) have pharmacologic activity (mostly immunosuppressive), inhibit *de novo* purine synthesis, and may contribute to some of the adverse effects of mercaptopurine, generally hepatotoxicity.[2,8,9] Individuals who inherit two loss-of-function *TPMT* alleles (homozygous or compound heterozygous *TPMT* deficient individuals) are at very high risk for life-threatening myelosuppression, due to very high TGNs, if given conventional doses of mercaptopurine (or azathioprine). Despite having higher TGNs than wild-type patients, only about 30–60% of *TPMT* heterozygotes cannot tolerate full doses of mercaptopurine or azathioprine.[8,10,11] Good thiopurine tolerance in some heterozygotes may be because, although they have higher TGNs than homozygous wild-type patients, they have lower concentrations (and, thus, fewer toxic effects) of the MeMPNs than do normal metabolizers, which may offset the toxic effects of having higher TGNs. Thus, there is less of a consensus over how to dose azathioprine and mercaptopurine in patients who are heterozygous for *TPMT* compared with those who are homozygous, although they are at a higher risk for toxicity compared with patients carrying two normal function alleles.[12]

Although there is lower affinity between thioguanine and TPMT than between mercaptopurine and TPMT, TPMT significantly affects thioguanine pharmacokinetics and its cytotoxic effects.[12–16] Thioguanine is directly metabolized by TPMT to inactive methylthioguanine base, leaving less drug available for anabolism by HPRT and other enzymes to active TGN metabolites. There is not a pharmacologically active secondary metabolite

of thioguanine to undergo activation via TPMT (i.e., there are no methyl-TIMP or methylmercaptopurine nucleotides). As a result, patients receiving thioguanine are able to tolerate substantially higher TGN concentrations than do those receiving mercaptopurine or azathioprine.[15] Within each TPMT phenotypic group, the initial recommended relative dosage decreases are similar for thioguanine, mercaptopurine, and azathioprine (**Table 2**).

NUDT15 background. Through agnostic genomewide association studies, variants in *NUDT15* have been identified that strongly influence thiopurine tolerance in patients with acute lymphoblastic leukemia (ALL)[17] and those with inflammatory bowel diseases.[18] As a nucleoside diphosphatase, NUDT15 catalyzes the conversion of cytotoxic thioguanine triphosphate (TGTP) metabolites to the less toxic thioguanine monophosphate. Defects in NUDT15-mediated degradation of TGTP result in more TGTP available for incorporation into DNA (DNA-TG; the primary antileukemic metabolite[19]), thus allowing for DNA damage and apoptosis. The SNP (rs116855232; c.415C>T) causing p.R139C was the first *NUDT15* variant linked to thiopurine toxicity. It was shown that this amino acid change results in a nearly complete loss of enzymatic activity and protein stability *in vitro*. Patients carrying this allele showed excessive DNA-TG and severe myelosuppression.[20] In children with ALL, patients homozygous for the p.R139C variant allele tolerated only 8% of the standard dose of mercaptopurine, whereas tolerated dose intensity was 63% and 83.5% for those heterozygous and wildtype for this SNP, respectively.[17] Although most clinical studies focused on mercaptopurine, *in vitro* experiments using laboratory models indicated similar influence of NUDT15 on the cytotoxicity of azathioprine and thioguanine.[20] Additional variant alleles have been identified with varying prevalence among differing ancestral groups and varying degrees of functional effects (***NUDT15* Allele Functionality Table and Frequency Table**)[21]. The variant p.R139C has been studied most extensively in patients receiving thiopurine therapy, thus providing the strongest evidence for clinical implementation. Subsequent studies reported additional variants, most of which are rare, and their associations with clinical thiopurine toxicity do not rise to clinical actionability at this point, even though some showed decreased NUDT15 activity in *in vitro*. For this reason, these variants (**4–*9*) are designated as unclear function but may be clarified as more data emerge.

Inherited TPMT deficiency is the primary genetic cause of thiopurine intolerance in Europeans and Africans, whereas risk alleles in *NUDT15* explain the majority of thiopurine-related myelosuppression in Asians and are also common in Hispanics.

