ONE MINUTE CONSULT

BRIEF ANSWERS TO SPECIFIC CLINICAL QUESTIONS

Q: Should thiopurine methyltransferase (TPMT) activity be determined before prescribing azathioprine, mercaptopurine, or thioguanine?

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A: The thiopurines azathioprine, mercaptopurine, and thioguanine are prodrugs that are converted to active thioguanine nucleotide metabolites or methylated by thiopurine methyltransferase (TPMT) to compounds with less pharmacologic activity. In the absence of TPMT activity, patients are likely to have higher concentrations of thioguanine nucleotides, which can pose an increased risk of severe life-threatening myelosuppression. Determining TPMT activity, either directly by phenotyping or indirectly by determining the specific genetic allele (different alleles have different enzymatic activity), can help identify patients at greater risk of severe myelosuppression. Therefore, we recommend that TPMT testing be strongly considered before initiating therapy with a thiopurine.

THIOPURINES AND TPMT

Azathioprine, mercaptopurine, and thioguanine are used for treating autoimmune and inflammatory diseases[1–3] and certain types of cancer such as leukemias and lymphomas.[1,4–6] Typically, azathioprine is used to treat nonmalignant conditions, thioguanine is used to treat malignancies, and mercaptopurine can be used to treat both malignant and nonmalignant conditions.

Although the exact mechanism of action of these drugs has not been completely elucidated, the active thioguanine nucleotide metabolites are thought to be incorporated into the DNA of leukocytes, resulting in DNA damage that subsequently leads to cell death and myelosuppression.[7–9]

Variants of the *TPMT* gene may alter the activity of the TPMT enzyme, resulting in individual variability in thiopurine metabolism. Compared with people with normal (high) TPMT activity, those with intermediate or low TPMT activity metabolize the drugs more slowly, and are likely to have higher thioguanine nucleotide concentrations and therefore an increased risk of myelosuppression.

One of the earliest correlations between TPMT activity and thiopurine-induced myelosuppression was described in a pediatric patient with acute lymphocytic leukemia.[10] After being prescribed a conventional mercaptopurine dosage (75 mg/m^2 daily), the patient developed severe myelosuppression and was observed to have a thioguanine nucleotide metabolite concentration seven times the observed population median. TPMT phenotyping demonstrated that the patient had low TPMT activity. Reducing the mercaptopurine dose by approximately 90% resulted in normalization of thioguanine nucleotide metabolite concentrations, and the myelosuppression subsequently resolved.

Approximately 10% of the population has intermediate TPMT activity and 0.3% has low or absent TPMT activity, though these percentages vary depending on ancestry.[1] Research has demonstrated that approximately 30% to 60% of

We recommend that TPMT testing be strongly considered before starting a thiopurine

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TABLE 1

Select *TPMT* genotype results and associated TPMT phenotypes

TPMT result				Predicted phenotype
*1/*1				Normal (high) activity
*1/*2	*1/*3A[a]	*1/*3B	*1/*3C	Intermediate activity
*2/*2 *3A/*3A *3B/*3B *3C/*3C	*2/*3A *3A/*3B *3B/*3C	*2/*3B *3A/*3C	*2/*3C	Low or absent activity

[a] There is a rare possibility (< 1:100,000) that the patient may have a genotype of *TPMT*3B/*3C* and would therefore have low or absent TPMT activity.

those with intermediate TPMT activity cannot tolerate a full thiopurine dose (eg, azathioprine 2–3 mg/kg/day or mercaptopurine 1.5 mg/kg/day).[1] Almost all patients with low TPMT activity will develop life-threating myelosuppression if prescribed a full thiopurine dose.[1]

About 10% of people have intermediate TPMT activity, and 0.3% have low or absent TPMT activity

SHOULD TPMT ACTIVITY BE DETERMINED FOR EVERY PATIENT PRESCRIBED A THIOPURINE?

Although determining TPMT activity in thiopurine-naïve patients will assist clinicians in selecting a thiopurine starting dose or in deciding if an alternative agent is warranted, there are instances when a clinician may elect to not perform a TPMT genotype or phenotype test. For example, determining TPMT activity is not recommended for patients who previously tolerated thiopurine therapy at full steady-state doses.

