Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Thiopurine Dosing Based on *TPMT* and *NUDT15* Genotypes: 2025 Update

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Thiopurine methyltransferase (TPMT) and Nudix hydrolase 15 (NUDT15) are key enzymes that catabolize thiopurines. Decreased or no-function alleles in *TPMT* and *NUDT15* are associated with reduced or no enzyme activity and predictive of pronounced adverse effects, including severe myelosuppression, that may occur among individuals treated with standard doses of thiopurines. Genetic variants in these genes are present in all world populations; however, their frequency varies by ancestry. In this updated guideline, we provide recommendations for adjusting starting doses of mercaptopurine, thioguanine, and azathioprine based on *TPMT* and *NUDT15* genotypes, including for individuals with variants in both genes (updates on www.clinpgx.org).

This document is an update to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for thiopurine dosing based on *TPMT* and *NUDT15* genotypes, which was updated last in November 2018.[1] The guideline text, evidence tables and recommendations have been updated to reflect new evidence. Specifically, this guideline updates recommendations by each thiopurine drug and introduces recommendations for a novel TPMT/NUDT15 compound intermediate metabolizer phenotype. In addition, we revised the classification of certain alleles' functionality based on literature data reviewed by CPIC's pharmacogenomics curation expert panel. 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 treated with thiopurines, given the pharmacokinetic nature of the genotype/phenotype associations. CPIC guidelines are published and periodically updated on www.clinpgx.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. *TPMT* and *NUDT15* respective Allele Definition, Allele Functionality, and Allele Frequency Tables are available online at https://www.clinpgx.org/guideline/PA166251442. Detailed guidelines for selection of specific variants to include in clinical genetic testing have been published.[2]

This CPIC guideline is intended to help clinicians understand how a genetic test result may be used to adjust thiopurine dosing, rather than provide guidance on whether the test should be ordered. Resources are available to guide clinicians in this clinical decision, including FDA-label annotations for thiopurines (available on clinpgx.org) and recommendations by professional societies (e.g., for particular diseases).[3–5]

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FOCUSED LITERATURE REVIEW

A systematic literature review focused on *TPMT* and *NUDT15* genotypes and thiopurine use was conducted (details in **Supplemental Material**).

DRUGS: THIOPURINES

Background

Three thiopurines are used clinically: azathioprine, mercaptopurine, and thioguanine. Although all three medications share many of the same pharmacologic effects, mercaptopurine and azathioprine are generally used for nonmalignant immunologic disorders, and mercaptopurine and thioguanine are used for hematological malignancies. Because azathioprine is converted to mercaptopurine in cells, the two drugs can be considered to have identical interactions with TPMT and NUDT15. Recommendations for individuals with functional 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[6,7] (**Figure S1**). Three *TPMT* single nucleotide variants (SNVs), which result in unstable proteins and enhanced TPMT protein degradation,[8] account for over 90% of low activity phenotypes in current literature and are the most common inactivating alleles studied to date: c.238G>C (*TPMT *2*), c.460G>A (**3B*), and c.719A>G (**3C*).[9–11] Inherited TPMT deficiency is the primary genetic cause of thiopurine intolerance in individuals of European and African descent.

TPMT is expressed in red blood cells, liver, and hematopoietic stem cells. TPMT catabolizes mercaptopurine to an inactive methylmercaptopurine base, leaving less parent drug available for potential anabolism to pharmacologically active thioguanine nucleotides (TGNs, **Figure 1**). The intermediate metabolite of mercaptopurine, thioinosine monophosphate (TIMP), is also a substrate for TPMT. MethylTIMP (MeTIMP) and its further phosphorylated metabolites (methylmercaptopurine nucleotides, MeMPNs, which include MeTIMP, MeTIDP, and MeTITP) have pharmacologic activity: they inhibit *de novo* purine synthesis and may contribute to some of the adverse effects of mercaptopurine, particularly hepatotoxicity.[12,13] Individuals who inherit two no-function *TPMT* alleles (homozygous or compound heterozygous TPMT-deficient individuals) are at high risk for life-threatening myelosuppression due to the generation of excessive TGNs if given standard doses of mercaptopurine (or azathioprine). There is also a growing body of evidence linking TPMT deficiency to the risk of hepatic sinusoidal obstructive syndrome in patients exposed to 6-thioguanine (6-TG) which appears to be highly dose dependent in acute lymphocytic leukemia (ALL) and inflammatory bowel disease (IBD) and influenced by the treatment regimen.[14,15]

Thirty to sixty percent of *TPMT* heterozygotes for a no-function allele (or TPMT intermediate metabolizers) cannot tolerate full doses of mercaptopurine or azathioprine.[12,16] Good thiopurine tolerance in some TPMT intermediate metabolizers may be explained by the fact that they produce less MeMPNs than do normal metabolizers.[17] The wide variability in thiopurine tolerance in IMs suggests that precise dosing in each patient cannot be determined by TPMT genotype alone.

Although TPMT has lower affinity for thioguanine than for mercaptopurine, TPMT significantly affects thioguanine pharmacokinetics and its cytotoxic effects.[18] Thioguanine is directly metabolized by TPMT to inactive methylthioguanine base, leaving less drug available for anabolism by hypoxanthine-guanine phosphoribosyltransferase (HPRT) and other enzymes to generate active TGN metabolites (**Figure 1**). In contrast to mercaptopurine, thioguanine is metabolized by HPRT into TGNs without involving intermediate metabolites such as TIMP (i.e. there are no methylTIMP produced from thioguanine). Within each TPMT phenotypic group, the initial recommended relative dosage decreases are similar for thioguanine, mercaptopurine, and azathioprine. Complementary biochemical laboratory tests that directly measure TPMT activity can be helpful adjuncts to genotyping tests, which is further discussed in the "Other Considerations" in this article. However, at present, similar biochemical assays are not readily available for NUDT15 activity (Other Considerations and **Supplement**).