Genetic test interpretation

Genetic testing analyzes the DNA sequence at specific SNP locations in the *TPMT* and *NUDT15* genes (**Supplement**). Each named star (*) allele is defined by the genotype at one or more specific loci (***TPMT* Allele Definition Table**[21,22] and ***NUDT15* Allele Definition Table**[21,23]) and is associated with a level of enzyme activity (***TPMT* Allele Functionality Table**[21,22] and ***NUDT15* Allele Functionality Table**[21,23]). **Table 1** summarizes the assignment of the likely TPMT and NUDT15 phenotypes, based on the most common * allele diplotypes, and these assignments are used to link genotypes with thiopurine prescribing recommendations. Of note, the phenotype of "possible intermediate metabolizer" has been introduced to this guideline to describe an individual carrying one uncertain/unknown function allele plus one known no function allele, as this individual should be treated with "at least" the same precautions as would apply to an intermediate metabolizer. Although inactivating *TPMT* and *NUDT15* alleles have been identified in multiple populations (***TPMT* Frequency Table**[21,22] and ***NUDT15* Frequency Table**[21,23]), one of the limitations inherent in a commercial genotype-only test is that rare or previously undiscovered variants may not be included.

Available genetic test options

See **Supplement** and the Genetic Testing Registry (https://www.ncbi.nlm.nih.gov/gtr/) for more information on commercially available clinical testing options.

Incidental findings

There are no diseases or phenotypic traits that have been linked to variation in *TPMT* or *NUDT15* in the absence of thiopurine treatment.[2]

Linking genetic variability to variability in drug-related phenotypes

There is substantial evidence linking *TPMT* and *NUDT15* genotype with phenotypic variability (see **Tables S1 and S2**). Pre-emptive dose adjustments based on *TPMT* genotype have reduced thiopurine-induced adverse effects without compromising desired antitumor and immunosuppressive therapeutic effects in several clinical settings (**Table S1**). Similarly, retrospective studies strongly indicate that patients with loss-of-function *NUDT15* alleles are at excessive risk of thiopurine toxicity if the standard dose is administered. This body of evidence, rather than randomized clinical trials, provides the basis for most of the dosing recommendations in **Tables 2 and 3**.

Therapeutic recommendations

Thiopurines are used to treat malignant and nonmalignant conditions, and, thus, the approach to dosing adjustments and the propensity to initiate therapy at higher vs. lower starting doses based on TPMT/NUDT15 status may differ depending on the clinical indication. Thiopurines have a unique role in the treatment of many malignancies. The "normal" starting doses of thiopurines are generally "high" because they have been derived from trials that have been heavily weighted by the ~ 90% of the population who are wildtype for *TPMT* and *NUDT15* and receive maximal tolerable doses by the standards of anticancer treatment (hence, full doses should be given to those who are normal metabolizers for TPMT and NUDT15; **Tables 2 and 3**). Because the level of thiopurine tolerance is highly correlated with genetic ancestry,[17] the "normal" starting doses can also vary by geographic regions and clinical practice.

TPMT *recommendation.* If starting doses are already high (e.g., 75 mg/m^2 of mercaptopurine), as is true in some ALL treatment

Table 1 Assignment of likely TPMT and NUDT15 phenotypes based on genotypes

Likely phenotype[a]	Genotypes	Examples of diplotypes
Assignment of likely TPMT phenotypes based on genotypes		
Normal metabolizer	An individual carrying two normal function alleles	*1/*1
Intermediate metabolizer	An individual carrying one normal function allele PLUS one no function allele	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4
Possible intermediate metabolizer	An individual carrying one uncertain/unknown function allele PLUS one no function allele	*2/*8, *3A/*7
Poor metabolizer	An individual carrying two no function alleles	*3A/*3A, *2/*3A, *3A/*3C, *3C/*4, *2/*3C, *3A/*4
Indeterminate	An individual carrying two uncertain/unknown function alleles OR one normal function allele plus one uncertain allele function allele	*6/*8 *1/*8
Assignment of likely NUDT15 phenotypes based on genotypes		
Normal metabolizer	An individual carrying two normal function alleles	*1/*1
Intermediate metabolizer	An individual carrying one normal function allele PLUS one no function allele	*1/*2, *1/*3
Possible intermediate metabolizer	An individual carrying one uncertain function allele PLUS one no function allele	*2/*5, *3/*6
Poor metabolizer	An individual carrying two no function alleles	*2/*2, *2/*3, *3/*3
Indeterminate	An individual carrying two 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
[a]See **TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table**[21–23] for estimates of phenotype frequencies among different ethnic/geographic groups and for a more comprehensive list of predicted metabolizer phenotypes.