The required starting dose of a thiopurine can influence the decision on whether or not to test for TPMT activity. TPMT genotyping or phenotyping may be of most benefit for patients requiring immediate full doses of a thiopurine.[11] Ideally, TPMT activity should be determined before prescribing immediate full doses of a thiopurine. This could be achieved by preemptively ordering a TPMT test in patients likely to require immunosuppression—for example, in patients diagnosed with inflammatory or autoimmune diseases. If therapy cannot be delayed and TPMT activity is unknown, ordering a TPMT test at the time of prescribing a full thiopurine dose is still of benefit. Depending on the clinical laboratory utilized for testing, TPMT phenotype results are usually reported in 3 to 5 days, and *TPMT* genotype results are usually reported in 5 to 7 days. Because most patients will not reach steady-state concentrations for 2 to 6 weeks, clinicians could initiate immediate full doses of a thiopurine and modify therapy based on TPMT test results before accumulation of thioguanine nucleotide metabolites occurs. Caution should be used with this approach, particularly in situations where the clinical laboratory may not return results in a timely manner.

For patients who are candidates for an initial low dose of a thiopurine, clinicians may choose to slowly titrate doses based on response and tolerability instead of determining TPMT activity.[11] Depending on the starting dose and how slowly titration occurs, initiating a thiopurine at a low dose and titrating based on response can be a feasible approach for patients with intermediate TPMT activity. Because drastic thiopurine dose reductions of approximately 10-fold are required for patients with low TPMT activity, which is a much smaller dosage than most clinicians will initially prescribe, the starting dosage will likely not be low enough to prevent myelosuppression in patients with low TPMT activity.[1,10]

Determining TPMT activity can help clinicians establish an appropriate titration schedule. Patients with normal TPMT activity will usually reach thiopurine steady-state concentrations in 2 weeks, and the dosage can be titrated based on response.[1] Alterations in TPMT activity influence the pharmacokinetic parameters of thiopurines, and the time to reach steady-state is extended to 4 or 6 weeks for those with intermediate or low TPMT activity.[1] Increasing the thiopurine dosage before reaching steady state can lead to the prescribing of doses that will not be tolerated, resulting in myelosuppression.

Factors to consider when deciding if TPMT activity should be assessed include the disease state being treated and corresponding starting dose, the need for immediate full doses, and previous documented tolerance

TABLE 2

Thiopurine dosing recommendations based on TPMT phenotype[a]

TPMT phenotype	Dosing recommendations
Normal (high) activity	Initiate thiopurine therapy with the usual starting dose. Adjust thiopurine dose based on disease-specific guidelines, and allow 2 weeks to reach steady state after each dose adjustment.
Intermediate activity	If disease treatment normally starts at the "full dose," consider reducing the initial azathioprine or mercaptopurine dose by 30%–70%, and consider reducing the initial thioguanine dose by 30%–50%. Titrate doses based on tolerance and disease-specific guidelines. Depending on other immunosuppressive therapy, emphasis should be on reducing thiopurine doses if myelosuppression occurs. After each dose adjustment, allow 2–4 weeks to reach steady state.
Low or absent activity	For nonmalignant conditions consider alternative agents. For malignancy, or if a thiopurine is used for a nonmalignant condition, consider reducing the "full dose" of thiopurine by 90% and administer three times per week instead of daily. Titrate doses based on tolerance and disease-specific guidelines. Depending on other immunosuppressive therapy, emphasis should be on reducing thiopurine doses if myelosuppression occurs. After each dose adjustment, allow 4–6 weeks to reach steady state.

[a] Dosing recommendations are based on the Clinical Pharmacogenetic Implementation Consortium TPMT-thiopurine dosing guideline.[1]

of thiopurines at steady-state doses. As with many aspects of medicine that have multiple options, coupled with an increase in patient access to healthcare information, the decision to test for TPMT activity may include shared decision-making between patients and providers. Although TPMT genotyping or phenotyping can help identify those at greatest risk of severe myelosuppression, such assays do not replace routine monitoring for myelosuppression, hepatotoxicity, or pancreatitis that may be caused by thiopurines.