NUDT15. Through agnostic genome-wide association studies, variants in *NUDT15* were identified that strongly influence thiopurine tolerance in patients with ALL[19] and IBD.[20] This hydrolase is expressed in most tissues. Decreased and no-function alleles in *NUDT15* explain the majority of severe thiopurine-related myelosuppression in patients of Asian descent and are common in patients with Amerindian genetic ancestry. The NUDT15 poor metabolizer phenotype is observed at a frequency of about 1 in every 100 patients of East Asian descent (*NUDT15* Frequency Table[21]), which is more common than the TPMT poor metabolizer phenotype in populations of European descent (estimated to be 0.19%, *TPMT* Frequency Table[21]). NUDT15 deficiency is also more prevalent in individuals who self-report as Hispanic, particularly those with high levels of Amerindian genetic ancestry.[19] Given the prevalence of *NUDT15* variants across populations, particularly among non-European populations, inclusion of *NUDT15* in genetic testing is of particular clinical importance to ensure equitable testing and treatment in admixed populations, such as that of the United States. As a nucleoside diphosphatase, NUDT15 catalyzes the conversion of cytotoxic metabolite thioguanine triphosphate (TGTP) to the noncytotoxic thioguanine monophosphate (TGMP). Defects in NUDT15-mediated degradation of TGTP result in higher concentrations of TGTP available for incorporation into DNA (DNA-TG, the primary antileukemic metabolite[22]), eventually leading to excessive DNA damage and apoptosis. The p.Arg139Cys variant (*NUDT15 *3*; rs116855232; c.415C>T) 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.[23] In children with ALL, patients homozygous for the p.Arg139Cys variant allele tolerated only 8% of the standard dose of mercaptopurine, whereas the tolerated dose intensity values were 63% and 83.5%