regimens, lower than normal starting doses should be considered in TPMT intermediate metabolizers[11,15,24,25] and markedly reduced doses (10-fold reduction) should be used in TPMT poor metabolizers[26] (**Table 2**). This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL.[27] 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).[4,26]

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,[28,29] and substantially reduced doses (or use of an alternative agent) in TPMT poor metabolizers (**Table 2**).[4,30]

Some of the clinical data upon which dosing recommendations are based (**Table 2**) rely on measures of TPMT phenotype rather than genotype; however, because *TPMT* genotype is strongly linked to TPMT phenotype,[5–7,31] 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.[17,18] In fact, the degree of thiopurine intolerance (e.g., for mercaptopurine) is largely comparable between carriers of *TPMT* vs. *NUDT15* decreased function alleles,[17] although 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*; **Table 2**), reduced starting doses should be considered to minimize toxicity, particularly if the starting doses are high (e.g., 75 mg/m^2/day for mercaptopurine). For NUDT15 poor metabolizers (e.g., *NUDT15*3/*3*), substantially reduced doses (e.g., 10 mg/m^2/day of mercaptopurine) or the use of an alternative agent should be considered (**Table 2**).[20]

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.[17,20] 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.[32,33]

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

Table 2 Recommended dosing of thiopurines by TPMT phenotype

	Mercaptopurine			Azathioprine		Thioguanine		
Phenotype	Implications for mercaptopurine and azathioprine phenotypic measures	Dosing recommendations for mercaptopurine	Classification of recommendations	Dosing recommendations for azathioprine	Classification of recommendations	Implications for thioguanine phenotypic measures	Dosing recommendations for thioguanine	Classification of recommendations[b]
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 normal starting dose[a] (e.g., 75 mg/m^2/day or 1.5 mg/kg/day) and adjust doses of mercaptopurine (and of any other myelosuppressive therapy) without any special emphasis on mercaptopurine compared with other agents. Allow at least 2 weeks to reach steady-state after each dose adjustment.[4,27,30]	Strong	Start with normal starting dose[a] (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.[4,30,37]	Strong	Lower concentrations of TGN metabolites, but note that TGN after thioguanine are 5–10 × higher than TGN after mercaptopurine or azathioprine. Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with normal starting dose[a] (e.g., 40–60 mg/m^2/day) and adjust doses of thioguanine and of other myelosuppressive therapy without any special emphasis on thioguanine. Allow 2 weeks to reach steady-state after each dose adjustment.[4,16]	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[a] is ≥ 75 mg/m^2/day or ≥ 1.5 mg/kg/day (e.g., start at 22.5–60 mg/m^2/day or 0.45–1.2 mg/kg/day) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing mercaptopurine over other agents.[4,11,15,24,25,27,30,38,39] If normal starting dose is already < 75 mg/m^2/day or < 1.5 mg/kg/day, dose reduction may not be recommended.	Strong	Start with reduced starting doses (30–80% of normal dose) if normal starting dose[a] 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.[4,30,37,38]	Strong	Moderate to high concentrations of TGN metabolites; but note that TGN after thioguanine are 5–10 × higher than TGN after mercaptopurine or azathioprine. Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with reduced doses (50–80% of normal dose) if normal starting dose[a] is ≥ 40–60 mg/m^2/day (e.g., 20–48 mg/m^2/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. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing thioguanine over other agents.[4,16]	Moderate

(Continues)

Table 2 (Continued)