WHAT TESTS ARE AVAILABLE TO DETERMINE TPMT ACTIVITY?

Patients with intermediate or low TPMT activity can be identified by either genotyping or phenotyping. There are considerations, though, that clinicians should be aware of before selecting a particular test.

TPMT genotyping

Four *TPMT* alleles, *TPMT*2*, **3A*, **3B*, and **3C*, account for over 90% of inactivating polymorphisms.[12] Therefore, most reference laboratories only analyze for those genetic variants. Based on the reported test result, a predicted phenotype (eg, normal, intermediate, or low TPMT activity) can be assigned. **Table 1** lists the predicted phenotypes for select genotyping results.

TPMT phenotyping

Phenotyping quantitates TPMT enzyme activity in erythrocytes, and based on the result, patients are classified as having normal, intermediate, or low TPMT activity. Because internal standards and other testing conditions may differ between reference laboratories, test results must be interpreted in the context of the laboratory that performed the assay.

***TPMT*2, *3A, *3B,* and **3C* account for > 90% of inactivating polymorphisms**

Which test is right for my patient?

In most cases, either the genotype or the phenotype test provides sufficient information to guide thiopurine therapy. There are certain circumstances, though, in which the genotype or phenotype test is less informative.

TPMT genotyping, when performed using a blood specimen, is not recommended in those with a history of allogeneic bone marrow transplantation, as the result would reflect the donor's genotype, not the patient's. In such instances, monitoring of white blood cell counts and thiopurine metabolites may be more beneficial.

TPMT phenotyping may be inaccurate if performed within 30 to 90 days of an erythrocyte transfusion, as the test result may be influenced by donor erythrocytes. If a patient is receiving erythrocyte transfusions, *TPMT* genotyping is preferable to phenotyping.

Test cost may also be a consideration when determining if the genotype or phenotype test

is best for your patient. Costs vary by laboratory, but phenotyping is generally less expensive than genotyping. The cost of genotyping, though, continues to decrease.[13] The approximate commercial cost is $200 for phenotyping and $450 for genotyping, but laboratory fees may be substantially higher. Several insurance plans, including Medicare, cover TPMT testing, but reimbursement and copayments vary, depending on the patient's specific plan.

There are conflicting data as to whether determining TPMT status is[11,14–18] or is not[19] cost-effective. Multiple studies suggest that the cost of genotyping a sufficient number of patients to identify a single individual at high risk of myelosuppression is cheaper than the costs associated with treating an adverse event. Additional cost-benefit studies are needed, particularly studies that consider how bundled payments and outcomes-based reimbursement influence cost-effectiveness.

MODIFYING THIOPURINE THERAPY BASED ON TPMT ACTIVITY

Patients with intermediate or low TPMT activity have an increased risk of myelosuppression on thiopurine therapy

There is a strong correlation between TPMT activity and tolerated thiopurine doses, with those having intermediate or low TPMT activity requiring lower doses.[10,20–23] Adjusting mercaptopurine doses based on TPMT activity to prevent hematopoietic toxicity has been successfully demonstrated in pediatric patients with acute lymphoblastic leukemia.[24] Furthermore, reducing initial thiopurine doses to avoid myelosuppression and titrating based on response has been shown to not compromise outcomes.[1,25,26] The Clinical Pharmacogenetic Implementation Consortium (CPIC) has developed an evidence-based guideline on how to adjust thiopurine doses based on TPMT activity,[1] summarized in **Table 2**. These dosing recommendations are classified as "strong."

Patients with normal TPMT activity should be prescribed the usual thiopurine starting dose as indicated by disease-specific guidelines.

For those with intermediate TPMT activity, the CPIC guideline recommends reducing the initial targeted full dose of azathioprine and mercaptopurine by 30% to 70% and reducing the targeted full dose of thioguanine by 30% to 50%. The percentage of dose reduction depends on the targeted full dose. Siegel and Sands[27] suggested that for those who are diagnosed with inflammatory bowel disease and have intermediate TPMT activity, azathioprine should be initiated at a low dose and titrated to 1.25 mg/kg and mercaptopurine should be initiated at a low dose and titrated to 0.75 mg/kg. Based on these titration goals, if the targeted full dose for mercaptopurine is 1 mg/kg, then a dose reduction of approximately 30% would be more appropriate. If the targeted full dose is 1.5 mg/kg, a dose reduction of approximately 50% would be more appropriate. Thiopurine doses should be titrated based on response and disease-specific guidelines, allowing 2 to 4 weeks to reach steady state before dose titration.