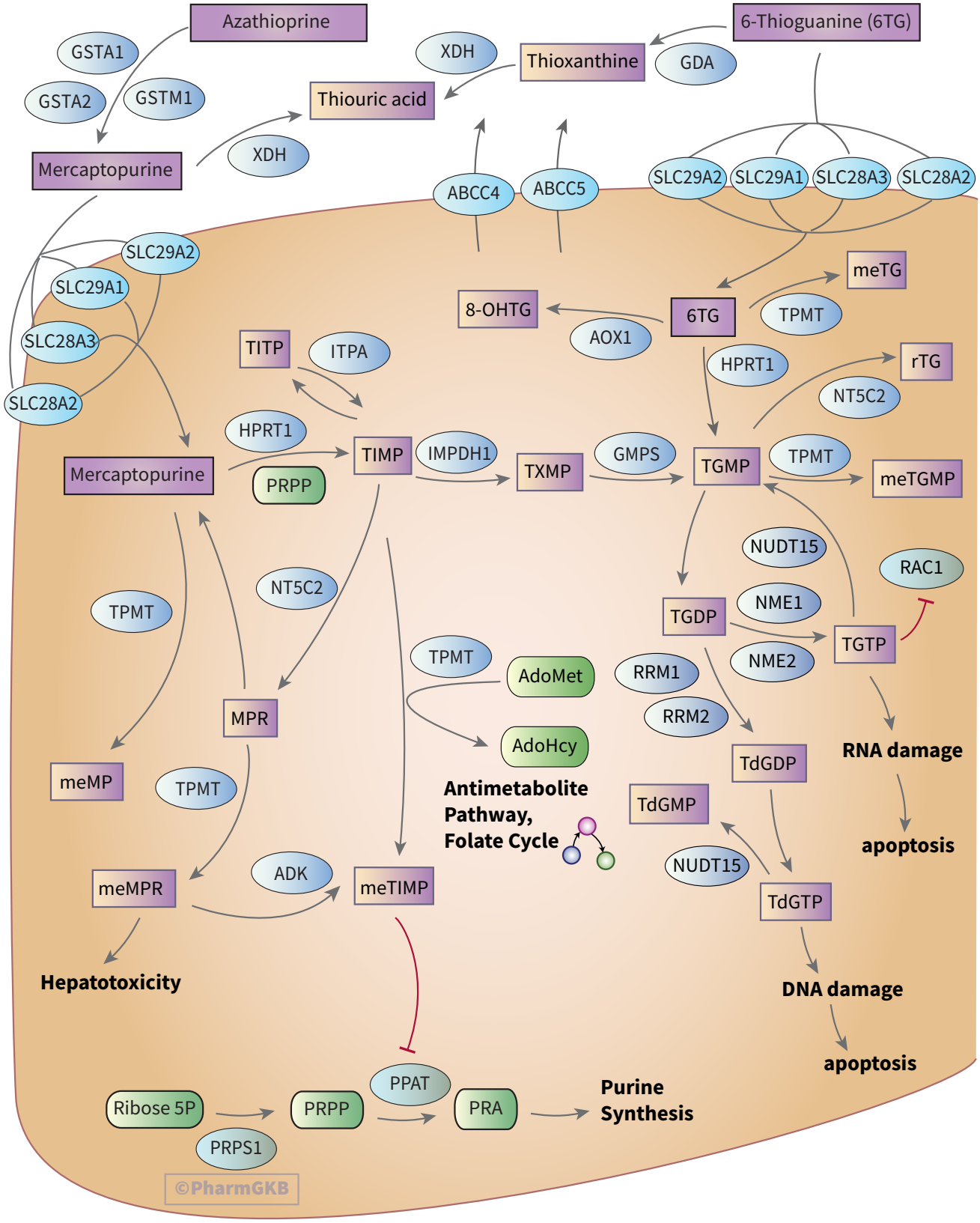

Figure 1 Diagrammatic representation of the intracellular metabolism of thiopurine drugs. Azathioprine, mercaptopurine, and thioguanine are all inactivated by thiopurine methyltransferase (TPMT). All three agents share the same active thioguanine nucleotides (TGN) metabolites. Thioguanine mono-, di-, and triphosphate (TGMP, TGDP, and TGTP, respectively) collectively constitute TGNs, with TGTP having the greatest activity. NUDT15 prevents TGTP incorporation into DNA (as DNA-TG) by dephosphorylating this cytotoxic metabolite into inactive TGMP. Pathway images and data are available under a Creative Commons BY-SA 4.0 license (https://www.clinpgx.org/pathway/PA2040).

for those heterozygous and wild-type for this SNP, respectively.[19] Similar findings were obtained in patients treated with mercaptopurine or azathioprine for IBD.[24] While most clinical studies focused on mercaptopurine and azathioprine due to their more common use, *in vitro* experiments using laboratory models indicated similar influence of NUDT15 on the cytotoxicity of thioguanine.[23] The variant p.Arg139Cys has been studied most extensively in patients receiving thiopurine therapy, thus providing the strongest evidence for clinical implementation. Additional variant alleles have been identified with varying prevalence among different ancestral groups and varying degrees of functional effects (*NUDT15* Allele Functionality Table and Frequency Table[21]). Most *NUDT15* variants are rare and lack definitive clinical impact to rise to actionability, even though some clearly exhibited decreased NUDT15 activity *in vitro*. Nevertheless, the accumulation of preclinical and clinical data associating some rare variants with thiopurine toxicity motivated their re-assignment as clinically actionable in this update (e.g., *NUDT15* **4* and **9*).[24,25]

Genetic test interpretation

Genetic testing analyzes the DNA sequence at specific nucleotides in the *TPMT* and *NUDT15* genes (**Supplement**). Each named star (*) allele is defined by the genotype at one or more specific loci.

Allele function and resulting diplotype-to-phenotype assignments were derived using CPIC's established consensus-based framework for allele function classification.[26]

The genotypes that constitute the haplotypes for *TPMT* and *NUDT15* are described in the *TPMT* Allele Definition Table and *NUDT15* Allele Definition Table.[21] The impact of these haplotypes on enzyme activity is described in the *TPMT* Allele Functionality Table and *NUDT15* Allele Functionality Table.[21]

Table 1 summarizes the assignment of the predicted TPMT and NUDT15 phenotypes, based on example star allele diplotypes, and these assignments are used to link genotypes with thiopurine prescribing recommendations. Flowcharts describing phenotype assignment based on *TPMT* and *NUDT15* genotypes are available in **Supplemental Figures S2** and **S3**, respectively. Of note, the phenotype of "possible intermediate metabolizer," introduced in the previous guideline, describes an individual carrying one uncertain/unknown function allele PLUS one no-function allele, as this individual should be treated with "at least" the same precautions as would apply to an intermediate metabolizer. This current guideline provides specific dosing recommendations for the *TPMT*/*NUDT15* "compound intermediate metabolizer" phenotype that describes an individual who is both a TPMT intermediate metabolizer and NUDT15 intermediate metabolizer (e.g., carrying one copy of no-function alleles in both genes) and thus requires greater dose reduction than intermediate metabolizers for a single gene. It should also be noted that we have now annotated some alleles as "decreased function" because the corresponding variant proteins retain residual enzymatic activity and their phenotype designation is added to the Allele Functionality Tables.[21]

Although decreased and no-function *TPMT* and *NUDT15* alleles have been identified in multiple populations (*TPMT* Frequency Table and *NUDT15* Frequency Table[21]), one of the limitations inherent in a clinical genotyping test is that rare or previously undiscovered variants are typically not included. The use of sequencing-based methods may provide more complete information; however, rare alleles appear to account for less than 10% of the variation in TPMT activity.[11] Even when sequencing-based methods are used, rare variants may not be reported due to a lack of systematic guidelines to classify pharmacogenomic variants at the time of detection in a clinical laboratory setting.

Available genetic test options

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

Incidental findings

There are no diseases or health traits that have been linked to variation in *TPMT* or *NUDT15* in the absence of thiopurine treatment.[8,27]

Linking genetic variability to variability in drug-related phenotypes

There is substantial evidence linking *TPMT* and *NUDT15* genotypes with phenotypic variability in thiopurine response (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**). Some of the clinical data upon which dosing recommendations are based (**Tables 2–4**) rely on measures of TPMT phenotype rather than genotype; however, because *TPMT* genotype is strongly linked to TPMT phenotype,[9–11] these recommendations apply regardless of the method used to assess TPMT status. In addition, retrospective studies strongly indicate that patients with decreased or no-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 2–4**.

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. In addition, because the level of thiopurine tolerance is related to genetic ancestry,[19] the standard starting doses can vary by geographic regions.