	Mercaptopurine			Azathioprine		Thioguanine		
Phenotype	Implications for mercaptopurine and azathioprine phenotypic measures	Dosing recommendations for mercaptopurine	Classification of recommendations	Dosing recommendations for azathioprine	Classification of recommendations	Implications for thioguanine phenotypic measures	Dosing recommendations for thioguanine	Classification of recommendations[b]
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 malignancy, start with drastically reduced doses (reduce daily dose[a] by 10-fold and reduce frequency to thrice weekly instead of daily (e.g., 10 mg/m^2/day given just 3 days/week) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing mercaptopurine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.[4,26,30,38]	Strong	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignancy, start with drastically reduced doses (reduce daily dose[a] by 10-fold and dose thrice 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.[28,30,37,38,40]	Strong	Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease. Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.	Start with drastically reduced doses[16] (reduce daily dose[a] by 10-fold and dose thrice weekly instead of daily) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing thioguanine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.[4]	Strong

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

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

[b]Rating scheme described in **Supplemental Material**.

Table 3 Recommended dosing of thiopurines by NUDT15 phenotype

	Mercaptopurine			Azathioprine		Thioguanine	
Phenotype	Implications for thiopurine phenotypic measures	Dosing recommendations for mercaptopurine	Classification of recommendations	Dosing recommendations for azathioprine	Classification of recommendations	Dosing recommendations for thioguanine	Classification of recommendations[b]
NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression	Start with normal starting dose[a] (e.g., 75 mg/m^2/day or 1.5 mg/kg/day) and adjust doses of mercaptopurine (and of any other myelosuppressive therapy) without any special emphasis on mercaptopurine compared with other agents. Allow at least 2 weeks to reach steady-state after each dose adjustment.[4,27,30]	Strong	Start with normal starting dose[a] (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.[4,30,37]	Strong	Start with normal starting dose[a] (40–60 mg/m^2/day). Adjust doses of thioguanine and of other myelosuppressive therapy without any special emphasis on thioguanine. Allow 2 weeks to reach steady-state after each dose adjustment.[4,16]	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[a] is ≥ 75 mg/m^2/day or ≥ 1.5 mg/kg/day (e.g., start at 22.5–60 mg/m^2/day or 0.45–1.2 mg/kg/day) and adjust doses of mercaptopurine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing mercaptopurine over other agents.[4,11,15,24,25,27,30,38,39] If normal starting dose is already < 75 mg/m2/day or < 1.5 mg/kg/day, dose reduction may not be recommended.	Strong	Start with reduced starting doses (30–80% of normal dose) if normal starting dose[a] 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.[4,30,37,38]	Strong	Start with reduced doses (50–80% of normal dose) if normal starting dose[a] is ≥ 40–60 mg/m^2/day (e.g., 20–48 mg/m^2/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. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing thioguanine over other agents.[4,16]	Moderate

(Continues)

Table 3 (Continued)

	Mercaptopurine		Azathioprine		Thioguanine	
Phenotype	Implications for thiopurine phenotypic measures	Dosing recommendations for mercaptopurine	Classification of recommendations	Dosing recommendations for azathioprine	Classification of recommendations	Dosing recommendations for thioguanine	Classification of recommendations[b]
NUDT15 poor metabolizer	Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression	For malignancy, initiate dose at 10 mg/m^2/day and adjust dose based on myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing mercaptopurine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.[4,26,30,38]	Strong	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses[a] (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.[28,30,37,38,40]	Strong	Reduce doses to 25% of normal dose[a] and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady-state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing thioguanine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.[4]	Strong

[a]Normal starting doses vary by race/ethnicity and treatment regimens. If standard dose is below normal recommended dose, dose reduction might not be recommended for intermediate metabolizers.
[b]Rating scheme described in **Supplemental Material**.