For those with low TPMT activity, alternative therapy should be considered for nonmalignant conditions because of the risk of severe myelosuppression. For malignancy, or if a thiopurine is warranted for a nonmalignant condition, consider a 90% dose reduction and give the drug three times per week instead of daily. For example, acute lymphoblastic leukemia patients with low TPMT activity can be started on mercaptopurine 10 mg/m^2 three times per week instead of the usual starting dose.[10] Thiopurine doses should be titrated based on response and disease-specific guidelines, allowing 4 to 6 weeks to reach steady state before dose titration.

RECOMMENDATIONS

Individuals with intermediate or low TPMT activity have an increased risk of myelosuppression. Because of the elevated risk for morbidity and death, especially for patients with low TPMT activity, multiple guidelines and regulatory agencies recommend TPMT genotyping or phenotyping if a thiopurine is prescribed.[25,28–32] Although additional cost-benefit analysis studies are needed, evidence suggests testing for TPMT activity may be cheaper than the costs associated with treating myelosuppression.

In view of treatment guidelines, the recommendations of regulatory agencies, cost-benefit analyses, and the availability of gene-based dosing recommendations, we consider the benefits of testing for TPMT activity to greatly outweigh any associated risks. Therefore, we recommend that TPMT testing be strongly considered before initiating therapy with a thiopurine. ■

