Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 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 (C PIC) 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 reviews1–4 were relied upon to summarize much of the earlier literature.

**DRUGS: THIOPURINES**

**Background.** Three thiopurines are used clinically: azathioprine (a produg 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 produg 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
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.

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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.
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. Within each TPMT phenotypic group, the initial recommended relative dosage decreases are similar for thioguanine, mercaptopurine, and azathioprine (Table 2).

**NUDT15.** Through agnostic genomewide association studies, variants in *NUDT15* have been identified that strongly influence thiopurine tolerance in patients with acute lymphoblastic leukemia (ALL) and those with inflammatory bowel diseases. 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), 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. 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. Although most clinical studies focused on mercaptopurine, *NUDT15* variants studied most extensively in patients receiving thiopurine therapy, provides the basis for most of the dosing 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 and *NUDT15* Frequency Table), 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


### 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.

### 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 antineoplastic 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, 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/m² of mercaptopurine), as is true in some ALL treatment...
Table 1 Assignment of likely TPMT and NUDT15 phenotypes based on genotypes

<table>
<thead>
<tr>
<th>Likely phenotype</th>
<th>Genotypes</th>
<th>Examples of diplotype phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal metabolizer</td>
<td>An individual carrying two normal function alleles</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>An individual carrying one normal function allele PLUS one no function allele</td>
<td>*1/*2, *1/*3A, *1/*3B, *1/*3C, *1/*4</td>
</tr>
<tr>
<td>Possible intermediate metabolizer</td>
<td>An individual carrying one uncertain/unknown function allele PLUS one no function allele</td>
<td>*2/*8, *3A/*7</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>An individual carrying two uncertain/unknown function alleles OR one normal function allele plus one uncertain allele function allele</td>
<td>*6/*8, *1/*8</td>
</tr>
</tbody>
</table>

Assignment of likely NUDT15 phenotypes based on genotypes

<table>
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</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>An individual carrying one normal function allele PLUS one no function allele</td>
<td>*1/*2, *1/*3</td>
</tr>
<tr>
<td>Possible intermediate metabolizer</td>
<td>An individual carrying one uncertain function allele PLUS one no function allele</td>
<td>*2/*5, *3/*6</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>An individual carrying two no function alleles</td>
<td>*2/*2, *2/*3, *3/*3</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>An individual carrying two uncertain function alleles OR one normal function allele plus one uncertain function allele</td>
<td>*4/*5, *5/*6</td>
</tr>
</tbody>
</table>

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

*See TPMT and NUDT15 Frequency Table and Diplotype-Phenotype Table* 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 and markedly reduced doses (10-fold reduction) should be used in TPMT poor metabolizers (Table 2). 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 (Table 2).

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, 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/m²/ day for mercaptopurine). For NUDT15 poor metabolizers (e.g., NUDT15*3/*3), substantially reduced doses (e.g., 10 mg/m²/day of mercaptopurine) or the use of an alternative agent should be considered (Table 2).

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 dose 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
<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implications for mercaptopurine and azathioprine phenotypic measures</th>
<th>Dosing recommendations for mercaptopurine</th>
<th>Classification of recommendations</th>
<th>Dosing recommendations for azathioprine</th>
<th>Classification of recommendations</th>
<th>Implications for thioguanine phenotypic measures</th>
<th>Dosing recommendations for thioguanine</th>
<th>Classification of recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT normal metabolizer</td>
<td>Lower concentrations of TGN metabolites, higher MeTIMP, this is the “normal” pattern. Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression.</td>
<td>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})</td>
<td>Strong</td>
<td>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.(^{3,30,37})</td>
<td>Strong</td>
<td>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.</td>
<td>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})</td>
<td>Strong</td>
</tr>
<tr>
<td>Moderate to high concentrations of TGN metabolites; low concentrations of MeTIMP. Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.</td>
<td>Start with reduced starting doses (30–80% of normal dose) if normal starting dose(^e) is (\geq 75) mg/m(^2)/day or (\geq 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.(^{4,11,15,24,25,30,36,39})</td>
<td>Strong</td>
<td>Start with reduced starting doses (30–80% of normal dose) if normal starting dose(^e) 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})</td>
<td>Strong</td>
<td>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.</td>
<td>Start with reduced doses (50–80% of normal dose) if normal starting dose(^e) is (\geq 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})</td>
<td>Moderate</td>
<td></td>
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</table>