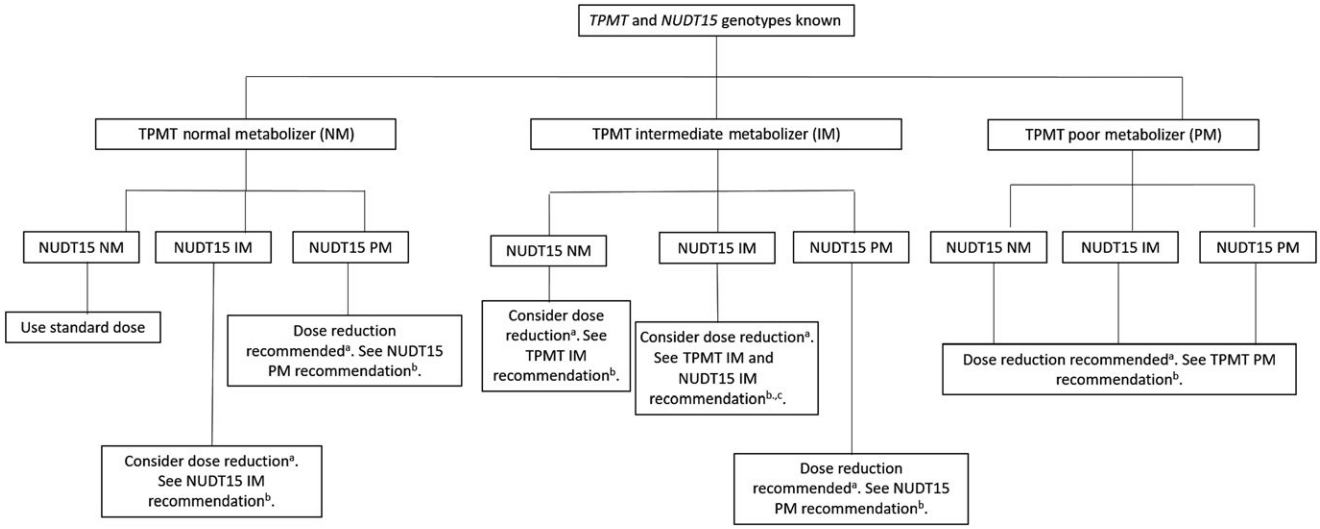

Figure 2 Recommended starting doses of thiopurines by thiopurine methyltransferase (TPMT) and NUDT15 phenotype. [a]Whether a dose reduction is recommended from the starting dose depends on the level of the standard starting dose; for example, if the standard starting dose of mercaptopurine is 75 mg/m^2/day or higher, then a lower starting dose may be considered in intermediate metabolizers and would be recommended in poor metabolizers, whereas if the starting dose is 50 mg/m^2/day or lower, a reduced starting dose may not be necessary in intermediate metabolizers. [b]See **Table 2** for recommendation. [c]For patients who are intermediate metabolizers for both TPMT and NUDT15, further dose reduction might be needed compared with those who are only intermediate metabolizers with respect to one gene (*TPMT* or *NUDT15*).

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.[17]

TPMT *and* NUDT15 *recommendation.* **Figure 2** outlines the recommended course of action if both *TPMT* and *NUDT15* genotypes are known. There have been reports of patients with intermediate metabolizer status for both TPMT and NUDT15 (i.e., compound intermediate metabolizers), and there was a trend for a lower thiopurine tolerance in these individuals compared with intermediate metabolizers for only TPMT or NUDT15. The two genes are independent: the likelihood of an individual being an intermediate metabolizer for both genes depends upon the population frequencies for variant alleles. For example, given an estimate of no function alleles for *NUDT15* of 11% and of no function alleles for *TPMT* of 2%, the frequency of the compound intermediate phenotype would be estimated to be 0.2%. However, the evidence for a different starting dosage recommendation for the compound intermediate metabolizers remains limited.

Recommendations for incidental findings

Not applicable.

Other considerations

If test results are available for only one gene (*TPMT* or *NUDT15*, but not both), prescribing recommendations based on that gene's results may be implemented with the caveat that the other gene's results are missing and may have important implications. The higher frequency of decreased function *NUDT15* variants among individuals of Asian and Hispanic backgrounds and of TPMT variants in those with European and African backgrounds should be considered. In addition, there may be other reasons underlying poor tolerance to thiopurines that are not related to *TPMT* or to *NUDT15* genetic variation.