Thiopurines have a unique role in the treatment of many malignancies (e.g., ALL). The standard starting doses of thiopurines are generally derived from trials that have predominantly included patients who are TPMT and NUDT15 normal metabolizers (who represent ~90% of the general populations) and received 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 2–4**). Thiopurines are also used in nonmalignant conditions for their

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

Predicted phenotype	Genotypes	Examples of diplotypes[a]
Assignment of predicted TPMT phenotypes based on genotypes		
Normal metabolizer	An individual carrying two normal function alleles OR one normal function allele PLUS one decreased function allele	*1/*1 *1/*8
Intermediate metabolizer	An individual carrying one normal function allele PLUS one no-function allele OR two decreased function alleles	*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4 *8/*8
Possible intermediate metabolizer	An individual carrying one uncertain/unknown function allele PLUS one no-function allele OR one no-function allele PLUS one decreased function allele	*2/*9, *3A/*12 *3A/*8
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 one normal function allele PLUS one uncertain/unknown function allele OR one decreased function allele PLUS one uncertain/unknown function allele OR two uncertain/unknown function alleles	*1/*19 *8/*30 *19/*40
Assignment of predicted NUDT15 phenotypes based on genotypes		
Normal metabolizer	An individual carrying two normal function alleles OR one normal function allele PLUS one decreased function allele	*1/*1 *1/*5
Intermediate metabolizer	An individual carrying one normal function allele PLUS one no-function allele OR an individual carrying two decreased function alleles	*1/*2, *1/*3 *5/*5
Possible intermediate metabolizer	An individual carrying one uncertain/unknown function allele PLUS one no-function allele	*2/*15, *3/*21
Poor metabolizer	An individual carrying two no-function alleles OR one no-function allele PLUS one decreased function allele	*2/*2, *2/*3, *2/*4 *3/*5
Indeterminate	An individual carrying one normal function allele PLUS one uncertain/unknown function allele OR one decreased function allele PLUS one uncertain/unknown function allele OR two uncertain/unknown function alleles	*1/*12 *5/*20 *11/*14

[a]Please refer to the *TPMT* and *NUDT15* Diplotype-Phenotype Table online for a complete list. For allele function, please refer to the *TPMT* and *NUDT15* Allele Functionality Table.[21]

immune suppressive activity (e.g., for rheumatoid arthritis, systemic lupus erythematosus, vasculitis, IBDs, autoimmune dermatologic disorders, and myasthenia gravis). The dosing strategy for nonmalignancy differs in that the thiopurine (azathioprine or mercaptopurine) is often initiated at a relatively low dose followed by gradual increases to reach the maximal tolerable dose specified in the protocol. For these conditions, dose adjustments should be made based on close monitoring of clinical myelosuppression (or disease-specific guidelines) and/or the measurement of thiopurine metabolites, such as TGNs or MeMPNs, especially in patients with TPMT deficiency.

Major changes from the 2018 guideline. In this update, we have shifted from providing gene-specific dosing recommendations to harmonizing guidance by drug (i.e., mercaptopurine, thioguanine, and azathioprine). Dosing recommendations are now provided for each drug by TPMT/NUDT15 phenotype, in their specific indication (i.e., malignant or nonmalignant conditions). **Figure 2** outlines the recommended course of action for each thiopurine drug if *TPMT* and/or *NUDT15* genotypes are known.

We also introduced specific dosing recommendations for the compound-deficient phenotypes (i.e., intermediate or poor metabolizers for both TPMT and NUDT15) in this guideline update. There have been reports of patients with intermediate metabolizer status for both TPMT and NUDT15 (i.e., TPMT/NUDT15 compound intermediate metabolizers). These individuals tolerated lower doses of thiopurines compared to intermediate metabolizers for only TPMT or NUDT15,[28] indicating that the decreased activity in both enzymes results in additive effects on drug toxicity. 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 in each gene (see **Supplementary Tables**). For example, given estimates of no-function alleles for *NUDT15* of 11% and

Table 2 Mercaptopurine dosing recommendations based on TPMT and/or NUDT15 phenotypes for malignant and nonmalignant conditions

