



Azathioprine Therapy and *TPMT* Genotype

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Introduction

Azathioprine is an immunosuppressant that belongs to the drug class of thiopurines. It is used in combination with other drugs to prevent kidney transplant rejection and in the management of rheumatoid arthritis when other treatments have not been effective (1). In addition, off-label uses include the treatment of inflammatory bowel disease (2).

Azathioprine is a prodrug that must first be activated to form thioguanine nucleotides (TGNs), the major active metabolites. Thiopurine S-methyltransferase (*TPMT*) inactivates azathioprine, leaving less parent drug available to form TGNs.

An adverse effect of azathioprine therapy is bone marrow suppression, which can occur in any patient, is dose-dependent, and may be reversed by reducing the dose of azathioprine. However, patients who carry two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression when treated with azathioprine, due to high levels of TGNs. Patients who carry one nonfunctional *TPMT* allele may also be unable to tolerate conventional doses of azathioprine (3, 4).

The FDA recommends *TPMT* genotyping or phenotyping before starting treatment with azathioprine. This allows patients who are at increased risk for toxicity to be identified and for the starting dose of azathioprine to be reduced, or for an alternative therapy to be used (1).

The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published recommendations for *TPMT* genotype-based azathioprine dosing. These recommendations include:

Consider an alternate agent or extreme dose reduction of azathioprine for patients with low or deficient *TPMT* activity. Start at 30-70% of target dose for patients with intermediate enzyme activity (see Table 1) (2-4).

Table 1. *TPMT* phenotypes and the therapeutic recommendations for azathioprine therapy, adapted from CPIC

| Phenotype | Phenotype details | <i>TPMT</i> Genotype | Examples of diplotypes | Therapeutic recommendations for azathioprine |
|---------------------------------|--|---|--|--|
| Homozygous wild-type (“normal”) | High enzyme activity. Found in ~86–97% of patients. | Two or more functional <i>TPMT</i> alleles | *1/*1 | Start with normal starting dose (e.g., 2–3 mg/kg/d) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment. |
| Heterozygous | Intermediate enzyme activity. Found in ~3–14% of patients. | One functional <i>TPMT</i> allele plus one nonfunctional <i>TPMT</i> allele | *1/*2 *1/*3A *1/*3B *1/*3C *1/*4 | If disease treatment normally starts at the “full dose”, consider starting at 30–70% of target dose (e.g., 1–1.5 mg/kg/d), and titrate based on tolerance. Allow 2–4 weeks to reach steady state after each dose adjustment. |
| Homozygous variant | Low or deficient enzyme activity. Found in ~1 in 178 to 1~3736 patients. | Two nonfunctional <i>TPMT</i> alleles | *3A/*3A *2/*3A *3C/*3A *3C/*4 *3C/*2 *3A/*4 | Consider alternative agents. If using azathioprine start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. Azathioprine is the likely cause of myelosuppression. |

The strength of therapeutic recommendations is “strong” for all phenotypes.

Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. *Clinical pharmacology and therapeutics*. 2011;89(3):387–91 (3, 4).

Drug Class: Thiopurines

Thiopurines are used as anticancer agents and as immunosuppressants in inflammatory bowel disease, rheumatoid arthritis, and other autoimmune conditions. Three thiopurines are used clinically: thioguanine, mercaptopurine, and azathioprine (a prodrug for mercaptopurine). All three agents have similar effects but are typically used for different indications. Thioguanine is most commonly used in the treatment of myeloid leukemias, mercaptopurine is used for lymphoid malignancies, and mercaptopurine and azathioprine are used for immune conditions.

Thiopurines are either activated to form TGNs (the major active metabolite) or deactivated by *TPMT*. Individuals who carry two non-functional *TPMT* alleles (“*TPMT* homozygotes”) universally experience life-threatening bone marrow suppression because of high levels of TGNs when treated with conventional doses. Individuals who carry one non-functional *TPMT* allele (“*TPMT* heterozygotes”) may also be unable to tolerate conventional doses of thiopurines due to increased levels of TGNs.

Drug: Azathioprine

Azathioprine is an immunosuppressive agent that is used in combination with other drugs to prevent the rejection of kidney transplants. It is also used in the management of active rheumatoid arthritis (1).

An off-label use of azathioprine is in the treatment of inflammatory bowel disease (IBD). Along with the closely related drug mercaptopurine (azathioprine is metabolized to mercaptopurine), azathioprine is used as an

“immunomodulator” and as a “steroid-sparing agent” in the treatment of Crohn’s disease and ulcerative colitis (2).

Azathioprine is a slow-acting drug and for IBD, it typically takes at least three months of therapy before a therapeutic effect is observed. Therefore, azathioprine is used for the induction and maintenance of IBD remission rather than as a monotherapy for acute relapses (5). Because the discontinuation of azathioprine is associated with a high rate of relapse of IBD, azathioprine is usually continued long-term if there are no adverse effects (6, 7).

The use of azathioprine or the related drug mercaptopurine has been associated with a 4-fold increased risk of developing lymphoma, which does not persist after discontinuation of therapy (8, 9).

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

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

Like all thiopurines, azathioprine is a purine analogue, and acts as an antimetabolite by interfering with nucleic acid synthesis and inhibiting purine metabolism. Azathioprine is first metabolized to mercaptopurine, which is then activated via HPRT1 (hypoxanthine phosphoribosyltransferase). This is followed by a series of reactions to form TGNs. The cytotoxicity of azathioprine is due, in part, to the incorporation of TGNs into DNA.