REFERENCES

1. **Relling MV, Gardner EE, Sandborn WJ, et al; Clinical Pharmacogenetics Implementation Consortium**. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clin Pharmacol Ther 2011; 89:387–391.
2. **Ansari A, Arenas M, Greenfield SM, et al**. Prospective evaluation of the pharmacogenetics of azathioprine in the treatment of inflammatory bowel disease. Aliment Pharmacol Ther 2008; 28:973–983.
3. **Beswick L, Friedman AB, Sparrow MP**. The role of thiopurine metabolite monitoring in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2014; 8:383–392.
4. **Gervasini G, Vagace JM**. Impact of genetic polymorphisms on chemotherapy toxicity in childhood acute lymphoblastic leukemia. Front Genet 2012; 3:249.
5. **Levinsen M, Rotevatn EØ, Rosthøj S, et al; Nordic Society of Paediatric Haematology, Oncology**. Pharmacogenetically based dosing of thiopurines in childhood acute lymphoblastic leukemia: influence on cure rates and risk of second cancer. Pediatr Blood Cancer 2014; 61:797–802.
6. **Adam de Beaumais T, Jacqz-Aigrain E**. Pharmacogenetic determinants of mercaptopurine disposition in children with acute lymphoblastic leukemia. Eur J Clin Pharmacol 2012; 68:1233–1242.
7. **Derijks LJ, Gilissen LP, Hooymans PM, Hommes DW**. Review article: thiopurines in inflammatory bowel disease. Aliment Pharmacol Ther 2006; 24:715–729.
8. **Fairchild CR, Maybaum J, Kennedy KA**. Concurrent unilateral chromatid damage and DNA strand breakage in response to 6-thioguanine treatment. Biochem Pharmacol 1986; 35:3533–3541.
9. **Karran P**. Thiopurines, DNA damage, DNA repair and therapy-related cancer. Br Med Bull 2006; 79–80:153–170.
10. **Evans WE, Horner M, Chu YQ, Kalwinsky D, Roberts WM**. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia. J Pediatr 1991; 119:985–989.
11. **Gardiner SJ, Gearry RB, Barclay ML, Begg EJ**. Two cases of thiopurine methyltransferase (TPMT) deficiency—a lucky save and a near miss with azathioprine. Br J Clin Pharmacol 2006; 62:473–476.
12. **Relling MV, Gardner EE, Sandborn WJ, et al**. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther 2013; 93:324–325.
13. **Altman RB**. Pharmacogenomics: "noninferiority" is sufficient for initial implementation. Clin Pharmacol Ther 2011; 89:348–350.
14. **van den Akker-van Marle ME, Gurwitz D, Detmar SB, et al**. Cost-effectiveness of pharmacogenomics in clinical practice: a case study of thiopurine methyltransferase genotyping in acute lymphoblastic leukemia in Europe. Pharmacogenomics 2006; 7:783–792.
15. **Clunie GP, Lennard L**. Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine. Rheumatology (Oxford) 2004; 43:13–18.
16. **Dubinsky MC, Reyes E, Ofman J, Chiou CF, Wade S, Sandborn WJ**. A cost-effectiveness analysis of alternative disease management strategies in patients with Crohn's disease treated with azathioprine or 6-mercaptopurine. Am J Gastroenterol 2005; 100:2239–2247.
17. **Winter J, Walker A, Shapiro D, Gaffney D, Spooner RJ, Mills PR**. Cost-effectiveness of thiopurine methyltransferase genotype screening in patients about to commence azathioprine therapy for treatment of inflammatory bowel disease. Aliment Pharmacol Ther 2004; 20:593–599.
18. **Marra CA, Esdaile JM, Anis AH**. Practical pharmacogenetics: the cost effectiveness of screening for thiopurine s-methyltransferase polymorphisms in patients with rheumatological conditions treated with azathioprine. J Rheumatol 2002; 29:2507–2512.
19. **Donnan JR, Ungar WJ, Mathews M, Hancock-Howard RL, Rahman P**. A cost effectiveness analysis of thiopurine methyltransferase testing for guiding 6-mercaptopurine dosing in children with acute lymphoblastic leukemia. Pediatr Blood Cancer 2011; 57:231–239.
20. **Lennard L, Gibson BE, Nicole T, Lilleyman JS**. Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia. Arch Dis Child 1993; 69:577–579.
21. **Hindorf U, Lindqvist M, Hildebrand H, Fagerberg U, Almer S**. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. Aliment Pharmacol Ther 2006; 24:331–342.
22. **Relling MV, Hancock ML, Rivera GK, et al**. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 1999; 91:2001–2008.
23. **Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE**. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood 1999; 93:2817–2823.
24. **Pui CH, Pei D, Sandlund JT, et al**. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia 2010; 24:371–382.
25. **Ford LT, Berg JD**. Thiopurine S-methyltransferase (TPMT) assessment prior to starting thiopurine drug treatment; a pharmacogenomic test whose time has come. J Clin Pathol 2010; 63:288–295.
26. **Schmiegelow K, Forestier E, Hellebostad M, et al; Nordic Society of Paediatric Haematology and Oncology**. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 2010; 24:345–354.
27. **Siegel CA, Sands BE**. Review article: practical management of inflammatory bowel disease patients taking immunomodulators. Aliment Pharmacol Ther 2005; 22:1–16.
28. **Mayberry JF, Lobo A, Ford AC, Thomas A**. NICE clinical guideline (CG152): the management of Crohn's disease in adults, children and young people. Aliment Pharmacol Ther 2013; 37:195–203
29. **Mowat C, Cole A, Windsor A, et al; IBD Section of the British Society of Gastroenterology**. Guidelines for the management of inflammatory bowel disease in adults. Gut 2011; 60:571–607.
30. **Turner D, Levine A, Escher JC, et al; European Crohn's and Colitis Organization; European Society for Paediatric Gastroenterology, Hepatology, and Nutrition**. Management of pediatric ulcerative colitis: joint ECCO and ESPGHAN evidence-based consensus guidelines. J Pediatr Gastroenterol Nutr 2012; 55:340–361.
31. **Bernstein CN, Fried M, Krabshuis JH, et al**. World Gastroenterology Organization Practice Guidelines for the diagnosis and management of IBD in 2010. Inflamm Bowel Dis 2010; 16:112–124.
32. **Becquemont L, Alfirevic A, Amstutz U, et al**. Practical recommendations for pharmacogenomics-based prescription: 2010 ESF-UB Conference on Pharmacogenetics and Pharmacogenomics. Pharmacogenomics 2011; 12:113–124.

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