TPMT/NUDT15 phenotype	Implications for toxicity	Implications for phenotypic measures[a]	Therapeutic recommendations[b]	Classification of recommendation[c]
TPMT and NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia and myelosuppression	TPMT NMs have lower erythrocyte concentrations of TGN metabolites and higher concentrations of MeMPNs compared to TPMT IMs and TPMT PMs. This is the 'normal' pattern.	Initiate therapy with standard starting dose of mercaptopurine (e.g., 75 mg/m^2/day for malignancy or 1.5 mg/kg/day for nonmalignancy). During therapy, adjust the doses of myelosuppressive agents, as per standard clinical practice. It usually takes at least 2 weeks of stable dosing to reach steady state after each dose adjustment.	Strong
TPMT normal metabolizer and NUDT15 (possible) intermediate metabolizer OR TPMT (possible) intermediate metabolizer and NUDT15 normal metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia and myelosuppression	TPMT IMs have moderate to high erythrocyte concentrations of TGN metabolites and low concentrations of MeMPNs compared to TPMT NMs when receiving standard dose.	Initiate therapy with decreased starting doses (30–80% of standard starting dose) if starting dose is ≥75 mg/m^2/day (for malignancy) or ≥1.5 mg/kg/day (for nonmalignancy) If starting dose is already below standard starting dose, dose reduction might not be necessary. During therapy, adjust mercaptopurine doses based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 2–4 weeks of stable dosing to reach steady state after each dose adjustment. If myelosuppression occurs, and the patient is on combination therapy, emphasis should be on reducing mercaptopurine over other agents.	Strong
Any combination with TPMT poor metabolizer and/or NUDT15 poor metabolizer[d]	Greatly increased risk of thiopurine-related leukopenia, neutropenia and myelosuppression. Fatal toxicity possible without dose decrease	TPMT PMs have extremely high erythrocyte concentrations of TGN metabolites and no MeMPN compared to TPMT NMs.	*For malignancy*: initiate therapy with drastically reduced starting doses. Reduce starting dose by 10-fold and reduce frequency to thrice weekly instead of daily (e.g., 10 mg/m^2/day given 3 days/week) During therapy, adjust mercaptopurine doses based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 4–6 weeks of stable dosing to reach steady state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing mercaptopurine over other agents *For nonmalignancy*: consider alternative nonthiopurine immunosuppressant therapy	Strong
TPMT intermediate metabolizer and NUDT15 intermediate metabolizer (i.e., TPMT/NUDT15 compound intermediate metabolizer)	Increased risk of thiopurine-related leukopenia, neutropenia and myelosuppression. Higher risk of toxicity compared to single IM	TPMT IMs have moderate to high erythrocyte concentrations of TGN metabolites and low concentrations of MeMPNs compared to TPMT NMs.	Initiate therapy with decreased starting doses (20–50% of standard starting dose) if starting dose is ≥75 mg/m^2/day (for malignancy) or ≥1.5 mg/kg/day (for nonmalignancy) If starting dose is already below standard starting dose, dose reduction might not be necessary. During therapy, adjust mercaptopurine doses based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 2–4 weeks of stable dosing to reach steady state after each dose adjustment. If myelosuppression occurs, and the patient is on combination therapy, emphasis should be on reducing mercaptopurine over other agents.	Strong

IM, intermediate metabolizer; MeMPN, methylmercaptopurine nucleotide; NM, normal metabolizer; NUDT15, Nudix hydrolase 15; PM, poor metabolizer; TGN, thioguanine nucleotide; TPMT, thiopurine methyltransferase. [a]This section primarily addresses the effects of *TPMT* genotype, as there are currently no clinically available pharmacological phenotypes associated with *NUDT15* genotype. [b]Standard starting doses may vary by patient characteristics and/or treatment regimens and according to local clinical practice. [c]Rating scheme described in the **Supplementary Material**. [d]This includes being NM, IM or possible IM for one gene and PM for the other gene, as well as being PM for both genes.

Table 3 Thioguanine dosing recommendations based on TPMT and/or NUDT15 phenotypes for malignant conditions

TPMT/NUDT15 phenotype	Implications for toxicity	Implications for phenotypic measures[a]	Therapeutic recommendations[b]	Classification of recommendation[c]
TPMT and NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia, and myelosuppression	TPMT NMs have lower erythrocyte concentrations of TGN metabolites compared to TPMT IMs and TPMT PMs. This is the 'normal' pattern.	Initiate therapy with standard starting dose of thioguanine (e.g., 40 mg/m^2/day for malignancy). During therapy, adjust the doses of myelosuppressive agents, as per standard clinical practice. It usually takes at least 2 weeks of stable dosing to reach steady state after each dose adjustment.	Strong
TPMT normal metabolizer and NUDT15 (possible) intermediate metabolizer OR TPMT (possible) intermediate metabolizer and NUDT15 normal metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia, and myelosuppression	TPMT IMs have moderate to high erythrocyte concentrations of TGN metabolites compared to TPMT NMs.	Initiate therapy with decreased starting doses (30–80% of standard starting dose) if standard starting dose is ≥40 mg/m^2/day. If starting dose is already below standard starting dose, dose reduction might not be necessary. During therapy, adjust the doses of thioguanine based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 2–4 weeks of stable dosing to reach steady state after each dose adjustment. If myelosuppression occurs, and the patient is on combination therapy, emphasis should be on reducing thioguanine over other agents.	Moderate
Any combination with TPMT poor metabolizer and/or NUDT15 poor metabolizer[d]	Greatly increased risk of thiopurine-related leukopenia, neutropenia, and myelosuppression. *Fatal toxicity possible without dose decrease*	TPMT PMs have extremely high erythrocyte concentrations of TGN metabolites compared to TPMT NMs.	Initiate therapy with drastically reduced starting doses. Reduce daily dose by 10-fold and reduce frequency to thrice weekly instead of daily During therapy, adjust thioguanine doses based on degree of myelosuppression and disease-specific guidelines. It usually takes at least 4–6 weeks of stable dosing to reach steady state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing thioguanine over other agents.	Strong
TPMT intermediate metabolizer and NUDT15 intermediate metabolizer (i.e., TPMT/NUDT15 compound intermediate metabolizer)	Increased risk of thiopurine-related leukopenia, neutropenia, and myelosuppression. Higher risk of toxicity compared to single IM	TPMT IMs have moderate to high erythrocyte concentrations of TGN metabolites compared to TPMT NMs.	Initiate therapy with decreased starting doses (20–50% of standard starting dose) if standard starting dose[a] is ≥40 mg/m^2/day If starting dose is already below standard starting dose, dose reduction might not be necessary. During therapy, adjust the doses of thioguanine based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 2–4 weeks of stable dosing to reach steady state after each dose adjustment. If myelosuppression occurs, and the patient is on combination therapy, emphasis should be on reducing thioguanine over other agents.	Moderate