Inactivation of azathioprine occurs via two different pathways, via methylation (by TPMT) or via oxidation (by xanthine oxidase). TPMT activity is highly variable in patients because of genetic polymorphism in the *TPMT* gene.

One of the most frequent adverse reactions to azathioprine is myelosuppression, which can occur in any patient, and can usually be reversed by decreasing the dose of azathioprine. However, all patients who carry two nonfunctional *TPMT* alleles (approximately 0.3%) experience life-threatening myelosuppression after starting treatment with conventional doses of azathioprine due to high levels of TGNs.

Individuals who are heterozygous for nonfunctional *TPMT* alleles (approximately 10%) are at a significantly higher risk for toxicity than individuals with two functional alleles. However, some of these individuals, approximately 40–70%, can tolerate the full dose of azathioprine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as MeMPN (methylmercaptopurine nucleotides), than homozygous-deficient individuals (3, 4).

Approximately 90% of individuals have normal TPMT activity with two functional alleles; however, all individuals receiving azathioprine require close monitoring (3, 4, 10, 11). One study reports that in patients with IBD receiving thiopurine therapy, TPMT polymorphisms are associated with the overall incidence of adverse reactions and with bone marrow toxicity, but not with other adverse reactions, such as liver damage and pancreatitis. Therefore, although determining *TPMT* genotype is helpful before initiating therapy, regular blood tests to monitor for side effects are needed during therapy (12, 13).

The other azathioprine inactivation pathway is via oxidation, which is catalyzed by xanthine oxidase. If this pathway is inhibited, for example, in patients taking allopurinol (an inhibitor of xanthine oxidase), the decreased break down of azathioprine can lead to azathioprine toxicity (13). However, some studies have found that the co-administration of allopurinol, with a reduced dose of azathioprine (or mercaptopurine), can help optimize the treatment response in patients with IBD (14, 15).

Gene: *TPMT*

The *TPMT* gene encodes one of the important enzymes of phase II metabolism, thiopurine S-methyltransferase. *TPMT* is one of the main enzymes involved in the metabolism of thiopurines, such as azathioprine. *TPMT* activity is inherited as a co-dominant trait, as the *TPMT* gene is highly polymorphic with over 40 reported variant alleles (16-19).

The wild-type *TPMT**1 allele is associated with normal enzyme activity. Individuals who are homozygous for *TPMT**1 (*TPMT* normal metabolizers) are more likely to have a typical response to azathioprine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) (3, 4).

Individuals who are *TPMT* poor (approximately 0.3%) or intermediate (approximately 3–14%) metabolizers carry variant *TPMT* alleles that encode reduced or absent enzyme activity. Three variant *TPMT* alleles account for over 90% of the reduced or absent activity *TPMT* alleles (16, 17):

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

The frequency of *TPMT* alleles varies among different populations. In the United States, the most common low-activity allele in the Caucasian population is *TPMT**3A (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently (18, 19).

In East Asian, African-American, and some African populations, the most common variant is *TPMT**3C (~2%), although *TPMT**8 may be more common in African populations than previously thought (~2%). In general, *TPMT**2 occurs much less commonly, and *TPMT**3B occurs rarely (18, 20).

Genetic Testing

Genetic testing is available for several *TPMT* variant alleles, which most commonly includes *TPMT**2, *3A, and *3C as they account for >90% of inactivating alleles. Of note, rare and/or previously undiscovered variants will not be detected by variant-specific genotyping methods (3, 4, 21-24).

TPMT phenotype enzyme activity testing is also available by measuring *TPMT* activity in red blood cells directly. However, the results will not be accurate in patients who have received recent blood transfusions (13) and *TPMT* activity will also be falsely low in patients with leukemia, because of atypical hematopoiesis (25).

One study reported that *TPMT* genotyping was more reliable than phenotyping in identifying patients at risk of adverse reactions from thiopurine treatment (26). In addition, several studies report that the *TPMT* genotype is a better indicator than *TPMT* activity for predicting TGN accumulation or treatment outcome (11, 27-29).

Therapeutic Recommendations based on Genotype

This section contains excerpted¹ information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2015 Statement from the US Food and Drug Administration (FDA): *TPMT* TESTING CANNOT SUBSTITUTE FOR COMPLETE BLOOD COUNT (CBC) MONITORING IN PATIENTS RECEIVING AZATHIOPRINE. *TPMT* genotyping or phenotyping can be used to identify patients with absent or reduced

¹ The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

TPMT activity. Patients with low or absent TPMT activity are at an increased risk of developing severe, life threatening myelotoxicity from azathioprine if conventional doses are given. Physicians may consider alternative therapies for patients who have low or absent TPMT activity (homozygous for non-functional alleles). Azathioprine should be administered with caution to patients having one non-functional allele (heterozygous) who are at risk for reduced TPMT activity that may lead to toxicity if conventional doses are given. Dosage reduction is recommended in patients with reduced TPMT activity. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction.

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

2013 Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for *TPMT* status is recommended prior to starting azathioprine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, either an alternative agent should be used, or the doses of azathioprine should be drastically reduced. In heterozygous individuals, depending on the disease being treated, starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

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

Nomenclature

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

The TPMT Nomenclature Committee defines the nomenclature and numbering of novel TPMT variants: <http://www.imh.liu.se/tpmtalleles>

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): <http://www.hgvs.org/content/guidelines>

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Version History

To view an earlier version of this summary (Update: March 18, 2013), please [click here](#).

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