\(^{a}\) Normal starting dose for mercaptopurine: 75 mg/m\(^2\)/day or 1.5 mg/kg/day. Normal starting dose for azathioprine: 2–3 mg/kg/day. Normal starting dose for thioguanine: 40–60 mg/m\(^2\)/day.
Table 2 (Continued)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implications for mercaptopurine and azathioprine phenotypic measures</th>
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<th>Implications for thioguanine phenotypic measures</th>
<th>Classification of recommendations</th>
<th>Dosing recommendations for thioguanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT poor metabolizer</td>
<td>Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease; no MeTIMP metabolites. Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.</td>
<td>Strong</td>
<td>For malignancy, start with drastically reduced doses (reduce daily dose by 10-fold and reduce frequency to thrice weekly instead of daily (e.g., 10 mg/m²/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.</td>
<td>Strong</td>
<td>For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.</td>
<td>Strong</td>
<td>Extremely high concentrations of TGN metabolites; fatal toxicity possible without dose decrease. Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression.</td>
<td>Strong</td>
<td>Start with drastically reduced doses (reduce daily dose 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.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Implications for thiopurine phenotypic measures</th>
<th>Mercaptopurine</th>
<th>Azathioprine</th>
<th>Thioguanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUDT15 normal metabolizer</td>
<td>Normal risk of thiopurine-related leukopenia, neutropenia, myelosuppression</td>
<td>Start with normal starting dose&lt;sup&gt;a&lt;/sup&gt; (e.g., 75 mg/m²/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.&lt;sup&gt;4,27,30&lt;/sup&gt;</td>
<td>Strong</td>
<td>Start with normal starting dose&lt;sup&gt;a&lt;/sup&gt; (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.&lt;sup&gt;4,30,37&lt;/sup&gt;</td>
</tr>
<tr>
<td>NUDT15 intermediate metabolizer OR NUDT15 possible intermediate metabolizer</td>
<td>Increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression</td>
<td>Start with reduced starting doses (30–80% of normal dose) if normal starting dose&lt;sup&gt;a&lt;/sup&gt; is ≥ 75 mg/m²/day or ≥ 1.5 mg/kg/day (e.g., start at 22.5–60 mg/m²/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.&lt;sup&gt;4,11,13,24,25,27,30,38,39&lt;/sup&gt;</td>
<td>Strong</td>
<td>Start with reduced starting doses (30–80% of normal dose) if normal starting dose&lt;sup&gt;a&lt;/sup&gt; 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.&lt;sup&gt;4,30,37,38&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> If normal starting dose is already < 75 mg/m²/day or < 1.5 mg/kg/day, dose reduction may not be recommended.
Mercaptopurine

**Phenotype**
NUDT15 poor metabolizer

**Implications for thiopurine phenotypic measures**
Greatly increased risk of thiopurine-related leukopenia, neutropenia, myelosuppression

**Dosing recommendations for mercaptopurine**
For malignancy, initiate dose at 10 mg/m²/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.¹,²,⁶,30,38

**Classification of recommendations**
Strong

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Azathioprine

**Dosing recommendations for azathioprine**
For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses (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. In setting of myelosuppression, emphasis should be on reducing thioguanine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.²⁸,³⁰,³⁷,³⁸,⁴⁰

**Classification of recommendations**
Strong

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Thioguanine

**Dosing recommendations for thioguanine**
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.¹

**Classification of recommendations**
Strong

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**Notes**
- 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.
- Rating scheme described in Supplemental Material.

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**Table 3 (Continued)**

<table>
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<tr>
<th>Phenotype</th>
<th>Mercaptopurine</th>
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<td>NUDT15 poor metabolizer</td>
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<td><strong>Dosing recommendations for thioguanine</strong></td>
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<tr>
<td></td>
<td>Greatly increased risk of thiopurine-related leukopenia, neutropenia,</td>
<td>For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses (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. In setting of myelosuppression, emphasis should be on reducing thioguanine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.²⁸,³⁰,³⁷,³⁸,⁴⁰</td>
<td>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.¹</td>
</tr>
<tr>
<td></td>
<td>myelosuppression</td>
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</table>
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 thio purine 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, 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 mercaptopurine20 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.cptjournal.com) and https://cpicpgx.org/guidelines/guideline-for-thiopurines-and-tpmt/.


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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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