IM, intermediate metabolizer; NM, normal metabolizer; NUDT15, Nudix hydrolase 15; PM, poor metabolizer; TGN, thioguanine nucleotide; TPMT, thiopurine methyltransferase. [a]This section primarily addresses the effects of TPMT genotype, as there are currently no clinically available pharmacological phenotypes associated with NUDT15 genotype. [b]Standard starting doses may vary by patient characteristics and/or treatment regimens and according to local clinical practice. [c]Rating scheme described in the **Supplementary Material**. [d]This includes being NM, IM, or possible IM for one gene and PM for the other gene, as well as being PM for both genes.

of no-function alleles for *TPMT* of 2% in the global population, the frequency of carrying no-function alleles in both genes is approximately estimated at 0.2% overall. The growing body of clinical data on thiopurine tolerance in patients who are compound intermediate metabolizers points to the need for specific dosage recommendation for this group of patients. Although rare, individuals who are intermediate metabolizers for one enzyme and poor metabolizers for the other, or poor metabolizers for both

Table 4 Azathioprine dosing recommendations based on TPMT and/or NUDT15 phenotypes for nonmalignant conditions

TPMT/NUDT15 phenotype	Implications for toxicity	Implications for phenotypic measures[a]	Therapeutic recommendations[b]	Classification of recommendation[c]
TPMT and NUDT15 normal metabolizer	Normal risk of thiopurine-related leukopenia, neutropenia and myelosuppression	TPMT NMs have lower erythrocyte concentrations of TGN metabolites and higher concentrations of MeMPNs compared to TPMT IMs and TPMT PMs. This is the 'normal' pattern.	Initiate therapy with standard starting dose (e.g., 2 mg/kg/day for autoimmune diseases). During therapy, adjust doses of azathioprine based on disease-specific guidelines. It usually takes at least 2 weeks to reach steady state after each dose adjustment.	Strong
TPMT normal metabolizer and NUDT15 (possible) intermediate metabolizer OR TPMT (possible) intermediate metabolizer and NUDT15 normal metabolizer	Increased risk of thiopurine-related leukopenia, neutropenia and myelosuppression	TPMT IMs have moderate to high erythrocyte concentrations of TGN metabolites and low concentrations of MeMPNs compared to TPMT NMs.	Initiate therapy with reduced starting doses (30–80% of standard starting dose) if standard starting dose is ≥2 mg/kg/day If starting dose is already below standard starting dose, dose reduction might not be necessary. During therapy, adjust the doses of azathioprine based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 2–4 weeks of stable dosing to reach steady state after each dose adjustment.	Strong
Any combination with TPMT poor metabolizer and/or NUDT15 poor metabolizer[d]	Greatly increased risk of thiopurine-related leukopenia, neutropenia and myelosuppression. *Fatal toxicity possible without dose decrease*	TPMT PMs have extremely high erythrocyte concentrations of TGN metabolites and no MeMPN compared to TPMT NMs.	Consider alternative nonthiopurine immunosuppressant therapy	Strong
TPMT intermediate metabolizer and NUDT15 intermediate metabolizer (i.e., TPMT/NUDT15 compound intermediate metabolizer)	Increased risk of thiopurine-related leukopenia, neutropenia and myelosuppression Higher risk of toxicity compared to single IM	TPMT IMs have moderate to high erythrocyte concentrations of TGN metabolites and low concentrations of MeMPNs compared to TPMT NMs.	Initiate therapy with reduced starting doses (20–50% of standard starting dose) if standard starting dose[a] is ≥2 mg/kg/day If starting dose is already below standard starting dose, dose reduction might not be necessary. During therapy, adjust the doses of azathioprine based on the degree of myelosuppression and disease-specific guidelines. It usually takes at least 2–4 weeks of stable dosing to reach steady state after each dose adjustment.	Moderate

IM, intermediate metabolizer; MeMPN, methylmercaptopurine nucleotide; NM, normal metabolizer; NUDT15, Nudix hydrolase 15; PM, poor metabolizer; TGN, thioguanine nucleotide; TPMT, thiopurine methyltransferase. [a]This section primarily addresses the effects of TPMT genotype, as there are currently no clinically available pharmacological phenotypes associated with NUDT15 genotype. [b]Standard starting doses may vary by patient characteristics and/or treatment regimens and according to local clinical practice. [c]Rating scheme described in the **Supplementary Material**. [d]This includes being NM, IM, or possible IM for one gene and PM for the other gene, as well as being PM for both genes.

enzymes, should be treated extremely cautiously, with decreased reduction in dosage at least as large as those recommended for poor metabolizers of a single gene.

Mercaptopurine therapeutic recommendations. Tolerated mercaptopurine dosage is correlated with the number of no-function alleles that the patient has in both *TPMT* and *NUDT15*.[9,10,19,20] The degree of mercaptopurine intolerance is largely comparable between carriers of *TPMT* vs. *NUDT15* no-function alleles.[19]

Genotype-guided prescribing recommendations apply primarily to starting doses of mercaptopurine. For TPMT and NUDT15 normal metabolizers, the starting dose does not need to be altered. Variability has been observed in the tolerated thiopurine dosages among TPMT or NUDT15 intermediate metabolizers, with a minority of individuals who do not seem to require significant dose reduction.[19,23,29–32] If starting doses of mercaptopurine are high (e.g., 75 mg/m^2 in ALL or 1.5 mg/kg/day for nonmalignant condition), lower than standard starting doses should be considered in TPMT or NUDT15 (possible) intermediate