Complementary clinical laboratory tests are available to measure thiopurine metabolites in erythrocytes: TGNs (for mercaptopurine, azathioprine, and thioguanine) and MeMPNs (or MeTIMP) for those on mercaptopurine or azathioprine (see **Supplement** for details on associations with TPMT). Erythrocyte TGNs or MeMPNs are not related to *NUDT15* genotypes[34–36], but there is evidence that intermediate and poor metabolizers for NUDT15 accumulate higher levels of DNA-TG than normal metabolizers given the same mercaptopurine dosage.[20] Thus, currently available erythrocyte therapeutic drug monitoring tests do not distinguish NUDT15 metabolizer phenotypes.

Implementation of this guideline

The guideline supplement contains resources that can be used within electronic health records to assist clinicians in applying genetic information to patient care for the purpose of drug therapy optimization (see *Resources to incorporate pharmacogenetics into an electronic health record with clinical decision support* sections of the **Supplement**).

POTENTIAL BENEFITS AND RISKS FOR THE PATIENT

The benefits of pre-emptive *TPMT* testing are that doses that are customized based on TPMT status reduce the likelihood of acute myelosuppression without compromising disease control.[4,8,24,25] The risks would be that a proportion of TPMT intermediate metabolizers may spend a period of time at lower thiopurine doses than they can eventually tolerate, because only ~30–60% of *TPMT* heterozygous patients receiving conventional thiopurine doses experience severe myelosuppression.[4,8,11] However,

because steady state is reached in 2–4 weeks, any period of "underdosing" should be short, and, using this approach, at least in ALL and in inflammatory bowel disease, outcomes were not compromised.[4,8,24,25,28]

Similar benefits are expected with pre-emptive *NUDT15* genotyping, especially for Asian patients, given that these variants have comparable effects as risk alleles in *TPMT*. At least in ALL, leukemia cells with no function *NUDT15* alleles are also more sensitive to mercaptopurine[20] and, thus, in theory, *NUDT15* genotype guided dosing would not compromise antileukemic efficacy of this drug.

A possible risk to the patient is an error in genotyping.[4] As shown in preclinical models, some *TPMT* and/or *NUDT15* variants may not be included in the genotype test used, and patients with these variants may be assigned a "wildtype" (**1*) genotype by default. Thus, an assigned "wildtype" allele could potentially harbor a no or decreased function variant. Because genotypes are life-long test results, any such error could stay in the medical record for the life of the patient.

CAVEATS: APPROPRIATE USE AND/OR POTENTIAL MISUSE OF GENETIC TESTS

Most of the time, thiopurines are given orally daily for a period of at least several months. Genotype-based starting doses are just that—starting doses, and, in most diseases, titration to the desired degree (or lack thereof) of myelosuppression is required. Thus, clinicians must continue to evaluate markers of disease progression and/or of myelosuppression to adjust thiopurine doses up or down from the genotype-directed starting doses. One caveat is that some serious long-term adverse effects (secondary tumors) have been associated with defective TPMT activity without necessarily causing serious acute myelosuppression; whether capping doses of thiopurines in those with a TPMT defect will decrease the risk of the late effect of secondary cancer is not known (see **Supplement** for additional information). Some adverse reactions to thiopurines, such as pancreatitis and hepatotoxicity, are not related to low TPMT activity.

The discovery and clinical implementation of *NUDT15* variants in thiopurine dosing is relatively recent, and the exact impact of *NUDT15* genotype-guided dose adjustments on toxicity and efficacy are less clear compared with *TPMT*.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com) and https://cpicpgx.org/guidelines/guideline-for-thiopurines-and-tpmt/.

Supplement. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

DISCLAIMER

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines reflect expert consensus based on clinical evidence and peer-reviewed literature available at the time they are written and are intended only to assist clinicians in decision making, as well as to identify questions for further research. New evidence may have emerged since the time a guideline was submitted for publication. Guidelines are updated periodically on https://cpicpgx.org/guidelines/ and it is the responsibility of the guideline user to consult this website for updates. Guidelines are limited in scope and are not applicable to interventions or diseases not specifically identified. Guidelines do not account for all individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It remains the responsibility of the healthcare provider to determine the best course of treatment for the patient. Adherence to any guideline is voluntary, with the ultimate determination regarding its application to be solely made by the clinician and the patient. CPIC assumes no responsibility for any injury to persons or damage to property related to any use of CPIC's guidelines, or for any errors or omissions.

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