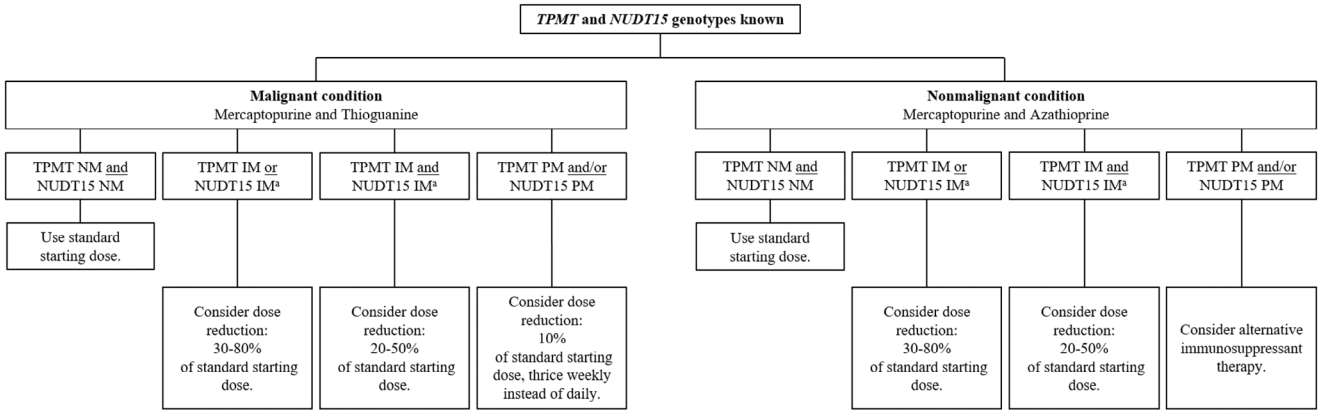

Figure 2 Recommended starting doses of thiopurines based on TPMT and NUDT15 phenotypes. [a]Also applies for TPMT or NUDT15 possible IM.

metabolizers[29–34] (**Table 2**). On average, TPMT or NUDT15 IM patients can tolerate ~60% of the standard dose of thiopurine, even though it ranges from 30% to 80% across patients.[19] We acknowledge that our recommended dose range (30–80%) is broad but also believe it is necessary to account for the significant interindividual variability in thiopurine tolerance observed in the IM patient population. In contrast, a full dose of mercaptopurine poses a severe risk of prolonged hematopoietic toxicity in TPMT or NUDT15 poor metabolizers and pre-emptive dose reductions are strongly recommended.[16,35–37] Thus, markedly reduced doses (10-fold reduction and reduced frequency of drug administration) should be used in TPMT and/or NUDT15 poor metabolizers.[23,38,39] This approach has decreased the risk of acute toxicity without compromising relapse rate in ALL[40] or efficacy in IBD.[34,41] Of note, 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).[39,42] Patients with a TPMT/NUDT15 compound intermediate metabolizer phenotype are at increased risk of thiopurine-induced toxicity and require further reduction in starting dose compared to intermediate metabolizers for either enzyme.[28] In nonmalignant conditions, due to the availability of alternative agents, this guideline recommends alternative nonthiopurine agents for TPMT poor metabolizers and/or NUDT15 poor metabolizers rather than administering reduced doses of mercaptopurine.

Thioguanine therapeutic recommendations. Thioguanine is mainly used for the treatment of malignant diseases, e.g., acute myeloid leukemia and to a lesser extent ALL. The degree of intolerance is expected to be comparable between carriers of *TPMT* vs. *NUDT15* no-function alleles, although there is limited evidence for thioguanine tolerance in *NUDT15* variant carriers. The dosing recommendations are similar to those of mercaptopurine (**Figure 2**). For TPMT and NUDT15 normal metabolizers, the starting dose does not need to be altered (**Table 3**). In TPMT intermediate metabolizers, the erythrocyte concentration of TGNs is increased.[43] Starting dose may be adjusted if the protocol dose is considered as high (≥ 40 mg/m^2/day); otherwise, dose reduction might not be necessary. TPMT/NUDT15 compound intermediate metabolizers will require further dose reduction compared to single gene intermediate metabolizers, although there are limited data available to confirm the lower tolerance to thioguanine in this group of patients. A full dose of thioguanine exposes TPMT and/or NUDT15 poor metabolizers to fatal toxicity due to extremely high erythrocyte concentration of TGNs and excessive levels of dose-normalized DNA-TG; drastic dose reduction is required to mitigate the risk of thioguanine-induced myelosuppression in this group of patients (**Table 3**).

Azathioprine therapeutic recommendations. Azathioprine interacts with TPMT and NUDT15 in a similar manner to mercaptopurine. The degree of intolerance is largely comparable between carriers of *TPMT* vs. *NUDT15* no-function alleles.[44] Azathioprine is mainly prescribed for autoimmune diseases for which the maximal tolerated dose will depend on the clinical indication. For TPMT and NUDT15 normal metabolizers, the starting dose does not need to be altered (**Table 4**). If starting doses of azathioprine are high (e.g., ≥ 2 mg/kg/day as considered for IBD), lower than standard starting doses should be considered in TPMT and/or NUDT15 intermediate metabolizers. If the starting dose is already low, dose reduction might not be necessary.[45] TPMT/NUDT15 compound intermediate metabolizers will require further dose reduction compared to single gene intermediate metabolizers, although there are limited data available to confirm the lower tolerance to azathioprine in this group of patients. A high dose of azathioprine exposes TPMT and/or NUDT15 poor metabolizers to extremely high concentrations of TGNs and potentially fatal myelosuppression. Clinicians should consider alternative immunosuppressant therapy in TPMT and/or NUDT15 poor metabolizers rather than a decreased dose of azathioprine.

Pediatrics. Because thiopurines are essential for the treatment of both childhood malignancies (e.g., ALL) and immune suppressive disorders (e.g., IBD, mostly diagnosed in adults), much of the evidence supporting the original dosing recommendations

(summarized in **Tables S1 and S2**) was derived from studies including pediatric and adult patients. Thus, the guideline dosing recommendations are intended for use in both age groups.

Biogeographic groups. This guideline aims to provide compelling information on ancestry-related differences in the prevalence of no-function phenotypes of TPMT and NUDT15. Initial investigations into TPMT phenotype–genotype correlations predominantly focused on populations of European ancestry, largely due to the higher frequency of well-characterized no-function alleles within this group.[9] For NUDT15, early studies found that *NUDT15*'s most common no-function allele (i.e., *NUDT15 *3*) was predominantly detected in individuals of Asian descent and of Amerindian genetic ancestry.[19,20] For both genes, the inclusion of individuals from diverse ancestry groups in pharmacogenetic studies has facilitated the detection of rare population-specific variants, for example, *TPMT *8* in patients of African descent or *NUDT15 *4* in patients of Amerindian descent (TPMT Frequency Table and NUDT15 Frequency Table[21]). Nevertheless, given the robustness of the genotype–phenotype associations for both TPMT and NUDT15 across diverse populations, the impact of *TPMT* and *NUDT15* genetic variation on thiopurine exposure and treatment outcomes is likely to be consistent across biogeographic groups. Therefore, the recommendations are not limited to any specific ancestry group.

Recommendations for incidental findings

Recommendations for incidental findings are 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, with up to 10–15% of patients having actionable variants in the non-tested gene. 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 that measure thiopurine metabolites in erythrocytes and are used to monitor and adjust therapy: TGNs (for mercaptopurine, azathioprine, and thioguanine) and MeMPNs for those on mercaptopurine or azathioprine (see **Supplement** for details on associations with TPMT). Clinical assays to measure erythrocyte TGN levels typically do not differentiate among the mono-, di-, and triphosphate forms of active TGNs and do not inform the potential toxicity among individuals with *NUDT15* variants.[46,47] In some patients, elevated TPMT activity leads to excessive MeMPN levels,[13] increasing the risk of hepatotoxicity while reducing active TGN, although this relationship has not been definitively established.[48,49] The potential impact of *NUDT15* variants on MeMPN has not been well-characterized. There is evidence that intermediate and poor metabolizers for NUDT15 accumulate higher levels of DNA-TG in leukocytes than normal metabolizers given the same thiopurine dosage, highlighting its potential to inform NUDT15 metabolizer status.[23]

Monitoring of erythrocyte TGNs and/or MMPNs can inform clinicians on thiopurine exposure, particularly in case of unexpected clinical response/toxicity or suspected noncompliance, and help further optimize thiopurine dosing. Unlike measuring thiopurine metabolites in erythrocytes, DNA-TG in leukocytes is not yet implemented in clinical practice.

Implementation of this guideline. The guideline supplement contains resources that can be used within electronic health records (EHRs) 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 supplement).

POTENTIAL BENEFITS AND RISKS FOR THE PATIENT

The benefits of pre-emptive *TPMT* testing are that doses that are customized based on TPMT phenotype reduce the likelihood of acute myelosuppression without compromising disease control.[12,30,31,42] 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 intermediate metabolizers receiving conventional thiopurine doses experience severe myelosuppression.[12,32,42] However, because steady state is reached in 2–4 weeks, any period of "under-dosing" should be short, and using this approach, at least in ALL and in IBD, outcomes were not compromised.[12,30,31,33,42] Furthermore, metabolite monitoring may be used to optimize the thiopurine dose.

Similar benefits are expected with pre-emptive *NUDT15* genotyping, especially for populations with a high prevalence of NUDT15 deficiency (such as for patients of Asian or Amerindian genetic ancestry), given that these variants have comparable effects as no-function alleles in *TPMT*. At least in ALL, leukemia cells with no-function *NUDT15* alleles are also more sensitive to mercaptopurine,[23] and thus, in theory, *NUDT15* genotype-guided dosing would not compromise the antileukemic efficacy of this drug. As with *TPMT*, thiopurine dose adjustments based on the *NUDT15* genotype should be followed by close monitoring of tolerance. If the drug is well tolerated, the dose should be gradually increased, especially if the initial dose reduction was significant (which is the case for intermediate metabolizers with a broad dose reduction range), to avoid under-dosing and ensure treatment efficacy.[41,50]

A possible risk of genotyping is that the patient may have a rare variant that is not included in the test design, which would result in the patient being assigned a "wild-type" (*1) genotype by default. Thus, the assigned normal metabolizer phenotype would not reflect the patient's actual TPMT or NUDT15 activity. Use of a biochemical TPMT activity assay or pharmacologic measures of thiopurine metabolites may mitigate this risk by identifying the patient's decreased activity, but such tests are not available or informative for NUDT15.

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. Other nongenetic variables also contribute to TPMT and NUDT15 enzyme activity and response to thiopurine therapy. 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 secondary cancers have been associated with long-term exposure to thiopurines and eventually TPMT deficiency[51,52] and to a lesser extent with NUDT15 deficiency[53]; whether capping doses of thiopurines in those with TPMT and/or NUDT15 deficiency will decrease this risk of secondary cancer is not known. In addition, some adverse reactions to thiopurines, such as flu-like syndrome, pancreatitis, and liver toxicity, are not related to TPMT nor NUDT15 deficiency.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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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://www.clinpgx.org/cpic/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 health care 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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